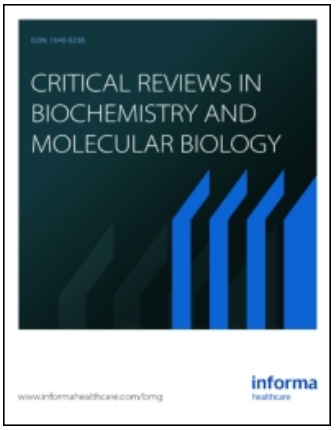

CRC Critical Reviews in Biochemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ibmg19

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Published online: 09 Apr 2015.

To cite this article: H. P. C. Driessen, W. W. De Jong, G. I. Tesser & H. Bloemendal (1985) The Mechanism of N-Terminal Acetylation of Protein, CRC Critical Reviews in Biochemistry, 18:4, 281-325

To link to this article: http://dx.doi.org/10.3109/10409238509086784

THE MECHANISM OF N-TERMINAL ACETYLATION OF PROTEINS

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I. INTRODUCTION

N-terminal blocking of proteins is a widespread phenomenon in eukaryotes, prokaryotes, and viruses.[1-3] Formyl, acetyl, pyruvoyl, α-ketobutyryl, glucuronyl, α-aminoacyl, pyroglutamyl, murein, glucose,[2,3] and carbon dioxide[4] have been recognized as N^{α}-acylating groups. Some of these blocking groups are extremely rare. The first polypeptide with an N^{α}-acetyl group was discovered in 1958 by Narita.[5] Thereafter, numerous proteins followed. Brown and Roberts provided evidence that about 80% of the soluble proteins from Ehrlich ascites cells are N^{α}-acetylated.[6] Approximately 90% of the proteins from mouse L cells seem, also, to be N^{α}-acetylated.[7] In lower eukaryotic organisms (*Saccharomyces fragilis, Neurospora crassa*), about 50% of the soluble proteins are acetylated.[8] These data demonstrate that N^{α}-acetyl is a very important blocking group.

The blocking nature of the N^{α}-acetyl group becomes apparent during protein sequencing, since the Edman degradation is prevented. The group can only be removed from the N-terminus of a protein under conditions which are harmful to the polypeptide chain itself. The general strategy to solve this problem is to isolate, after enzymatic digestions of the protein, the peptide which contains the blocked N-terminus. Several methods have been used to both detect the acetyl group and the sequence of the blocked peptide,[5,9-12] but nowadays this is most often done by mass spectrometry.[13-15]

Another feature of a protein blocked by an N^{α}-acetyl group is its protection from the action of aminopeptidases. This aspect will be discussed when we describe some current ideas about the function of N^{α}-acetylation. Furthermore, we shall deal with the mechanistic aspects of the attachment of the acetyl group to the protein and the structural characteristics of the N-termini of proteins which are N^{α}-acetylated.

II. WHY ARE PROTEINS N^{α}-ACETYLATED?

It has been suggested that a general function for N^{α}-acetylation is the protection of proteins from proteolytic degradation by aminopeptidases.[16] Experiments to sustain this hypothesis

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have not shown significant differences in the turnover rate of acetylated and nonacetylated forms of feline β-globin[17] and of proteins from mouse L cells.[7] However, these cases have to be interpreted with great care, since the acetylated and nonacetylated proteins were not of the same type. In *Dictyostelium discoideum*, a minor nonacetylated form of cytoplasmic actin with a rapid turnover was found next to a major and more stable acetylated form.[18] However, this turnover is probably not caused by proteolytic breakdown, but rather reflects a precursor-product relationship. For the acetylated form of cytoplasmic actin from cultured *Drosophila* cells[19] and the in vitro acetylated terminal octapeptide of cholecystokinin[20] it has been shown that the acetyl group protects against N-terminal degradation by kidney leucine aminopeptidase and soluble peptidases from the gall bladder, respectively. In the case of the proteins of the eye lens, which have to remain intact during the lifespan of the organ, αA_2-crystallin has an N^{α}-acetyl group which is not essential for protection against N-terminal degradation by lens leucine aminopeptidase.[21] Here the three-dimensional structure of the native protein seems to be a more important factor. Although the N^{α}-acetyl group by its very nature protects against exopeptidases, no decisive evidence has been presented for such a protective role of N^{α}-acetyl groups in vivo.

Although a clear general function for N^{α}-acetylation has not been assessed with certainty, some specific effects for a small number of proteins have been observed. Contrary to normal nonacetylated hemoglobin, feline hemoglobin with N^{α}-acetylated β-chain amino termini is insensitive to the modifying influence on oxygen affinity of organic phosphates.[22] A similar effect has been noted for the human mutant hemoglobin Raleigh (β_1 valine→acetylalanine)[23] and the minor human fetal hemoglobin F_1.[24] N^{α}-acetylation of two of the products from the precursor protein proopiomelanocortin has a profound regulatory effect on the biological activity of these polypeptides: the opioid activity of β-endorphin is completely suppressed,[25,26] while the melanotropic effect of α-MSH is increased.[27] In the first case N^{α}-acetylation is possibly a reversible process. Both acetylated and nonacetylated cytoplasmic actin from cultured *Drosophila* cells participate in the assembly of microfilaments,[19] the latter, however, with less efficiency. A mutant of *Escherichia coli*, in which ribosomal protein S5 is not acetylated, exhibits thermosensitivity.[28] Nonacetylated NADP-specific glutamate de-hydrogenase in a mutant of *Neurospora crassa* is heat-unstable, in contrast to the acetylated form.[29] Moreover, it has been suggested that N^{α}-acetylation is an additional way of acetyl-group handling in the cell,[30] in which case it would resemble other α-amino-group modifications by compounds that reflect high physiological concentrations (e.g., CO_2). This assumption may explain the consistent variation in acetylation during physiological conditions in the yeast alcohol dehydrogenase system, where no effect of acetylation on enzyme activity has been observed.[30]

III. HOW ARE PROTEINS N^{α}-ACETYLATED?

A. At Which Stage?

It was initially thought that different N^{α}-acetyl aminoacyl-tRNAs might serve as the initiators of protein synthesis in various eukaryotic systems[31-33] and that, therefore, N^{α}-acetylated proteins with different N-terminal amino acids should be produced. However, soon it became evident that methionine is the initiating residue of eukaryotic proteins as it is in prokaryotes;[34-37] even on direct search no N^{α}-acetylseryl-tRNA could be detected in rat liver.[38] Moreover, for some N^{α}-acetylated proteins it was shown unequivocally that initiation starts with methionine, e.g., for α-crystallin,[39] ovalbumin,[40] and histones.[41] In many proteins the initiator Met is removed. In *Escherichia coli* the responsible methionine aminopeptidase appears to have a preference for cleaving the Met-X peptide bond if X is Ala or Ser.[42] A ribosomal-bound aminopeptidase has been isolated from this organism which preferentially removes N-terminal methionine from peptides.[43] However, if position 2 is occupied by an

Asp, no hydrolysis takes place. For yeast there is evidence that the methionine aminopeptidase hydrolyzes the Met-X bond very efficiently in cases where X is Ala or Thr, less efficiently if X is Val, and not at all when X is Leu, Ile, or Arg.[44] For immunoglobins and hemoglobin it has been shown that Met-X is cleaved if X is Ala or Val, but not if X is Glu or Asp.[45] These observations clearly demonstrate the important role of the amino acid at position 2. However, the overall conformation of the N-terminal region may, also, be an important factor for removal of the N-terminal Met. It is now thought that for most proteins acetylation takes place at the level of the peptidyl-tRNA, which means that it represents a post-initiational process. It is an enzymatic process that requires acetyl coenzyme A as acetyl donor.

Bloemendal and Strous showed that αA_2 crystallin is acetylated in vitro when a nascent chain of about 25 residues protrudes from the ribosome.[46-48] Acetylation is complete at a length of 50 residues. In ovalbumin, the initiator methionine is removed in vitro when the nascent chain is 20 amino acids long;[49] acetylation takes place at a length of 44 residues. In vitro the β-chain of cat hemoglobin B is acetylated when the nascent chain is about 30 amino acid residues long.[50] That acetylation is a post-initiational process has, also, been suggested for translation of brome mosaic viral RNA in vitro[51] and for rat liver polyribosomes in vitro.[52-54] Acetylation, thus starts when the nascent chain begins to emerge from the ribosome at a length of about 25 residues, or somewhat later if the initiator methionine has to be removed first. Since it is assumed that protein chains are not yet completely folded when emerging from the ribosome, the N-terminal amino acid sequence of a chain may be expected to be an important factor in N^{α}-acetylation.

B. The Enzyme

1. Properties

N^{α}-acetylation is an enzymatic process, and the enzyme responsible has been demonstrated and studied in *E. coli* for ribosomal protein L12,[55] for ribosome-associated proteins from rat liver,[53,54,56] in calf lens,[57,58] rat pituitary,[59-61] ox pituitary,[61] hen's oviduct,[62] and in a ribosomal fraction of wheat germ.[63]

The enzyme from hen's oviduct has been partially purified.[62] It has a pH optimum of 7.2 and a relative molecular mass of about 250,000. Extensive purification was difficult because of the instability of the enzyme. Using N-terminal ACTH-fragments as substrates and acetyl coenzyme A as acetyl donor, this enzyme in vitro preferentially acetylates the α-NH_2 group, as does the enzyme from rat pituitary.[59,60] The latter enzyme is localized predominantly in subcellular fractions sedimenting above 10,000 g; it has a broad pH optimum with a maximum near 7.6 and is very unstable. Glembotski showed that bovine acetyltransferase activity, which is believed to be specific for the formation of α-MSH and N^{α}-Ac-endorphin, is present in the secretory granules from the intermediate pituitary.[61] However, the activity is not membrane-bound. The bovine enzyme has a pH optimum of 7.0. The enzyme from wheat germ is associated with the ribosome fraction and catalyzes the transfer of acetyl groups from acetyl coenzyme A to the N terminus of synthetic des-N^{α}-Ac-thymosin α_1.[63] The pH optimum is below 7.5. The enzyme is activated considerably by KCl concentrations as high as 3.0 *M*. The bovine lens N^{α}-acetyltransferase acetylates amino-terminal ACTH-fragments exclusively at the N terminus using acetyl coenzyme A.[57] The enzyme has a pH optimum near 7.4, an apparent relative molecular mass of 170,000, and an isoelectric point of about 5.[58] This enzyme has been purified 760-fold, although the purification was difficult to reproduce due to extreme instability of the protein.

Acetyl coenzyme A has an apparent Km of 2.2 μ*M* for the rat pituitary enzyme (270 μ*M* ACTH-(1-24)),[60] 8μ*M* for the ox pituitary enzyme (160 μ*M* ACTH-(1-13)-NH_2)[61], and 4.6 μ*M* for the ox lens enzyme (310 μ*M*[Nle^4]-ACTH-(1-10)).[58] These values are roughly 1/10 of the estimated intracellular acetyl coenzyme A concentration.[64]

In conclusion, the nature of the enzymes responsible for N^{α}-acetylation of proteins is still

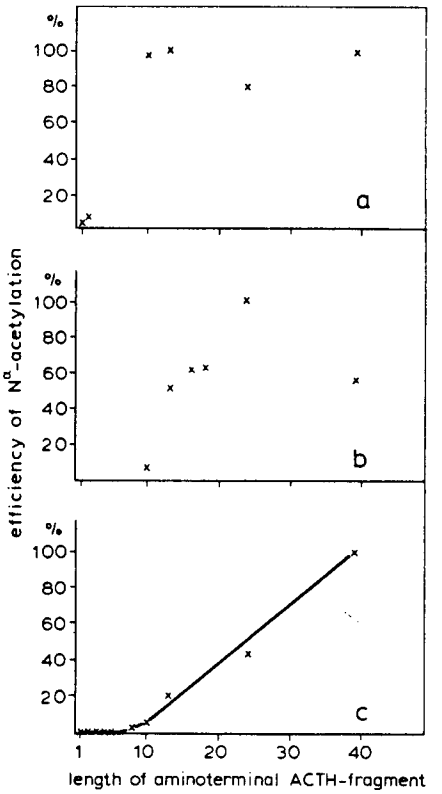

FIGURE 1. Substrate specificity of N^{α}-acetyltransferases from rat pituitary (a), ox intermediate pituitary secretory granule (b), and ox lens (c) with regard to the length of the aminoterminal ACTH-fragments used as substrates. Efficiency is expressed relative to the highest activity measured.

largely unknown. This is due to their low concentration and extreme instability after purification.

2. Substrate Specificity

The substrate specificity of several acetyltransferases has been explored, especially with regard to the chain length of the substrate. The enzyme from rat pituitary[59] needs the N-terminal serine of amino-terminal ACTH-fragments and works equally well with ACTH-(1-10), ACTH-(1-13)-NH_2, ACTH-(1-24), and ACTH-(1-39) (Figure 1a). Free serine and ACTH-(1-2) are not acetylated. Further study corroborated this picture[60] suggesting that the information required for N^{α}-acetylation resides in the first ten amino acids of ACTH-related molecules.

Glembotski[61] carried out extensive kinetic measurements on the enzyme from ox intermediate pituitary secretory granules (Figure 1b). Peptides longer than 18 amino acids showed complex non-Michaelis-Menten kinetics. The presence of the N-terminal Ser-Tyr sequence seems to be required for binding to the catalytic site, but is not sufficient to allow acetylation. Actually a more distant binding site, that needs the residues at approximately position 11 through 18 of ACTH-related peptides, has to be occupied to allow N^{α}-acetylation.

The bovine lens enzyme showed a different behavior compared to amino-terminal ACTH-fragments as substrates[58] (Figure 1c). The length of the fragment is minimally 8 residues. Thereafter, the rate of N^{α}-acetylation increases roughly linearly up to a length of at least 39

Table 1
DISTRIBUTION OF N^{α}-ACETYLTRANSFERASE ACTIVITY IN VARIOUS ORGANS OF THE RAT. RESULTS HAVE BEEN CORRECTED FOR ACETYLATION OF ENDOGENOUS SUBSTRATES IN THE ENZYME PREPARATION[59]

Source	Relative specific activity
Pituitary	100
Lens	18
Heart	11
Lung	24
Kidney	15
Liver	18
Muscle	31
Brain (except pituitary)	41
Serum	1

residues. The reason for this behavior is not known. However, one has to keep in mind that the ACTH-fragments are not natural substrates for the lens enzyme. Replacing the serine at position 1 by the corresponding D-amino acid in ACTH-(1-10) results in a substrate which is not acetylated.[57] The stereochemistry of the first residue is, therefore, a very important factor in N^{α}-acetylation.

The information available at the moment indeed stresses the importance of the first residue and shows that in addition a minimal length of the peptide is necessary.

3. Distribution and Similarity of N^{α}-Acetyltransferases

The distribution of N^{α}-acetyltransferase activity in various organs of rat has been investigated by Woodford et al.[59] (Table 1), using ACTH-(1-10)-decapeptide as a substrate. The extracts of lung, muscle, and brain have a considerable activity, although none is as efficient as that of the pituitary. The lens has a lower specific activity due to its high protein content. It is clear, however, that some N^{α}-acetyltransferase activity is present in all organs but not in serum. Erythrocytes have not been investigated in this study. Several reports have provided evidence that acetylation of proteins can take place in heterologous systems. Berns et al. showed that calf lens α-crystallin A_2 mRNA directs the synthesis of α-crystallin A_2 polypeptides which become acetylated in frog oocytes[65] and in a reticulocyte lysate.[66] Brome mosaic virus RNA produces N^{α}-acetylated coat protein in a cell-free system from wheat germ. The latter system is also capable of acetylating synthetic N^{α}-desacetyl thymosin α_1[63] and rat cytoplasmic actins.[67] Likewise, the reticulocyte lysate gives acetylated cytoplasmic actin of *Drosophila*[19] and *Dictyostelium discoideum*.[68,69] In experiments with isolated N^{α}-acetyltransferases, it has been shown that the enzymes from hen's oviduct[62] and bovine lens[57] acetylate the N terminus of amino-terminal ACTH-fragments, the (1-13)-sequence of which is normally acetylated by pituitary N^{α}-acetyltransferase to produce α-MSH.[61,70] Thus, it is most likely that eukaryotic cells possess one or more N^{α}-acetyltransferases capable of acetylating various proteins with different N-terminal regions. Apparently, they are very similar with respect to substrate specificity.

IV. WHEN ARE PROTEINS N^{α}-ACETYLATED?

In 1975, Jörnvall examined the then known 40 N-terminally acetylated proteins for structural characteristics of the N-terminal region.[16] He established that in the acetylated N-terminal position serine and alanine are predominant as compared with the distribution of N-terminal residues, in general. Branched-chain residues would be more frequently present in the N-terminal region of acetylated proteins. These properties were observed regardless of origin and function of the proteins. Therefore, it seems reasonable to assume that N^{α}-acetyltransferases have very specific requirements as far as the structure of the N-terminal region of proteins is concerned, although the substrate specificity of the enzymes from various tissues and species is very similar. Here we describe in more detail the structural and other requirements for N^{α}-acetylation on the basis of the hitherto known sequences of acetylated and nonacetylated proteins.

A. Compilation of N^{α}-Acetylatled Proteins

The primary structures of proteins as compiled by Dayhoff (1972 to 1978)[1] have been used. Moreover, for N^{α}-acetylated proteins published between 1977 and 1982 the literature has been screened. Only the first ten amino acids were taken into account. Care was taken to check the evidence given for the presence of an N^{α}-acetyl group. Acetyl groups assumed on the basis of homology were allowed if the reference protein had been determined unambiguously. We did not consider an assumed acetyl group of those cases in which the first amino acid is not identical to that of the reference protein. Incidentally, we included a protein which had been decribed in the literature as being N-terminally blocked. This seemed to be reasonable when a homologous N^{α}-acetylated sequence had been reported previously. The complete compilation of N^{α}-acetylated proteins, listing 361 entries, is given in the Appendix. The proteins have been ordered according to their function or origin. No attempts have been made to place homologous proteins together as done by Dayhoff.[1]

When comparing the (1-10)-decapeptide sequences of N^{α}-acetylated and nonacetylated proteins, we used the following seven less stringent rules in order to have available as much structural information as possible.

1. The sequence of at least the first five amino acids must be known. Asx, Glx, or undetermined positions are not allowed.
2. If Asx, Glx, or undetermined positions occur in the second five residues, the sequence up to that residue is considered.
3. For proteins for which identical sequences are known in more species, only one species is taken into account. However, a variant is accepted as a new entry. Shortened sequences, which are N-terminally identical to complete (1-10)-sequences, are not accepted.
4. For proteins in which there is tissue-specificity, gene multiplicity, allelic variation, or species-dependent diversity, every tissue, gene, allele, or species product is considered as a protein of its own.
5. Precursor proteins with the same N-terminus as the product are not accepted.
6. Acetylated proteins with incomplete blocking ($\leq$60%) are not accepted.
7. In the category of nonacetylated proteins all otherwise N-terminally blocked proteins are included.

For the determination of hydrophobicity values we used the "hydropathy" index for amino acids of Kyte and Doolittle,[71] which is based on water-vapor transfer free energies and the interior-exterior distribution of side chains from amino acids. Values vary from -4.5 for Arg through -0.4 for Gly to $+4.5$ for Ile. For every protein the hydrophobicity

was averaged over the number of positions involved (span length) and is, thus, given per residue. Moreover, the hydrophobicity per residue was averaged over all proteins.

In order not to overemphasize structures of which many species variants are known, it was necessary to use some means of selection. For comparison of the N-terminal amino acids only, we used four stringent selection rules:

1. The N-terminal amino acid must be known.
2. In the case of homologous proteins only a single representative sequence is taken into account. However, when there is reasonable evidence for products of duplicated or allelic genes, one product of each orthologous gene is considered.[72] This is the case for tissue-specific proteins, isozymes, and proteins which are only found in a restricted group of species. The product of each unique gene is thus included in our analysis.
3. However, if a species variant has an N-terminal amino acid differing from the reference protein it is taken into account.
4. Precursors which give a product having the same N-terminal sequence are not taken into account.

These rules are not always unambiguous, since it is not known in each case if gene products are orthologous or paralogous. Therefore, incidentally, the decision has been arbitrary. After selection there is still some bias, because of the presence of related gene products.

We estimated the total number and the nature of all sequences known up to 1978 by applying criterion 2 and 4 of the less stringent rules on the cumulative index of Supplement 3 of Dayhoff (1978).[1]

B. The N-Terminal Residue of Proteins, in General

From experiments by Waller,[73] Horikoshi and Doi,[74] and Brown[75] it is known that methionine, alanine, and serine are the main N-terminal amino acids of the soluble unblocked proteins of prokaryotes, and that in eukaryotes alanine and serine are predominant. When all protein sequence data from Dayhoff (1972 to 1978)[1] were assessed for uniqueness with the stringent selection rules, 582 proteins were left. Table 2 lists the N-terminal residues of these proteins and their relative amounts. When Ala, pyrrolidone carboxylic acid (π-Glu), Met, and Ser are taken together, they amount to 46.5% of the total. On basis of the average amount of these amino acids in proteins (Dayhoff, 1978),[1] this should have been around 20%. Obviously, these amino acids are found much more frequently at the N terminus of proteins than expected. This is, also, clearly demonstrated by the ratio between these N-terminal amino acids and the average presence of these residues. Only Ala, π-Glu, Met, and Ser have a ratio higher than 1. Since the protein sequence data set from Dayhoff contains proteins, both soluble and insoluble, from eukaryotic, prokaryotic, viral, and bacteriophageal origin, it is clear that the N-terminal amino acids determined in soluble unblocked proteins, from either eukaryotic or prokaryotic source, as described above, do not have to fit exactly the protein data of Dayhoff. Immunoglobulins, some contractile system proteins, etc. are not present in prokaryotes and lower eukaryotes which were used for the survey by Waller,[73] Horikoshi and Doi,[74] and Brown.[75] However, the predominance of Ala and Ser in the proteins listed by Dayhoff (1972 to 1978)[1] is quite consistent with these determinations.

The relative abundance of Met at the N terminus may be explained by the fact that this probably is the initiator methionine still present in the chain. Considering the genetic origin of the other three, Ala, Ser, and π-Glu (the latter being assumed to be derived from Gln[76]), it appears that Ala is encoded by nucleotide triplets starting with G (4x), Ser from codons with U (4x) and A (2x), and Gln from C (2x). Assuming an equal probability for the use of each codon, we find that there is a 50% probability that the first nucleotide of the codon used for these N-terminal residues is A or G. However, Manderschied et al. reported that

Table 2
N-TERMINAL AMINO ACID OF PROTEINS COMPARED WITH THE AVERAGE PRESENCE IN PROTEINS (DAYHOFF, 1978).[1] THE NATURE OF THE BLOCKING GROUP, IF ANY, IS INDICATED

N-terminal amino acid	Number of proteins	Presence in N-terminal position (%)	Average presence (%)	Presence on N-terminus/average presence	Precentage of N-terminal blocking and nature of the blocking group
Ala	101	17.4	8.6	2.0	3.4 Ac + 0.2 Me
Arg	25	4.3	4.9	0.9	
Asn	10	1.7	4.3	0.4	0.2 Ac
Asp	27	4.6	5.5	0.8	0.3 Ac
Cys	15	2.6	2.9	0.9	
Glu	30	5.1	6.0		
Gln	3	0.5	3.9	1.6	
π-Glu	59	10.2			10.2 π-Glu
Gly	33	5.7	8.4	0.7	0.5 Ac + 0.2 F
His	8	1.4	2.0	0.7	
Ile	18	3.1	4.5	0.7	
Leu	29	5.0	7.4	0.7	
Lys	21	3.6	6.6	0.5	
Met	46	7.9	1.7	4.7	1.2 Ac + 0.3 F + 0.2 Me
Phe	9	1.5	3.6	0.4	
Pro	19	3.3	5.2	0.6	0.2 F + 0.2 Me
Ser	64	11.0	7.0	1.6	4.3 Ac
Thr	24	4.1	6.1	0.7	
Trp	2	0.3	1.3	0.2	
Tyr	9	1.5	3.4	0.4	
Val	30	5.2	6.6	0.2	
	Total: 582				Total: 21.4%

in mRNA sequences the initiation triplet AUG is very often followed by a codon starting with a purine nucleotide.[77] They found that the formation of *Escherichia coli* 30S initiation complex with initiator-tRNA occurred far more readily with the tetranucleotides AUGA and AUGG than with AUG itself or AUGU. This suggested that the initiator-tRNA, which has a U-residue following the anticodon, recognizes the initiation site through four nucleotides. In a search for the information necessary for an optimal binding of an mRNA to *E. coli* ribosomes, it was found that in the optimal fit an A follows the AUG codon.[78] However, the mRNA sequences showed that actually about 70% of the nucleotides following AUG is A or G. It is not known if these rules are also valid for eukaryotic mRNAs. For many proteins the initiator Met is removed, especially if the second residue is Ala or Ser.[42,45] In most cases the N-termini of proteins correspond with the second codon of the mRNA involved. If the hypothesis of Manderschied et al. had general validity, N-terminal Ala and Ser would fit reasonably well. However, it does not give an explanation of why the other amino acids encoded by triplets starting with an A and G are present much less frequently at the N-terminus of proteins than are Ala and Ser.

Upon examination of known N-terminal residues it appears that 21.4% are blocked, either by a formyl (0.7%), a methyl (0.6%), a pyroglutamyl (10.2%), or an acetyl group (9.9%). Of the 21.4% blocked N-terminal amino acids 19.8% are represented by just four species: Ala, π-Glu, Met, and Ser. Apparently, there is a strong preference for N-terminal blocking of exactly the same amino acids which are overrepresented at the N-terminus. No explanation for this phenomenon can be given at the moment.

C. Factors Which May Influence N^{α}-Acetylation

Several factors may influence N^{α}-acetylation of proteins. For instance, the distribution of the enzyme within a tissue is very important, as has been demonstrated for the products of the precursor protein proopiomelanocortin. ACTH, which has N-terminally the same sequence as α-MSH, is not acetylated, while α-MSH mostly is.[1] α-MSH is acetylated in *pars intermedia*, whereas it is not acetylated in other parts of the pituitary and the brain.[79] β-Endorphin, which normally has a free N-terminus necessary to exhibit its morphomimetic character, becomes acetylated only in *pars intermedia*.[25,26,80] The N^{α}-acetyltransferase responsible for the N-terminal acetylation of these two polypeptides is present only in secretory granules in the intermediate pituitary of rat and ox.[70] N^{α}-acetylation has been shown to be linked to α-MSH release from *pars intermedia* of the amphibian pituitary gland.[81] It may well be that this acetylation of products which only come into existence after processing of a precursor protein is an exception, since for most proteins up to now acetylation has been shown to be a post-initiational process.

On the other hand, a shortage of acetyl coenzyme A may be responsible for incomplete acetylation. Jörnvall et al. showed that two isozymes of yeast alcohol dehydrogenase are synthesized with a consistent variation in acetylation under physiological conditions. Isozyme I is almost completely acetylated; isozyme II, which may be nonacetylated up to 40%, is the predominant form during aeration, when acetyl coenzyme A might be rapidly removed during efficient cell respiration. It has also been suggested that the availability of the coenzyme is a major factor in partial acetylation of human fetal hemoglobin, which has to compete with carbonic anhydrases B and C (both are N^{α}-acetylated) for the available acetyl groups in reticulocytes, where the main source of such groups, the mitochondrion, begins to disappear.[82] However, this case is more complicated because of an enhanced activity of the responsible acetyltransferase in erythrocytes as compared with reticulocytes.[83] Moreover, there is evidence that in vitro human fetal hemoglobin can be acetylated nonenzymatically.[84] This acetylation can be increased by sodium butyrate.[85] The latter compound seems to inhibit the turnover of preformed HbF_1. It is suggested that this is caused by inhibition of deacetylating enzymes. This action of sodium butyrate has been proven for histone N^{ϵ}-acetylation.[86]

The presence of deacetylases might be a third factor. Since there is evidence that N^{α}-acetylated proteins turn over at the same rate as nonacetylated ones,[7] it is possible that there exist such enzymes, which would make the protein accessible for aminopeptidases. However, up to now no enzyme removing N^{α}-acetyl groups from proteins has been found. On the other hand, the existence of enzymes deacylating N^{α}-acylamino acids has been established fairly well.[87-92] Much less is known about enzymes deacylating peptides. There are reports of N^{α}-acylpeptide hydrolases in liver[93] and erythrocytes.[94] These enzymes catalyze the hydrolysis of N^{α}-acylamino acids from the N-terminus of small peptides only. Remarkably, the human erythrocyte enzyme only releases acetylalanine, while the bovine liver enzyme also hydrolyzes acetylmethionine, -glycine, -serine, and -phenylalanine. Therefore, it has been suggested that the removal of acetyl groups from proteins may be affected by proteases which release an aminoterminally acetylated peptide, followed by an acylpeptide hydrolase which catalyzes the release of the *N*-acetylamino acid from the peptide and a deacetylase which hydrolyzes the acetylamino acid to yield acetate and the terminal amino acid.[94] Such enzymes are presumably involved in the catabolism of acetylated proteins. At the moment, there is no conclusive evidence that N^{α}-acetylated proteins are enzymatically deacetylated. Therefore, it is not very likely that deacetylation plays a role in the actual acetylation status of proteins, in general. Yet, in specific cases other factors may be involved. An intriguing example has been described for *E. coli*. This bacterium has a ribosomal protein which is found in both the N^{α}-acetylated (L7) and nonacetylated (L12) form.[95,96] Four copies of L7/L12 are present on the ribosome.[97] Ramagopal and Subramanian provided evidence that during early growth L12 is the major form present, while during the stationary phase L7 is

Table 3
NUMBER OF PROTEINS (UNIQUE GENE PRODUCTS) WHICH ARE N^{α}-ACETYLATED AND THE GROUPS TO WHICH THEY BELONG

Structural Proteins		48	40.7%
Actins	6		
Tropomyosin	2		
Intermediary filament proteins	2		
Keratins	8		
Virus coat proteins	9		
Crystallins	3		
Ribosomal proteins	4		
Histones	10		
Myelin proteins	2		
Others	2		
Enzymes		23	19.5%
Oxidoreductases	10		
Transferases	5		
Hydrolases	4		
Lyases	3		
Isomerases	1		
Transfer proteins		16	13.6%
Cytochrome c	4		
Hemoglobins	12		
Ca- and metal-binding proteins		16	13.6%
Parvalbumins	4		
Troponin C	3		
Myosin L-4 chain	1		
Calmodulin	1		
Intestinal Ca-binding protein	2		
S100b	1		
Metallothioneins	3		
Ferritin	1		
Hormones		3	2.5%
α-MSH	1		
β-Endorphin	2		
Miscellaneous proteins		12	10.1%
	12		

the predominant form.[98] This shift apparently requires production of new ribosomes, as it does not depend on modification of preassembled ribosomes.[99] In *E. coli* at least two, but a maximum of five, subspecies of the large subunits exist with regard to the L7/L12 content.[97] The observed variance in the L7/L12 ratio might reflect continuous changes in the selective abundance of these subspecies in vivo during the growth cycle. Moreover, it has been calculated that the level of the responsible N^{α}-acetyltransferase in *E. coli* remains constant during the growth cycle.[100] The possibility of a depletion of the available acetyl donor due to a high metabolism during growth should be investigated.

In conclusion, when N^{α}-acetyltransferase and acetyl coenzyme A are present, the most important factor in N^{α}-acetylation is the primary structure of the N-terminal region of the polypeptide chain.[16]

D. Which Proteins are N^{α}-Acetylated?

Until 1982, 361 proteins were described to be N-terminally acetylated, many of which are species variations of the same protein. When only unique genes are taken into account (using stringent selection rules) 118 proteins are left. Table 3 lists the variety of classes to

Table 4
ORIGIN OF N^{α}-ACETYLATED PROTEINS AS COMPARED WITH ALL SEQUENCED PROTEINS. FOR THE COMPARISON ONLY UNIQUE GENE PRODUCTS HAVE BEEN USED. THE NUMBER OF ENTRIES IS GIVEN BETWEEN BRACKETS

	Acetylated proteins (%) (entries)	All sequenced proteins (%)
Eukaryotes	86 (101)	77
Prokaryotes	4 (5)	18
Viruses	10 (12)	1
Bacteriophages	0 (0)	4

which they belong. About 40% are structural proteins. From the cumulative index of Supplement 3 of Dayhoff (1978)[1] we estimate that about 20% of all unique sequences are accounted for by structural proteins. Thus, N^{α}-acetylated polypeptides appear to be more often structural proteins than expected statistically. A predominance of structural proteins might be expected, if the function of N^{α}-acetylation, indeed, were protection against aminopeptidases. However, since there are many acetylated proteins with different size, origin, and function, this general property does not seem to be a limiting factor for acetylation.[16]

Only a few proteins of noneukaryotic origin are N^{α}-acetylated (Table 4). There are no examples of acetylated mitochondrial proteins, coded for by the mitochondrial genome,[101,102] nor acetylated polypeptides from bacteriophages. There are only five prokaryotic proteins N^{α}-acetylated, four of which are ribosome-associated. For viruses, 12 proteins have been described to be acetylated. When these data are compared with the distribution of these groups, over all unique gene products comprised in Dayhoff,[1] it is obvious that predominantly eukaryotic and proteins of eukaryotic viruses are N^{α}-acetylated. At the moment they comprise 96% of all known acetylated proteins. Data for viruses have to be considered with care, since many sequences have become available for this group since 1978. They might be underrepresented in the list of sequenced proteins of Dayhoff.[1]

Jörnvall et al. remarked that there are isozymes of bovine heart malate dehydrogenase and aldehyde dehydrogenase, in which the mitochondrial form is free, while the cytoplasmic form is N^{α}-acetylated.[30] They suggest that this reinforces an apparent link between nonacetylation and an environment with efficient aerobic respiration. There is another enzyme with isozymic forms for which this phenomenon has also been established. Whereas mitochondrial aspartate aminotransferase of pig and chicken heart is nonacetylated,[103-107] the cytoplasmic form is N^{α}-acetylated in chicken,[108,109] but nonacetylated in pig.[110] Virtually all Cu/Zn-superoxide dismutases are confined to eukaryotes,[112] whereas the Mn- and Fe-superoxide dismutases are widely distributed in eukaryotes, prokaryotes, and mitochondria. The latter are all related to each other, but not to the Cu/Zn family of superoxide dismutases. Harris et al.[111] interpret this similarity and the distribution in the light of the endosymbiontic hypothesis, which suggests that mitochondria of eukaryotes have evolved from bacteria which existed as intracellular symbionts in primitive eukaryotes.[112,113] This theory holds that when the atmosphere became more aerobic, these symbionts were acquired as a means of confronting selective pressures that require oxidative pathways.[114] Probably, the Cu/Zn type of enzyme evolved after the prokaryote-eukaryote divergence. In this respect it is remarkable that almost all Cu/Zn dismutases are N^{α}-acetylated, while the Mn and Fe dismutases are not.[1,111]

In conclusion, N^{α}-acetylation is a phenomenon predominantly confined to eukaryotes.

Table 5
N^{α}-TERMINAL AMINO ACID OF $N\alpha$-ACETYLATED PROTEINS (UNIQUE GENE PRODUCTS ONLY)

N terminus	Unique gene products	Percentage
Ac-Ser	41	34.8
Ac-Ala	38	32.2
Ac-Met	21	17.8
Ac-Gly	4	3.4
Ac-Asp	4	3.4
Ac-Glu	3	2.5
Ac-Tyr	2	1.7
Ac-Thr	2	1.7
Ac-Val	2	1.7
Ac-Asn	1	0.8
	n = 118	100.0

E. The N-Terminal Residue of N^{α}-Acetylated Proteins

When the 361 N^{α}-acetylated proteins, described up to 1982, are selected according to the stringent rules, the remaining 118 unique gene products (Table 5) mainly have Ala, Ser, and Met at the N-terminus (84.8%). Ala and Ser together make up 67.0% of the total. This is somewhat less than the 75% reported by Jörnvall.[16] However, it is obvious that methionine is found very often as N-terminal residue in acetylated proteins. While Jörnvall mentioned only four other residues to occur at the N-terminus of acetylated proteins (i.e., Asp, Gly, Thr, Val), we encountered in the literature the description of other N^{α}-acetylated N-termini such as Asn, Glu, and Tyr. Although there is a clear preference for Ser, Ala, and Met, there are still about 15% with other acetylated N-termini. This means qualitatively that at the aminoterminus of acetylated proteins half of the 20 amino acids can be found. The three amino acids Ala, Met, and Ser are all neutral; Ala and Ser are very similar in size, while Met is considerably larger. It is not known if this different bulkiness can be accommodated for by the active site of just one N^{α}-acetyltransferase. Remarkably, the larger hydroxyl amino acid Thr and the smaller aliphatic residue Gly, as compared to Ser and Ala, respectively, are not often present at the N-terminus of acetylated proteins.

The importance of the N-terminal residue is corroborated by the fact that the presence of a D-Ser at the N-terminus of an ACTH-fragment does not lead to N^{α}-acetylation in vitro.[57] This importance can further be demonstrated with several proteins which are found to be either nonacetylated or N^{α}-acetylated, depending on the nature of the aminoterminal residue. A convincing example has been found for the human mutant hemoglobin Raleigh. In this protein the β_1Val has been replaced by Ac-Ala.[23] The other examples which follow are more complicated due to differences other than the N-terminal residue. α-Globin normally has an unblocked Val or Met at position 1.[1] It is N^{α}-acetylated in fish and amphibians. In amphibians the N-terminal amino acid is Ala[115-117] or Ser,[118] in fish Ser.[1,119-121] Normally, mammalian β-globin has Val or Met at the N-terminus with a free amino group.[1] In the Felidae family different β-globins exist, some of which are blocked at the N-terminus.[122] In domestic cat the amino acid at position 1 has been shown to be Gly in case of the unblocked β-chain, and Ac-Ser in the case of the blocked chain.[17,113] β-Globins of some reptiles have Ac-Ala at the N-terminus.[124] Tobacco mosaic virus has a coat protein with either Ac-Ala or Ac-Ser at the N-terminus. However, one strain variant is known with unblocked Pro.[1] In cytochrome

Table 6
NUMBER OF PROTEINS, ACETYLATED AND NONACETYLATED, BELONGING TO EITHER THE EUKARYOTES OR THE PROKARYOTES

	Eukaryotes (viruses, mitochondria)	Prokaryotes (bacteriophages)
H-Ala, H-Met, H-Ser	136	114
Ac-Ala, Ac-Met, Ac-Ser	133	3

c, mammals have Ac-Gly-Asp and plants Ac-Ala-Ser at their N-terminus.[1] All other eukaryotic cytochromes c are nonacetylated. Insects have N-terminal Gly-Val, lower organisms Pro-Ala, Thr-Gln, and Gly-Phe.

Since there are many nonacetylated proteins which have the same N-terminal amino acids as N^{α}-acetylated proteins, other factors must be important for N^{α}-acetylation.

F. The N-Terminal Region of N^{α}-Acetylated Proteins

From the preceding sections it may be concluded that catalysis of N^{α}-acetylation in eukaryotes and prokaryotes is not achieved by acetyltransferases which have identical substrate specificity. However, N^{α}-acetyltransferases originating from different species or tissues recognize each other's substrates. Assuming that this is the general rule, we wanted to determine to what extent the primary structure of the N-terminal region is a decisive criterion for the suitability of a protein to be a substrate for N^{α}-acetylation. We, therefore, examined the first ten residues of proteins.

To this end we made use of our compilation of N^{α}-acetylated proteins (361 entries), taking into account only those which have N-terminal Ala, Ser, or Met (261 entries). These three were chosen, because they are the most frequently occurring N-terminal residues of acetylated proteins. For the nonacetylated proteins with the same N-terminal amino acids use was made of Dayhoff's protein sequence data set (312 entries). Both protein data sets were then screened with the less stringent selection rules. This left 136 entries in the case of the acetylated proteins and 250 of the nonacetylated ones.

The residual data sets were split up into entries from prokaryotic (including bacteriophageal) and eukaryotic (including viral and mitochondrial) origin. Since only a limited number of proteins is coded for by the mitochondrial genome,[101,102] of which none is included in our data sets, we placed all other mitochondrial proteins, encoded in the nucleus of the eukaryote, in the eukaryotic section. An investigation of the structural aspects of acetylated proteins from prokaryotic origin is not feasible, since only three are left after the selection. On the other hand, a sufficient number of sequences is known for eukaryotic proteins (compare Table 6).

Both the acetylated and nonacetylated proteins from eukaryotic origin were ordered alphabetically and compared. For each of the three amino acids Ala, Ser, and Met the sequences of both categories are given in the one-letter code up to the residue where a difference between them exists (Table 7). For the protein sequences now known, apparently, the decisive information for N^{α}-acetylation is enclosed within the first four amino acids for N-terminal Ala, within three for Met, and five for Ser. In the case of Ac-Met, there is a conspicuous preference for an acidic residue at position 2. In this respect it is remarkable that the methionine aminopeptidase normally responsible for the removal of the initiator Met does not act if position 2 is occupied by an acidic residue.[45] Unfortunately, these data do not yet allow the unambiguous prediction of the presence of acetyl groups in amino acid sequences derived from DNA-sequencing.

When we calculate the percentage of occurrence of amino acid residues for positions 2

Table 7
N-TERMINAL REGION OF ACETYLATED (AC-) AND NONACETYLATED (H-) PROTEINS, STARTING WITH ALA, MET, AND SER. RESIDUES ARE INDICATED WITH ONE-LETTER CODE[a]

Ac-	H-	Ac-	H-	Ac-	H-	Ac-	H-	Ac-	H-
AAQ	AAK	AKD	AKE		MA	SAN	SAG	SSK	SSSQD
AASI	AAN		AKG	MDA	MDM	SAQ	SAK	SSQ	SST
	AAP	ALK	ALT	MDD		SCY		SSSQK	
	AASG		ALV	MDG		SDA	SDEA	STA	STSR
	AAY		ALY	MDI		SDEE	SDH	STD	
ACC	ACD	AMT	AMS	MDK		SDQA	SDN	STST	
ACN			AN	MDL			SDP		SV
ACR			AP	MDP			SDQS	SYN	SYC
ADE	ADD	AQK	AQI	MDV		SEAP	SEAE	SYS	SYD
ADF	ADG	AQP		MEA	MEC	SELE	SEF		SYE
ADK	ADI		AR	MEE		SETAP	SELT		
ADQ	ADL	ASF	ASA	MEI			SES		
ADW	ADR	ASN	ASD		MH		SETAA		
	ADS	ASP	ASY		MI	SFA	SFD		
	ADT	ASQ		MKR	MKL	SFS			
AED	AEI	ATF	ATD		ML	SFT			
AEVA	AES	ATK	ATT	MN		SGR	SHG		
	AET	ATL	ATY		MQ	SGV			
	AEVL	ATP			MR	SH			
	AEVQ	AVA	AVE		MT	SI			
AFA	AFE		AVK			SKI	SKA		
AFG	AFKV		AVL			SKP	SKQ		
AFKG	AFP		AVQ			SL			
AGW	AGC		AVS			SN			
	AGQ		AVT			SRP	SRE		
AHL	AHR		AW						
	AI	AYN	AYD						
		AYS	AYR						

Note: The sequence is shown up to the residue where a difference between both classes is apparent; thus, the N-terminal sequence Ala-Ala-Gln (AAQ) has been found to be acetylated, whereas Ala-Ala-Lys (AAK) is not acetylated. AAS is found both acetylated and nonacetylated as N-terminal sequence in different proteins, but AASI has been found acetylated and AASG nonacetylated. Vertical bars indicate groups of sequences where the first two residues are identical. The groups are arranged alphabetically according to the N-terminal sequence in the one-letter notation. Frequently, the number of entries in acetylated and nonacetylated proteins within a group differ remarkably. In some cases there is only one representative in either one of these classes.

[a] Explanation of the one-letter code:[1] A = Ala, C = Cys, D = Asp, E = Glu, F = Phe, G = Gly, H = His, I = Ile, K = Lys, L = Leu, M = Met, N = Asn, P = Pro, Q = Gln, R = Arg, S = Ser, T = Thr, V = Val, W = Trp, Y = Tyr.

to 5 and 6 to 10 for both the acetylated and nonacetylated proteins and compare the results with the average percentage of these residues in proteins, in general (Dayhoff, 1978),[1] there seems to be a preference for Asp and Ile in residues 2 to 5 of acetylated proteins, while Gly, Gln, Arg, and Val are underrepresented (Table 8). In the nonacetylated proteins, Asp and Arg are overrepresented, while Ala, Gly, and Asn are underrepresented. Other extremely variable residues are not seen. We do not find the predominance of Ile, Leu, and Val as reported by Jörnvall,[16] and do not perceive a consistent pattern in the differences between acetylated and nonacetylated proteins. Only for Arg is the predominance in nonacetylated proteins balanced by an underrepresentation in acetylated proteins. In residues 6 to 10 of

Table 8
AVERAGE PERCENTAGE OF RESIDUES AT POSITIONS 2 TO 5 AND 6 TO 10 OF ACETYLATED AND NONACETYLATED PROTEINS, COMPARED WITH THE AVERAGE PERCENTAGE OF RESIDUES IN PROTEINS, IN GENERAL (DAYHOFF, 1978[1]). AMINO ACID RESIDUES ARE INDICATED WITH THE ONE-LETTER CODE

	H-		Ac-		Proteins,
	2—5	6—10	2—5	6—10	in general
A	5.9	5.6	9.4	12.6	8.6
C	2.6	3.2	2.6	2.6	2.9
D	11.7	6.4	9.4	7.3	5.5
E	6.8	8.2	6.9	5.5	6.0
F	2.3	5.7	4.9	3.6	3.6
G	4.2	7.7	5.6	7.7	8.4
H	2.0	2.6	1.3	2.6	2.0
I	3.4	1.5	7.1	4.2	4.5
K	5.2	6.0	8.5	9.4	6.6
L	6.7	8.2	6.6	4.7	7.4
M	2.0	1.0	0.6	0.8	1.7
N	2.6	1.7	3.6	4.0	4.3
P	5.3	7.9	5.6	8.1	5.2
Q	3.7	5.3	2.2	5.7	3.9
R	6.5	4.3	2.9	1.8	4.9
S	9.4	10.1	9.6	6.4	7.0
T	8.3	6.8	7.7	3.2	6.1
V	5.9	5.0	2.5	5.9	6.6
W	0.5	0.8	1.1	2.2	1.3
Y	5.0	2.0	1.9	1.7	3.4

both acetylated and nonacetylated proteins there are also apparent differences which are somewhat lower than for residues 2 to 5. Thus, differences tend to level off on going further along the chain. However, variations in residues 2 to 5 of acetylated and nonacetylated proteins are not consistent for the sequences hitherto known.

When we compare broad categories of amino acid residues for these same regions (Table 9), there seems to be a slight predominance of hydroxyl amino acids and acidic residues at positions 2 to 5 of acetylated sequences compared with proteins, in general. However, the same phenomenon is apparent in nonacetylated proteins. Small aliphatic amino acids are underrepresented in nonacetylated polypeptides. We do not believe that it is possible to carry out statistical quantitation, because our data sets are still rather small and contain overemphasis of related gene products.

In conclusion, there is no clear difference in steric and charge properties of the amino acid residues of positions 2 to 5 and 6 to 10 of acetylated and nonacetylated proteins starting with Ala, Ser, and Met. However, proteins starting with Ac-Met often have an acidic residue at position 2. Therefore, there seems to exist a difference between the structural requirements for acetylation of proteins starting either with Ala and Ser or Met.

Table 9
AVERAGE PERCENTAGE OF GROUPS OF RESIDUES AT POSITIONS 2 TO 5 AND 6 TO 10 OF ACETYLATED AND NONACETYLATED PROTEINS, COMPARED WITH THE AVERAGE PERCENTAGE OF THESE GROUPS IN PROTEINS IN GENERAL (DAYHOFF, 1978[1]). AMINO ACID RESIDUES ARE INDICATED WITH THE ONE-LETTER CODE

		H-		Ac-		
		2—5	6—10	2—5	6—10	Proteins, in general
Small aliphatic	A + G	10.1	14.0	15.0	20.3	16.9
Hydroxyl	S + T	17.7	16.9	17.3	9.6	13.1
Acidic	D + E	18.5	14.6	16.3	12.8	11.6
Acidic + acid amide	D + E + N + Q	24.8	21.6	22.1	22.5	19.8
Basic	H + K + R	13.7	12.9	12.7	13.8	13.5
Hydrophobic	I + L + M + V	18.0	15.7	16.8	15.6	20.2
Aromatic	F + W + Y	7.8	8.5	7.9	7.5	8.3

G. The Local Hydrophobicity of the N-Terminal Region of N^{α}-Acetylated Proteins

Since no obvious steric and charge properties account for the determination of the suitability of the N-terminal region of proteins for N^{α}-acetylation, we decided to investigate the local hydrophobicity of the first five amino acids of acetylated proteins, as the information for acetylation seems to be located in this region (Table 7). For the calculations we used the same data sets of acetylated and nonacetylated proteins as in the preceding section. The hydrophobicity of the three N-terminal residues involved differs. Met (+ 1.9) and Ala (+ 1.8) are comparable, while Ser (− 0.8) is much more hydrophilic. In our calculations, we averaged the hydrophobicity over positions 1-2, 1-3, 1-4, and 1-5 for each protein. We then determined the average for all proteins. The results, expressed per residue, are summarized in Figure 2.

The most striking results are found for Met. While the N-terminal regions of proteins starting with H-Met show a relatively broad hydrophobicity range, the region of those with Ac-Met contains a moderate hydrophilic character for the first two positions ($\simeq -1.0$). Since Met itself has a value of + 1.9, this means that the second residue is always very hydrophilic. There are no obvious differences on going further along the sequence. Since there are many proteins also having a hydrophilic residue at position 2 without being acetylated, other factors must be important, too, as was already deduced from Table 7. Average values for the first 5 positions tend to be somewhat more hydrophilic for acetylated proteins as compared with nonacetylated proteins. However, the values for span length 5 are already very close to each other.

When Ala is in the N-terminal position, we find that the hydrophobicity range between the highest and lowest value is near 4 for acetylated proteins for span lengths up to 5, while being somewhat larger (4 to 5) for nonacetylated proteins. The hydrophobicity per amino acid residue for acetylated proteins seems to be between the values for Phe and Pro. Extremely hydrophilic or hydrophobic N-terminal regions of proteins starting with Ala are not suitable for enzymatic N^{α}-acetylation. The same holds true for Ser. Values range between the same boundaries for acetylated proteins.

In conclusion, the hydrophobicity of the N-terminal region seems to play a role in N^{α}-acetylation. For Ala and Ser both very hydrophilic and hydrophobic N-terminal regions are unfavorable, while for Met position 2 must be very hydrophilic. Thus, again a difference between Ala/Ser, on the one hand, and Met, on the other, has been established.

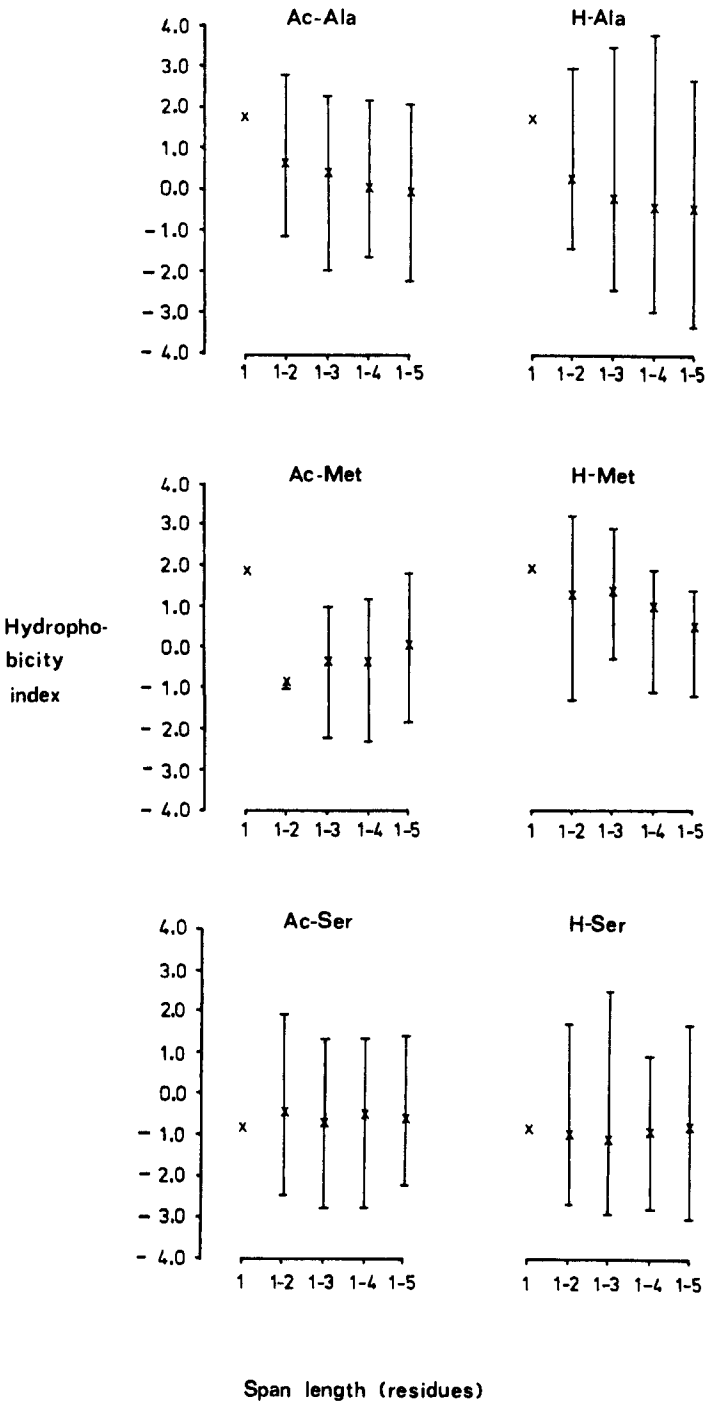

FIGURE 2. Hydrophobicity of the N-terminal region of proteins starting with Ac-Ala (n = 50), H-Ala (n = 87), Ac-Met (n = 27), H-Met (n = 19), Ac-Ser (n = 55), and H-Ser (n = 29). Results are shown per residue for span lengths of 2 up to 5. Average hydrophobicity for all proteins starting with a particular acetylated or nonacetylated residue is indicated with x. The highest and lowest values for any protein found are indicated with −.

H. The Two- and Three-Dimensional Structural Characteristics of N^{α}-Acetylated Proteins

For several of the known N^{α}-acetylated proteins information about the two- and three-dimensional structure is available. However, since N^{α}-acetylation mostly takes place with the nascent chain on the ribosome, when the chain starts to protrude at a length of 25 to 50 residues (mostly after removal of the initiator methionine), it is not clear to what extent, if any, the structure of the completed chain is of relevance for the N^{α}-acetylation process itself. In the completed chain the N-terminus is mostly present on the surface of the structure. The N^{α}-acetyl group is found at the beginning of an α-helix in dogfish lactate dehydrogenase M_4 isozyme[125] and at the beginning of a β-strand in lobster glyceraldehyde-3-phosphate dehydrogenase.[126] For the following proteins the N-terminal amino acid is separated by one or more residues from secondary structural characteristics: carp parvalbumin,[127] human glutathione reductase,[128] horse methemoglobin,[129] tuna ferricytochrome c,[130] human carbonic anhydrases B and C,[131,132] rabbit glycogen phosphorylase,[133] horse phosphoglycerate kinase,[134] horse liver alcohol dehydrogenase EE isozyme,[135] bovine Cu/Zn-superoxide dismutase,[136] and chicken muscle triosephosphate isomerase.[137] In the case of parvalbumin, the methyl carbon of the acetyl group is tucked back into the interior of the protein.

However, a completed three-dimensional structure does not have to be an impediment for efficient N^{α}-acetylation. Feline B-globin chains, synthesized in a rabbit reticulocyte lysate with prevention of acetylation[50] and isolated in normal tetrameric hemoglobin after addition of carrier protein, can be acetylated by incubation in the rabbit reticulocyte cell-free system. This has also been established for δ- and ϵ-actin, which are found in vitro and in vivo and are most likely nonacetylated precursors of cytoplasmic β- and γ-actin.[67,138] In vitro δ- and ϵ-actin can be acetylated very easily by incubation in cell-free systems in the presence of acetyl coenzyme A.

V. EXCEPTIONAL CASES OF N^{α}-ACETYLATION

As mentioned before, the products of proopiomelanocortin are acetylated post-translationally. The acetylation of α-MSH appears to be a very complicated process. α-MSH normally contains only one N^{α}-acetyl group. In 1979 Rudman et al. showed that in the pituitary of ox, rat, guinea pig, and rabbit one third of the melanotropic activity is represented by a structural variant of α-MSH with *N,O*-diacetylserine as its N-terminal residue.[139] When the pituitary gland is incubated in vitro, more than 90% of the melanotropic activity released in the medium is associated with the diacetylated compound. Physiological secretion of α-MSH may, therefore, be related to acetylation of the hydroxyl group of serine at position 1. In contrast to this assumption is the finding of Martens et al.[81] showing that in amphibian pituitary *pars intermedia* release of α-MSH is linked to N^{α}-acetylation. However, the latter authors did not verify the possibility that the compound, which was released, might be diacetylated. The separation of the mono- and diacetylated compound is very difficult.[139] The diacetylated compound is also present in porcine pituitary in equal amounts as in the other species.[140] Biological activity (melanotropic and lipolytic) is identical to that of α-MSH, so that the extra acetyl group on the hydroxyl oxygen of the N-terminal serine does not seem to have any effect in this respect. For bovine and rat pituitary the existence of this new melanotropin has been confirmed.[141] Glembotski has provided evidence that in rat *pars intermedia* ACTH is cleaved in CLIP (corticotropin-like intermediate lobe peptide) and ACTH-(1-13)-tridecapeptide (Figure 3).[70] The latter is then amidated C-terminally, followed by N^{α}-acetylation. Thereafter, the product, α-MSH, is acetylated at the hydroxyl group of the N-terminal serine. It is not known how many enzymes are responsible for these two acetylation steps.[61]

Another special case is actin. The N-terminal sequences of all actins are acidic and most likely acetylated[18,142,143] (compare the Appendix). In this respect actin is a conspicuous

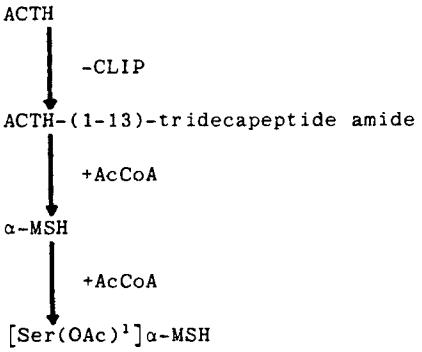

FIGURE 3. Processing of the N-terminus of ACTH in rat pituitary *pars intermedia*.

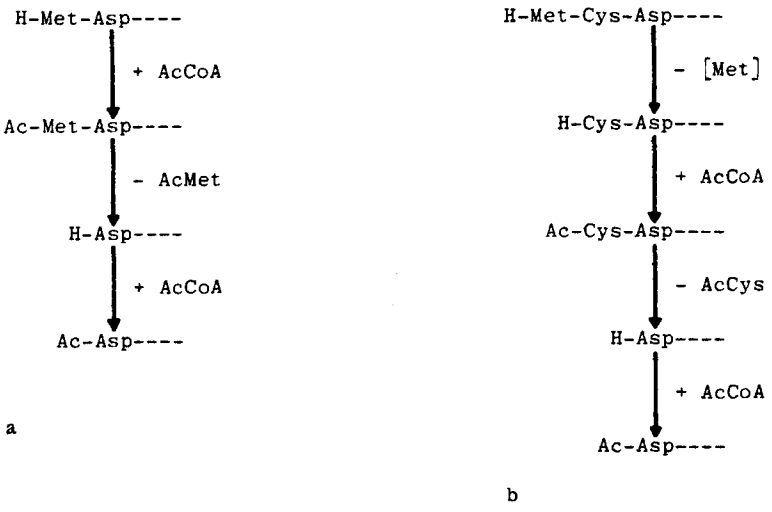

FIGURE 4. Processing of the N-terminus of actin from *Dictyostelium discoideum* (a) and *Drosophila melanogaster* (b) in vitro.

exception to the rules for N^{α}-acetylation as discussed in the preceding sections. As far as the processing of the N-terminal region of the actins is concerned, we have to discern between two groups of gene products. The first class comprises the cytoplasmic *Dictyostelium discoideum* and mammalian β-actins, where the initiator methionine immediately precedes the aspartic acid which is the N-terminal residue of the mature protein.[144-146] In the second class we find the mammalian striated muscle actins (cardiac and α-actin) and *Drosphila melanogaster* actin,[147-149] in which there are precursor proteins starting with Met-Cys-Asp. Since the mature proteins commence with the aspartic acid residue, the Met-Cys moiety has to be removed.

The N-terminal sequence of cytoplasmic actin from *Dictyostelium discoideum* is acidic and comprises the following sequence: Ac-Asp-Gly-Glu-Asp-....[18,68,69,142] Generally, in eukaryotic systems, the initiator methionine is removed if the second residue is not acidic.[45] It is, therefore, surprising that this type of cleavage does take place in the *Dictyostelium* actin. Rubenstein et al. showed that *Dictyostelium* actin prepared in a rabbit reticulocyte lysate in vitro contains Ac-Met at the N-terminus.[69] This product is rather stable. Post-translationally, Ac-Met is then removed (the acetyl group is necessary for this removal), followed by renewed acetylation.[68] It has been suggested[68] that this scheme (Figure 4A) is

also applicable for brain actin.[150] Interestingly, Met-Asp-Gly is a sequence which is often present among N^{α}-acetylated proteins with Met at the N-terminus (Table 7). The processing of *Drosophila melanogaster* actin in a reticulocyte lysate is somewhat different (Figure 4B).[151] At first, the initiator methionine is removed followed by N^{α}-acetylation of cysteine which possibly occurs while the nascent polypeptide is still attached to the ribosome. Further processing follows completion of the synthesis of the polypeptide. In a time-dependent fashion, Ac-Cys is removed, generating actin with an exposed aminoterminal aspartic acid which is subsequently acetylated to produce the mature form of actin.

Obviously, processing of actins in vitro has the novel feature of posttranslational removal of an N^{α}-acetylamino acid from the N-terminus of a completed peptide chain. Furthermore, the actins provide a second example of posttranslational acetylation besides the one already described for the products of proopiomelanocortin.

VI. CONCLUDING REMARKS

In summary, we have to admit that the mechanism of N^{α}-acetylation is still far from being fully understood. However, some features emerge from the data available:

- N^{α}-acetylation is a process virtually confined to eukaryotes.
- N^{α}-acetylation, in most cases, is a post-initiational process in which acetylation takes place when the nascent chain emerges from the ribosome.
- The nature of the N-terminal residue and its neighbors is crucial.
- N^{α}-acetyltransferases appear to have a narrow substrate specificity, which is almost identical for enzymes from different tissues and species.

In order to elucidate the precise function of N^{α}-acetylation further studies are of the utmost importance.

ACKNOWLEDGMENTS

As far as our own investigations discussed in this survey are concerned, they were carried out under auspices of The Netherlands Organization for Chemical Research (SON) and with financial aid of The Netherlands Organization for the Advancement of Pure Research (ZWO).

We thank Dr. C. Slingsby for the critical reading of the manuscript.

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APPENDIX. A COMPILATION OF N^{α}-ACETYLATED PROTEINS (1958 to 1981)

For all proteins the sequence of the first ten amino acids, if known, is given in the three-letter code. If no reference is made to the literature, a protein entry has been taken from Dayhoff.[1] The following symbols have been used:

- Ac- Indicates an N^{α}-acetylated N-terminus of which the acetyl group has been determined experimentally or has been assumed on the basis of homology
- Ac. Indicates an N^{α}-acetylated N-terminus of which the sequence of the amino acids following the acetyl group is not known
- b Blanks (flanked by - or .) indicate amino acids which have not been determined experimentally
- – Indicates a peptide bond which has been determined experimentally
- ~ Indicates that the sequence has been determined by homology with known primary structures
- , Indicates that the sequence is not known at all, or with great uncertainty
- / Indicates that the sequence is followed by amino acids of which the sequence is not known; they are not shown
- . Indicates the end of fragments, the order of which has not been determined experimentally

The use of these symbols is not always unambiguous, especially in the case of those entries from Dayhoff, for which the experimental details are scarce.

Oxidoreductases	
Alcohol dehydrogenase EC 1.1.1.1	
B1-chain, man	(2, 3) Ac-Ser-Thr-Ala-Gly-Lys. .Cys-Lys
B2-chain, man	(2, 3) Ac-Ser-Thr-Ala-Gly-Lys.Val-Ile-Lys.Cys-Lys
E-chain, horse	Ac-Ser-Thr-Ala-Gly-Lys-Val-Ile-Lys-Cys-Lys
S-chain, horse	Ac-Ser-Thr-Ala-Gly-Lys-Val-Ile-Lys-Cys-Lys
Alleloenzyme S, *Drosophila melanogaster*	(4) Ac-Ser-Phe-Thr-Leu-Thr-Asn-Lys-Asn-Val-Ile
Alleloenzyme UF, *D. melanogaster*	(4) Ac-Ser-Phe-Thr-Leu-Thr-Asn-Lys-Ala-Val-Ile
Alleloenzyme N-11, *D. melanogaster*	(4, 5) Ac-Ser-Phe-Thr-Leu-Thr-Asn-Lys-Asn-Val-Ile

Isozyme-I, yeast (*Saccharomyces cerevisiae*)	(1, 6, 7) Ac-Ser-Ile-Pro-Glu-Thr-Gln-Lys-Gly-Val-Ile[a]
Isozyme-II, yeast (*S. cerevisiae*)	(7) Ac-Ser-Ile-Pro-Glu-Thr-Gln-Lys-Ala-Ile-Ile[b]
Lactate dehydrogenase EC 1.1.1.27	
H-chain, pig	Ac-Ala-Thr-Leu-Lys-Glu-Lys-Leu-Ile-Ala-Pro
H-chain, chicken	(8) Ac-Ala-Thr
M-chain, pig	Ac-Ala-Thr-Leu-Lys-Asp-Gln-Leu-Ile-His-Asn
M-chain, rat	(9) Ac-Ala-Ala
M-chain, spiny dogfish (*Squalus acanthias*)	Ac-Ala-Thr-Leu-Lys-Asp-Lys-Leu-Ile-Gly-His
Glyceraldehyde-phosphate dehydrogenase EC 1.2.1.12	
Lobster	Ac-Ser-Lys-Ile-Gly-Ile-Asp-Gly-Phe-Gly-Arg
Glutamate dehydrogenase ($NADP^+$) EC 1.4.1.4	
Neurospora crassa	Ac-Ser-Asn-Leu-Pro-Ser-Glu-Pro-Glu-Phe-Glu
Glutathione reductase (NAD(P)H) EC 1.6.4.2	
Man	(10) Ac-Ala-Cys-Arg-Gln-Glu-Pro-Gln-Pro-Gln-Gly
Cytochrome c oxidase EC 1.9.3.1	
Subunit VI (≡ subunit VII), ox	(11—13) Ac-Ala-Glu-Asp-Ile-Gln-Ala-Lys-Ile-Lys-Asn
Monophenol monooxygenase (tyrosinase) EC 1.14.18.1	
N. crassa	(14, 15) Ac-Ser-Thr-Asp-Ile-Lys-Phe-Ala-Ile-Thr-Gly
Superoxide dismutase EC 1.15.1.1	
Cu-Zn enzyme, man	(16—18) Ac-Ala-Thr-Lys-Ala-Val-Cys-Val-Leu-Lys-Gly
Cu-Zn enzyme, ox	Ac-Ala-Thr-Lys-Ala-Val-Cys-Val-Leu-Lys-Gly
Cu-Zn enzyme, horse	(19) Ac-Ala-Leu-Lys-Ala-Val-Cys-Val-Leu-Lys-Gly
Transferases	
Glycogen phosphorylase EC 2.4.1.1	
Muscle, rabbit	(20, 21) Ac-Ser-Arg-Pro-Leu-Ser-Asp-Gln-Glu-Lys-Arg
Muscle, dogfish	(22) Ac-Ser-Lys-Pro-Lys-Ser-Asp-Met.Glu-Arg-Arg
Aspartate aminotransferase EC 2.6.1.1	
Cytoplasmic, chicken	(23, 24) Ac-Ala-Ala-Ser-Ile-Phe-Ala-Ala-Val-Pro-Arg
Phosphoglycerate kinase EC 2.7.2.3	
Man	(25, 26) Ac-Ser-Leu-Ser-Asn-Lys-Leu-Thr-Leu-Asp-Lys
Horse	(27, 28) Ac-Ser-Leu-Ser-Asn-Lys-Leu-Thr-Leu-Asp-Lys
Yeast	(29, 30) Ac-Ser-Gly
Arginine kinase EC 2.7.3.3	
Lobster	(31) Ac-Ala-Asx-Ala-Ala-Thr[c]
Adenylate kinase EC 2.7.4.3	
Isoenzyme AKi, man	Ac-Met-Glu-Glu-Lys-Leu-Lys-Lys-Thr-Lys-Ile
Pig	Ac-Met-Glu-Glu-Lys-Leu-Lys-Lys-Ser-Lys-Ile

Hydrolases		
Fructose-biphosphatase EC 3.1.3.11		
Sheep	(32)	Ac-Thr-Asp-Glu-Ala-Pro-Phe-Asp-Thr-Asn-Ile
Pig	(33)	Ac-Thr-Asp-Gln-Ala-Ala-Phe-Asp-Thr-Asn-Ile
Rabbit	(34—36)	Ac-Ala-Asp-Lys-Ala-Pro-Phe-Asp-Thr-Asp-Ile
Hyalonuronoglucosaminidase (hyalonuridase) EC 3.2.1.35		
Ox	(37)	Ac.Ala,Glu,Leu,Phe,Tyr
Calcium adenosine triphosphatase EC 3.6.1.3		
Rabbit	(38, 39)	Ac-Met-Glu-Ala-Ala-His-Ser-Lys-Ser-Thr-Glu
Acylphosphatase EC 3.6.1.7		
Horse	(40)	Ac-Ser-Thr-Ala-Arg-Pro-Leu-Lys-Ser-Val-Asp
Lyases		
Carbonate dehydratase (carbonic anhydrase) EC 4.2.1.1		
B(I) type, man		Ac-Ala-Ser-Pro-Asp-Trp-Gly-Tyr-Asp-Asp-Lys
B(I) type, chimpanzee		Ac-Ala-Ser-Pro-Glu-Trp-Gly-Tyr-Asp-Asp-Lys
B(I) type, orangutan		Ac-Ala-Ser-Pro-Glu-Trp-Gly-Tyr-Asp-Asp-Lys
B(I) type, rhesus monkey	(1, 41)	Ac-Ala-Ser-Pro-Asp-Trp-Gly-Tyr-Asp-Asp-Lys
B(I) type, baboon		Ac-Ala-Ser-Pro-Asp-Trp-Gly-Tyr-Asp-Asp-Lys
B(I) type, irus macaque		Ac-Ala-Ser-Pro-Asp-Trp-Gly-Tyr-Asp-Asp-Lys
B(I) type, green monkey		Ac-Ala-Ser-Pro-Glu-Trp-Gly-Tyr-Asp-Asp-Lys
C(II) type, man		Ac-Ser-His-His-Trp-Gly-Tyr-Gly-Lys-His-Asn
C(II) type, sheep		Ac-Ser-His-His-Trp-Gly-Tyr-Gly-Glu-His-Asn
C(II) type, ox		Ac-Ser-His-His-Trp-Gly-Tyr-Gly-Lys-His-Asx
C(II) type, rabbit	(1, 42)	Ac-Ser-His-His-Trp-Gly-Tyr-Gly-Lys-His-Asn
Enolase EC 4.2.1.11		
Rabbit	(43)	Ac-Ala
Isomerases		
Glucosephosphate isomerase EC 5.3.1.9		
Rabbit	(44)	Ac-Ala-
Contractile system and cytoskeleton proteins		
Actins		
Warm-blooded vertebrates		
Striated muscle, skeletal (α-actin), ox	(45—47)	Ac-Asp-Glu-Asp-Glu-Thr-Thr-Ala-Leu-Val-Cys
Striated muscle, skeletal (α-actin), rabbit	(1, 46, 48—51)	Ac-Asp-Glu-Asp-Glu-Thr-Thr-Ala-Leu-Val-Cys

Striated muscle, skeletal (α-actin), chicken	(45, 47, 52)	Ac-Asp-Glu-Asp-Glu-Thr-Thr-Ala-Leu-Val-Cys
Striated muscle, cardiac, ox	(45—47)	Ac-Asp-Asp-Glu-Glu-Thr-Thr-Ala-Leu-Val-Cys
Smooth muscle (type 1), ox	(45, 46)	Ac-Glu-Glu-Glu-Asp-Ser-Thr-Ala-Leu-Val-Cys
Smooth muscle (type 2), ox	(45)	Ac-Glu-Glu-Glu-Thr-Thr-Ala-Leu-Val-Cys-Asp
Smooth muscle (type 2), chicken	(45, 47, 52)	Ac-Glu-Glu-Glu-Thr-Thr-Ala-Leu-Val-Cys-Asp
Cytoplasmic (β-actin), ox	(45, 47, 51, 53)	Ac-Asp-Asp-Asp-Ile-Ala-Ala-Leu-Val-Val-Asp
Cytoplasmic (β-actin), rat	(47, 54, 55)	Ac-Asp-Asp-Asp-Ile-Ala-Ala-Leu-Val-Val-Asx[d]
Cytoplasmic (β-actin), mouse	(47, 51)	Ac-Asp-Asp-Asp-Ile-Ala-Ala-Leu-Val-Val-Asp
Cytoplasmic (β-actin), chicken	(45)	Ac-Asp-Asp-Asp-Ile-Ala-Ala-Leu-Val-Val-Asp
Cytoplasmic (γ-actin), ox	(47, 51, 53)	Ac-Glu-Glu-Glu-Ile-Ala-Ala-Leu-Val-Ile-Asp
Cytoplasmic (γ-actin), rat	(47, 54, 55)	Ac-Glu-Glu-Glu-Ile-Ala-Ala-Leu-Val-Ile-Asx[e]
Cytoplasmic (γ-actin), mouse	(47, 51)	Ac-Glu-Glu-Glu-Ile-Ala-Ala-Leu-Val-Ile-Asp
Amphibia		
Cytoplasmic (type 1), newt (*Triturus cristatus*)	(56)	Ac-Asp-Asp-Asp- - -Ala-Leu-Val- -Asp
Cytoplasmic (type 3), newt (*T. cristatus*)	(56)	Ac-Asp-Glu-Asp- - -Ala-Leu-Val- -Asp
Cytoplasmic (type 4), frog (*Rana pipiens*)	(56)	Ac-Glu-Asp-Asp- - -Ala-Leu-Val- -Asp
Cytoplasmic (type 4), *Xenopus laevis*	(56)	Ac-Glu-Asp-Asp- - -Ala-Leu-Val- -Asp
Cytoplasmic (type 5), frog (*R. pipiens*)	(56)	Ac-Asp-Glu-Glu- - -Ala-Leu-Val- -Asp
Cytoplasmic (type 5), *X. laevis*	(56)	Ac-Asp-Glu-Glu- - -Ala-Leu-Val- -Asp
Cytoplasmic (type 8), newt (*T. cristatus*)	(56)	Ac-Glu-Glu-Glu- - -Ala-Leu-Val- -Asp
Cytoplasmic (type 8), *X. laevis*	(56)	Ac-Glu-Glu-Glu- - -Ala-Leu-Val- -Asp
Nonvertebrates		
Muscle, larval stage (type I), *D. melanogaster*	(57)	Ac-[f]
Cytoplasmic (type II), *D. melanogaster*	(58)	Ac-[g]
Cytoplasmic, *Physarum polycephalum*	(45, 59)	Ac-Glu-Gly-Glu-Asp-Val-Gln-Ala-Leu-Val-Ile[h]
Cytoplasmic, *Dictyostelium discoideum*	(60—63)	Ac-Asp-Gly-Glu-Asp-Val-Gln-Ala-Leu-Val-Ile[i]
Tropomyosin		
α-Chain, skeletal muscle, rabbit		Ac-Met-Asp-Ala-Ile-Lys-Lys-Lys-Met-Gln-Met
β-Chain, skeletal muscle, rabbit	(64, 65)	Ac-Met-Asp-Ala-Ile-Lys-Lys-Lys-Met-Gln-Met
Cardiac muscle, rabbit	(64, 66)	Ac-Met-Asp-Ala-Ile-Lys-Lys-Lys-Met-Gln-Met
Troponin C		
Fast skeletal muscle, man		Ac-Asp-Thr-Gln-Gln-Ala-Glu-Ala-Arg-Ser-Tyr
Fast skeletal muscle, rabbit		Ac-Asp-Thr-Gln-Gln-Ala-Glu-Ala-Arg-Ser-Tyr
Fast skeletal muscle, frog (*R. esculenta*)	(1, 67)	Ac-Ala-Gln-Pro-Thr-Asp-Gln-Gln-Met-Asp-Ala
Cardiac muscle, ox		Ac-Met-Asp-Asp-Ile-Tyr-Lys-Ala-Ala-Val-Glu
Cardiac muscle, slow skeletal muscle, rabbit	(68)	Ac-Met-Asp-Asp-Ile-Tyr-Lys-Ala-Ala-Val-Glu
Troponin I		

Skeletal muscle, rabbit		Ac-Gly-Asp-Glu-Glu-Lys-Arg-Asn-Arg-Ala-Ile
Troponin T		
Skeletal muscle, rabbit		Ac-Ser-Asp-Glu-Glu-Val-Glu-His-Val-Glu-Glu
Myosin		
L-4 light chain, skeletal muscle, rabbit		Ac-Ser-Phe-Ser-Ala-Asp-Gln-Ile-Ala-Glu-Phe
L-4 light chain, skeletal muscle, chicken	(69)	Ac-Ser-Phe-Ser-Pro-Asp-Gln-Ile-Asp-Asp-Phe
Parvalbumin		
pI 4.45 (α/β-lineage), thornback ray (*Raja clavata*)		Ac-Ser-Ser-Lys-Ile-Thr-Ser-Ile-Leu-Asn-Pro
pI 5.50 (α-lineage), rabbit		Ac-Ala-Met-Thr-Glu-Leu-Leu-Asn-Ala-Glu-Asp
pI 5.00, III (α-lineage), pike (*Esox lucius*)		Ac-Ala-Lys-Asp-Leu-Leu-Lys-Ala-Asp-Asp-Ile
pI 4.5 (β-lineage), frog (*Rana esculenta*)		Ac-Ser-Ile-Thr-Asp-Ile-Val-Ser-Glu-Lys-Asp
pI 4.52, III (β-lineage), coelacanth (*Latimeria chalumnae*)	(70)	Ac-Ala-Val-Ala-Lys-Leu-Leu-Ala-Ala-Ala-Asp
pI 4.4 (β-lineage), cod (*Gadus callarias*)		Ac-Ala-Phe-Lys-Gly-Ile-Leu-Ser-Asn-Ala-Asp
pI 4.36 (β-lineage), hake		Ac-Ala-Phe-Ala-Gly-Ile-Leu-Ala-Asp-Ala-Asp
pI 4.10, II (β-lineage), pike (*E. lucius*)		Ac-Ser-Phe-Ala-Gly-Leu-Lys-Asp-Ala-Asp-Val
pI 4.5 (β-lineage), whiting		Ac-Ala-Phe-Ala-Gly-Ile-Leu-Ala-Asp-Ala-Asp
II (β-lineage), carp (*Cyprinus carpio*)	(71)	Ac-Ala-Tyr-Ser-Gly-Ile-Leu-Asn-Asp-Ala-Asp
pI 4.25, III (β-lineage), carp (*C. carpio*)		Ac-Ala-Phe-Ala-Gly-Val-Leu-Asn-Asp-Ala-Asp
pI 4.47 (β-lineage), carp (*C. carpio*)	(72)	Ac-Ala-Phe-Ala-Gly-Val-Leu-Asn-Asp-Ala-Asp
V (β-lineage), chub (*Leuciscus cephalus*)	(73)	Ac-Ala-Phe-Gly-Leu-Lys-Glu-Ala-Asp-Ile-Thr
Profilin		
Spleen, ox	(74)	Ac-Ala-Gly-Trp-Asn-Ala-Tyr-Ile-Asp-Asn-Leu
Intermediary filament proteins		
Keratin IF subunits (5—7), epidermis, ox	(75, 76)	Ac-Ser
IF subunits (3, one decamin), kidney cells, hamster	(76)	Ac-Ala
Calcium-binding proteins[j]		
Calmodulin		
Brain, uterus, ox	(1, 77, 78)	Ac-Ala-Asp-Gln-Leu-Thr-Glu-Glu-Gln-Ile-Ala
Phosphorylase kinase EC 2.7.1.38 δ subunit, rabbit	(79)	Ac-Ala~Asp~Gln.Leu-Thr-Glu-Glu-Gln-Ile-Ala
Testis, rat	(80)	Ac-Ala-Asp-Glu-Leu-Thr-Glu-Glu-Gln-Ile-Ala
Scallop (*Patinopecten*)	(81)	Ac-Ala-Asp-Gln-Leu-Thr-Glu-Glu-Gln-Ile-Ala
Sea anemone (*Metridium senile*)	(82)	Ac-Ala~Asx~Glx.Leu-Thr-Glx-Glx-Glx-Ile-Ala
Tetrahymena (*Tetrahymena pyriformis*)	(83)	Ac-Ala-Asp-Gln-Leu-Thr-Glu-Glu-Gln-Ile-Ala
Intestinal calcium-binding protein		
Major component, pig	(1, 84)	Ac-Ser-Ala-Gln-Lys-Ser-Pro-Ala-Glu-Leu-Lys
Minor component, pig	(1, 84)	Ac-Ala-Gln-Lys-Ser-Pro

Fibrous proteins		
Keratin		
Wool high-sulfur fraction B2 related		
Lincoln wool, SCMK-B2A, sheep		Ac-Ala-Cys-Cys-Ser-Thr-Ser-Phe-Cys-Gly-Phe
Lincoln wool, SCMK-B2B, sheep		Ac-Ala-Cys-Cys-Ser-Thr-Ser-Phe-Cys-Gly-Phe
Lincoln wool, SCMK-B2C, sheep		Ac-Ala-Cys-Cys-Ser-Thr-Ser-Phe-Cys-Gly-Phe
Merino wool, SCMK-B2, sheep		Ac-Ala-Cys-Cys-Ser-Thr-Ser-Phe
Wool high-sulfur fraction IIIB related		
Merino wool, SCMKB-IIIB2, sheep		Ac-Ala-Cys-Cys-Ala-Pro-Arg-Cys-Cys-Ser-Val
Merino wool, SCMKB-IIIB3, sheep		Ac-Ala-Cys-Cys-Ala-Arg-Leu-Cys-Cys-Ser-Val
Merino wool, SCMKB-IIIB4, sheep		Ac-Ala-Cys-Cys-Ala-Arg-Leu-Cys-Cys-Ser-Val
Mohair, IIIB2, South African angora goat		Ac-Ala-Cys-Cys-Ala-Pro-Arg-Cys-Cys-Ser-Val
Feather		
Rachis, emu (*Dromaius novae-hollandiae*)	(1, 85)	Ac-Ser-Cys-Tyr-Asn-Pro-Cys-Leu-Pro-Arg-Ser
Callus, rachis, goose (*Anser domesticus*)	(86)	Ac-Ser-Cys-Tyr
Calamus, silver gull		Ac-Ala-Cys-Asn-Asp-Leu-Cys-Gly-Pro-Cys-Gly
Electron-transfer proteins		
Cytochrome c-related proteins		
Cytochrome c		
Man		Ac-Gly-Asp-Val-Glu-Lys-Gly-Lys-Lys-Ile-Phe
Chimpanzee		Ac-Gly-Asp-Val-Glu-Lys-Gly-Lys-Lys-Ile-Phe
Rhesus monkey		Ac-Gly-Asp-Val-Glu-Lys-Gly-Lys-Lys-Ile-Phe
Erythrocebus patas		Ac-Gly-Asp-Val-Glu-Lys-Gly-Lys-Lys-Ile-Phe
Long-winged bat (*Miniopteris schreibersi*)		Ac-Gly-Asp-Val-Glu-Lys-Gly-Lys-Lys-Ile-Phe
Dog		Ac-Gly-Asp-Val-Glu-Lys-Gly-Lys-Lys-Ile-Phe
Elephant seal (*Mirounga leonina*)		Ac-Gly-Asp-Val-Glu-Lys-Gly-Lys-Lys-Ile-Phe
Sheep		Ac-Gly-Asp-Val-Glu-Lys-Gly-Lys-Lys-Ile-Phe
Ox		Ac-Gly-Asp-Val-Glu-Lys-Gly-Lys-Lys-Ile-Phe
Pig		Ac-Gly-Asp-Val-Glu-Lys-Gly-Lys-Lys-Ile-Phe
Guanaco (*Lama guanaco*)		Ac-Gly-Asp-Val-Glu-Lys-Gly-Lys-Lys-Ile-Phe
Camel (*Camelus dromedarius*)		Ac-Gly-Asp-Val-Glu-Lys-Gly-Lys-Lys-Ile-Phe
Major component, *Hippopotamus amphibius*	(87)	Ac-Gly-Asp-Val-Glu-Lys-Gly-Lys-Lys-Ile-Phe
Minor component, *H. amphibius*	(87)	Ac-Gly-Asp-Ile-Glu-Lys-Gly-Lys-Lys-Ile-Phe[k]
Horse		Ac-Gly-Asp-Val-Glu-Lys-Gly-Lys-Lys-Ile-Phe
Donkey		Ac-Gly-Asp-Val-Glu-Lys-Gly-Lys-Lys-Ile-Phe
Zebra (*Equus quagga*)		Ac-Gly-Asp-Val-Glu-Lys-Gly-Lys-Lys-Ile-Phe
California gray whale		Ac-Gly-Asp-Val-Glu-Lys-Gly-Lys-Lys-Ile-Phe

Rabbit		Ac-Gly-Asp-Val-Glu-Lys-Gly-Lys-Lys-Ile-Phe
Mouse		Ac-Gly-Asp-Val-Glu-Lys-Gly-Lys-Lys-Ile-Phe
Testis-specific, mouse		Ac-Gly-Asp-Ala-Glu-Ala-Gly-Lys-Lys.Ile-Phe
Gray kangaroo		Ac-Gly-Asp-Val-Glu-Lys-Gly-Lys-Lys-Ile-Phe
Pekin duck		Ac-Gly-Asp-Val-Glu-Lys.Gly-Lys.Lys~Ile~Phe
Pigeon		Ac-Gly-Asp-Val-Glu-Lys.Gly-Lys.Lys~Ile~Phe
Ostrich		Ac-Gly-Asp-Ile-Glu-Lys-Gly-Lys-Lys-Ile-Phe
King penguin		Ac-Gly-Asp-Ile-Glu-Lys-Gly-Lys-Lys-Ile-Phe
Chicken		Ac-Gly-Asp-Ile-Glu-Lys-Gly-Lys-Lys-Ile-Phe
Turkey		Ac-Gly-Asp-Ile-Glu-Lys-Gly-Lys-Lys-Ile-Phe
Emu		Ac-Gly-Asp-Ile-Glu-Lys-Gly-Lys-Lys-Ile-Phe
Snapping turtle		Ac-Gly-Asp-Val-Glu-Lys.Gly-Lys-Lys-Ile-Phe
Rattlesnake		Ac-Gly-Asp-Val-Glu-Lys-Gly-Lys-Lys-Ile-Phe
Bullfrog		Ac-Gly-Asp-Val-Glu-Lys-Gly-Lys-Lys-Ile-Phe
Puget Sound dogfish		Ac-Gly-Asp-Val-Glu-Lys-Gly-Lys-Lys-Val-Phe
Tuna fish		Ac-Gly-Asp-Val-Ala-Lys.Gly-Lys-Lys.Thr-Phe
Bonito		Ac-Gly-Asp-Val-Ala-Lys-Gly-Lys-Lys-Thr-Phe
Iso-1-cytochrome c, carp		Ac-Gly-Asp-Val-Glu-Lys.Gly-Lys-Lys.Val-Phe
Iso-2-cytochrome c, carp		Ac-Gly-Asp-Val-Asp-Lys.Gly-Lys-Lys.Val-Phe
Freshwater prawn (*Macrobrachium malcomsonii*)		Ac-Gly-Asp-Val-Glu-Lys-Gly-Lys-Lys-Ile-Phe
Pacific lamprey (*Entosphenus tridentatus*)		Ac-Gly-Asp-Val-Glu-Lys-Gly-Lys-Lys-Val-Phe
Ginkgo biloba		Ac-Ala-Thr-Phe-Ser-Glu-Ala-Pro-Pro-Gly-Asp
Elder (*Sambucus nigra*)		Ac-Ala-Ser-Phe-Ala-Glu-Ala-Pro-Pro-Gly-Asn
Box-elder (*Acer negunda*)		Ac-Ala-Ser-Phe-Ala-Glu-Ala-Pro-Pro-Gly-Asn
Castor		Ac-Ala-Ser-Phe-Asx-Glx-Ala-Pro-Pro-Gly-Asx
Sunflower		Ac-Ala-Ser-Phe.Ala-Glu-Ala-Pro-Ala-Gly-Asp
Major component, sesame		Ac-Ala-Ser-Phe-Asx-Glx-Ala-Pro-Pro-Gly-Asx
Minor component, sesame		Ac-Ala-Ser-Phe-Ser-Glx-Ala-Pro-Pro-Gly-Asx[1]
Cotton		Ac-Ala-Ser-Phe-Gln-Glu-Ala-Pro-Pro-Gly-Asn
Hemp (*Cannabis sativa*)		Ac-Ala-Ser-Phe-Asx-Glx-Ala-Pro-Pro-Gly-Asx
Rice (*Oryza sativa*)	(88, 89)	Ac-Ala-Ser-Phe-Ser-Glu-Ala-Pro-Pro-Gly-Asn
Wheat		Ac-Ala-Ser-Phe-Ser-Glu-Ala-Pro-Pro-Gly-Asn
Buckwheat		Ac-Ala-Thr-Phe-Ser-Glu-Ala-Pro-Pro-Gly-Asn
Parsnip (*Pastinaca sativa*)		Ac-Ala-Ser-Phe-Ala-Glu-Ala-Pro-Pro-Glv-Asp
Mung-bean		Ac-Ala-Ser-Phe-Asx-Glx-Ala-Pro-Pro-Gly-Asx
Pumpkin		Ac-Ala-Ser-Phe-Asx-Glu-Ala-Pro-Pro-Gly-Asx
Potato		Ac-Ala-Ser-Phe-Gly-Glu-Ala-Pro-Pro-Gly-Asn

Tomato		Ac-Ala-Ser-Phe-Asn-Glu-Ala-Pro-Pro-Gly-Asn
Leek (*Allium porrum*)		Ac-Ala-Thr-Phe.Ser-Glx-Ala-Pro-Pro-Gly-Asx
Rape		Ac-Ala-Ser-Phe-Asp-Glu-Ala-Pro-Pro-Gly-Asn
Cauliflower		Ac-Ala-Ser-Phe-Asp-Glu-Ala-Pro-Pro-Gly-Asn
Spinach (*Spinacea oleracea*)		Ac-Ala-Thr-Phe-Ser-Glu-Ala-Pro-Pro-Gly-Asn
Abutilon theophrasti		Ac-Ala-Ser-Phe.Gln-Glu-Ala-Pro-Pro-Gly-Asn
Love-in-a-mist (*Nigella damascena*)		Ac-Ala-Ser.Phe.Asx-Glx-Ala-Pro-Ala-Gly-Asx
Nasturtium (*Tropaeolum majus*)		Ac-Ala-Ser-Phe-Ala-Glu-Ala-Pro-Ala-Gly-Asp
Niger (*Guizotia abyssinica*)		Ac-Ala-Ser-Phe-Ala-Glu-Ala-Pro-Ala-Gly-Asp
Enteromorpha intestinalis		Ac-Ser-Thr-Phe-Ala-Asx-Ala-Pro-Pro-Gly-Asx
Euglena gracilis		Ac-Gly-Asp-Ala-Glu-Arg-Gly-Lys-Lys-Leu-Phe
Cytochrome C_{550}		
Paracoccus denitrificans (= *Micrococcus denitrificans*)		Ac-Asn-Glu-Gly-Asp-Ala-Ala-Lys-Gly-Glu-Lys
Heme carrier proteins		
Globins		
Hemoglobin α-chain		
Frog (*Rana esculenta*)	(90, 91)	Ac-Ala-Leu
Component of Hb III, bullfrog tadpole (*R. catesbeiana*)	(92)	Ac-Ser-Leu-Ser-Ala-Ser-Glu-Lys-Ala-Ala-Val
Toad (*Bufo bufo*)	(93)	Ac-Ala-Leu
Port Jackson shark (*Heterodontus portus jacksoni*)		Ac-Ser-Thr-Ser-Thr-Ser-Thr-Ser-Asp-Tyr-Ser
Carp		Ac-Ser-Leu-Ser-Asp-Lys-Asp-Lys-Ala-Ala-Val
Component of Hb I, trout (*Salmo irideus*)	(94, 95)	Ac-Ser-Leu-Thr-Ala-Lys-Asp-Lys-Ser-Val-Val
Component of Hb IV, trout (*S. irideus*)	(94)	Ac-Ser-Leu-Ser-Ala-Lys
Goldfish (*Carassius auratus*)	(96)	Ac-Ser-Leu-Ser-Asp-Lys-Asp-Lys-Ala-Val-Val
Desert sucker (*Catostomus clarkii*)		Ac-Ser-Leu-Ser-Asp-Lys-Asp-Lys-Ala-Asp-Val
Hemoglobin β-chain		
Component of Hb Raleigh (β_1-valine + acetylalanine), man	(97)	Ac-Ala-His.Leu~Thr~Pro~Glu~Glu~Lys.Ser~Ala
Component of Hb B, cat (*Felis catus*)	(98—100)	Ac-Ser-Phe-Leu,Ser,Ala,Glu,Glu,Lys
Component of Hb B type, fishing cat	(101)	Ac-
Component of Hb B type, jungle cat	(101)	Ac-
Component of Hb B type, caracal	(101)	Ac-
Component of Hb B type, cheetah	(101)	Ac-
Component of Hb B type, serval	(101)	Ac-
Component of Hb B type, tiger	(101)	Ac-
Component of Hb B type, puma	(101)	Ac-

Component of Hb B type, lion	(101) Ac–
Nile crocodile (*Crocodylus niloticus*)	(102) Ac-Ala-Ser-Phe-Asp-Pro-His-Glu-Lys-Gln-Leu
Alligator (*Alligator mississippiensis*)	(102) Ac-Ala-Ser-Phe-Asp-Ala-His-Glu-Arg-Lys-Phe
Hemoglobin γ-chain	
Component of Hb F_1, fetal Hb, man	(103) Ac-Gly-His-Phe-Thr-Glu-Glu-Asp-Lys-Ala-Thr^m
Component of fetal Hb, rhesus monkey (*Macaca mulatta*)	(104) Ac-Gly-His-Phe-Thr-Glu-Glu-Asp-Lys-Ala-Thr^m
Hemoglobin ζ-chain	
Component of embryonic Hb, man	(105, 106) Ac-Ser-Leu-Thr-Lys-Thr-Glu-Arg-Thr-Ile-Ile
Myoglobin	
Shark (*H. portusjacksoni*)	(107) Ac-Thr-Glu-Trp-Glu-His-Val-Asn-Lys-Val-Trp
Shark (*Galeorhinus australis*)	(108) Ac-Ala-Asp-Trp-Asp-Lys-Val-Asn-Ser-Val-Trp
Shark (*Mustelus antarcticus*)	(109) Ac-Val-Asp-Trp-Glu-Lys-Val-Asn-Ser-Val-Trp
Yellowfin tuna (*Thunnus albacares*)	(110, 111) Ac-Ala-Asp-Phe-Asp-Ala-Val-Leu-Lys-Cys-Trp
Invertebrate globins	
Globin, gastropod mollusc (*Aplysia limacina*)	Ac-Ser-Leu-Ser-Ala-Ala-Glu-Ala-Asp-Leu-Ala
Myoglobin, gastropod mollusc (*A. kurodai*)	(112) Ac-Ser-Leu-Ser-Ala-Ala-Glu-Ala-Asp-Leu-Val
Hemoglobin α-chain, mollusc (*Anadara trapezia*)	(113) Ac-Val-Ala-Asp-Ala-Val-Ala-Lys-Val-Cys-Gly
Leghemoglobin	
Leghemoglobin b, soybean	(114) Ac-Ala
Viral proteins	
Coat proteins	
Tobacco mosaic virus coat protein	
Strain ER	Ac-Ser-Tyr-Asn-Ile-Thr-Thr-Pro-Ser-Gln-Phe
Strain O6	Ac-Ser-Tyr-Ser-Ile-Thr-Thr-Pro-Ser-His-Phe
Strain *dahlemense*	Ac-Ser-Tyr-Ser-Ile-Thr-Ser-Pro-Ser-Gln-Phe
Strain HR (Holmes rib-grass)	Ac-Ser-Tyr-Asn-Ile-Thr-Asn-Ser-Asn-Gln-Tyr
Strain ORS (*Odontoglossum* ringspot)	Ac.Ser,Tyr,Ser,Ile,Thr,Thr,Pro,Ser,Glx,Leu
Strain *vulgare*	Ac-Ser-Tyr-Ser-Ile-Thr-Thr-Pro-Ser-Gln-Phe
Strain OM	Ac-Ser-Tyr-Ser-Ile-Thr-Thr-Pro-Ser-Gln-Phe
Strain O	Ac-Ser-Tyr-Ser-Ile-Thr-Thr-Pro-Ser-Gln-Phe
Strain Kokubu	Ac-Ser-Tyr-Ser-Ile-Thr-Thr-Pro-Ser-Gln-Phe
Strain cowpea	Ac-Ala-Tyr-Ser-Ile-Pro-Thr-Pro-Ser-Gln-Leu
Turnip yellow mosaic virus coat protein	
Coat protein	Ac-Met-Glu-Ile-Asp-Lys-Glu-Leu-Ala-Pro-Gln
Alfalfa mosaic virus coat protein	
Strain S	Ac-Ser-Ser-Ser-Gln-Lys-Lys-Ala-Gly-Gly-Lys

Strain 425		Ac-Ser-Ser-Ser-Gln-Lys-Lys-Ala-Gly-Gly-Lys
Strain VRU	(115)	Ac-Ser-Ser-Ser-Gln-Lys-Lys-Ala-Gly-Gly-Lys
Adenovirus type 2 coat(-associated) proteins		
Hexon protein	(116, 117)	Ac-Ala-Thr-Pro-Ser-Met-Met-Pro-Gln-Trp-Ser
Component IX (hexon associated)	(117)	Ac-Ser-Ala-Asn-Ser-Phe-Asp-Gly-Ser-Ile-Val
Fiber protein	(117)	Ac-Met-Lys-Arg-Ala-Arg-Pro-Ser-Glu-Asp-Thr
Other virus coat protein		
Cucumber virus 4	(118)	Ac-Ala-Tyr-Asn-Pro-Ile-Thr-Ser
Virus Xf	(119)	Ac-Ser-Gly-Val-Gly-Asp-Gly-Val-Asp-Val-Val
Envelope protein PE2, Sindbis virus	(120)	Ac- -Ala-Ala- -Leu- - -Ala-Met-Cys
Other viral proteins		
Gag precursor polyprotein ($Pr76^{gag}$), Rous sarcoma virus	(121)	Ac-Met-Glu-Ala-Val-Ile-Lys-Val-Ile- -
Small-t antigen, Simian virus 40	(122, 123)	Ac-Met-Asp-Lys-Val-Leu-Asn-Arg-Glu-Glu-Ser
Large-T antigen, Simian virus 40	(122, 123)	Ac-Met-Asp-Lys-Val-Leu-Asn-Arg-Glu-Glu-Ser
Eye-lens proteins		
α-Crystallin		
αA2-chain		
Man (*Homo sapiens*)	(1, 125)	Ac-Met-Asp-Val-Thr-Ile-Gln.His~Pro~Trp~Phe
Rhesus monkey (*Macaca mulatta*)	(1, 125)	Ac-Met-Asp-Val-Thr-Ile-Gln.His~Pro~Trp~Phe
Potto (*Perodicticus potto*)	(124)	Ac-Met~Asp~Val~Thr~Ile~Gln~His~Pro~Trp~Phe
Galago (*Galago crassicaudatus*)	(124)	Ac-Met~Asp~Val~Thr~Ile~Gln~His~Pro~Trp~Phe
Brown lemur (*Lemur fulvus*)	(124)	Ac-Met~Asp.Val~Thr.Ile~Gln~His~Pro~Trp.Phe
Treeshrew (*Tupaia belangeri*)	(124, 125)	Ac-Met~Asp~Val~Thr~Ile~Gln~His~Pro~Trp~Phe
European hedgehog (*Erinaceus europaeus*)	(124, 125)	Ac-Met~Asp.Val~Thr.Ile~Gln~His~Pro~Trp.Phe
Jamaican fruit-eating bat (*Artibeus jamaicensis*)	(124)	Ac-Met~Asp~Ile~Thr~Ile~Gln~His~Pro~Trp~Phe
Dog (*Canis familiaris*)	(1, 125)	Ac-Met-Asp-Ile-Ala-Ile-Gln-His-Pro-Trp-Phe
Gray seal (*Halichoerus grypus*)	(124)	Ac-Met~Asp~Ile~Ala~Ile~Gln~His~Pro~Trp~Phe
California sea lion (*Zalophus californianus*)	(124)	Ac-Met~Asp~Ile~Ala~Ile~Gln~His~Pro~Trp~Phe
American mink (*Mustela vison*)	(124)	Ac-Met~Asp.Ile~Ala~Ile~Gln~Gln~Pro~Trp.Phe
Cat (*Felis catus*)	(1, 124, 125)	Ac-Met-Asp-Ile-Ala-Ile-Gln-His-Pro-Trp-Phe
Sloth bear (*Melursus ursinus*)	(124)	Ac-Met~Asp~Ile~Ala~Ile~Gln~His~Pro~Trp~Phe
Malayan pangolin (*Manis javanica*)	(124)	Ac-Met~Asp~Ile~Ala~Ile~Gln~His~Pro~Trp~Phe
Ox (*Bos taurus*)	(1, 125)	Ac-Met-Asp-Ile-Ala-Ile-Gln-His-Pro-Trp-Phe
Pig (*Sus scrofa*)	(1, 125)	Ac-Met-Asp-Ile-Ala-Ile-Gln-His-Pro-Trp-Phe
Giraffe (*Giraffa camelopardalis*)	(124)	Ac-Met~Asp~Ile~Ala~Ile~Gln~His~Pro~Trp~Phe
Dromedary (*Camelus dromedarius*)	(124)	Ac-Met~Asp~Ile~Ala~Ile~Gln~His~Pro~Trp~Phe

Hippopotamus (*Hippopotamus amphibius*)	(124)	Ac-Met~Asp~Ile~Ala~Ile~Gln~His~Pro~Trp~Phe
Horse (*Equus caballus*)	(1, 125)	Ac-Met-Asp-Ile-Ala-Ile-Gln-His-Pro-Trp-Phe
White rhinoceros (*Ceratotherium simum*)	(125, 126)	Ac-Met~Asp~Ile~Ala~Ile~Gln~His~Pro~Trp~Phe
Malayan tapir (*Tapirus indicus*)	(124)	Ac-Met~Asp~Ile~Ala~Ile~Gln~His~Pro~Trp~Phe
Minke whale (*Balaenoptera acutorostrata*)	(125, 126)	Ac-Met~Asp~Ile~Ala~Ile~Gln~His~Pro~Trp~Phe
Common porpoise (*Phocaena phocaena*)	(124)	Ac-Met~Asp~Ile~Ala~Ile~Gln~His~Pro~Trp.Phe
African elephant (*Loxodonta africana*)	(125, 126)	Ac-Met~Asp.Val~Thr.Ile~Gln~His~Pro~Trp.Phe
Cape hyrax (*Procavia capensis*)	(125, 126)	Ac-Met~Asp.Val~Thr.Ile~Gln~His~Pro~Trp.Phe
Aardvark (*Orycteropus afer*)	(124, 127)	Ac-Met~Asp~Val~Thr~Ile~Gln~His~Pro~Trp~Phe
Brazilian manatee (*Trichechus inunguis*)	(124, 127)	Ac-Met~Asp~Val~Thr~Ile~Gln~His~Pro~Trp~Phe
Rabbit (*Oryctolagus cuniculus*)	(1, 125)	Ac-Met-Asp-Val-Thr-Ile-Gln-His-Pro-Trp-Phe
American pika (*Ochotona princeps*)	(124)	Ac-Met~Asp~Val~Thr~Ile~Gln~His~Pro~Trp~Phe
Rat (*Rattus norvegicus*)	(1, 125)	Ac-Met-Asp-Val-Thr-Ile-Gln-His-Pro-Trp-Phe
Golden hamster (*Mesocricetus auratus*)	(124)	Ac-Met~Asp~Val~Thr~Ile~Gln~His~Pro~Trp~Phe
Mongolian gerbil (*Meriones unguiculatus*)	(124)	Ac-Met~Asp~Val~Thr~Ile~Gln~His~Pro~Trp~Phe
Guinea pig (*Cavia porcellus*)	(124)	Ac-Met~Asp~Val~Thr~Ile~Gln~His~Pro~Trp~Phe
Springhaas (*Pedetes cafer*)	(124)	Ac-Met~Asp~Val~Thr~Ile~Gln~His~Pro~Trp~Phe
Two-toed sloth (*Choloepus hoffmanni*)	(124)	Ac-Met~Asp.Val~Thr.Ile~Gln~His~Pro~Trp.Phe
Three-toed sloth (*Bradypus variegatus*)	(124)	Ac-Met~Asp.Val~Thr.Ile~Gln~Gln~Pro~Trp.Phe
Ant bear (*Tamandua mexicana*)	(124)	Ac-Met~Asp~Val~Thr~Ile~Gln~His~Pro~Trp~Phe
Red kangaroo (*Macropus rufus*)	(1, 125)	Ac-Met-Asp-Ile-Thr-Ile-Gln-His-Pro-Trp-Phe
North American opossum (*Didelphis marsupialis*)	(1, 125)	Ac-Met-Asp-Ile-Thr-Ile-Gln-His-Pro-Trp-Phe
Chicken (*Gallus gallus*)	(124, 125)	Ac-Met-Asp-Ile-Thr-Ile-Gln-His-Pro-Trp-Phe
Frog (*Rana esculenta*)	(124, 125)	Ac-Met-Asp-Ile-Thr-Ile-Gln-His-Pro-Trp-Phe
Shark (*Squalus acanthias*)	(128)	Ac-Met-Asp-Leu-Ala-Ile-Gln-Tyr-Pro-Trp-Phe
αA^{ins}-chain		
Rat (*Rattus norvegicus*)	(129)	Ac-Met~Asp~Val~Thr~Ile~Gln~His~Pro~Trp~Phe
αB2-chain		
Man (*Homo sapiens*)		Ac-Met-Asp-Ile-Ala-Ile-His-His-Pro-Trp-Ile
Ox (*Bos taurus*)		Ac-Met-Asp-Ile-Ala-Ile-His-His-Pro-Trp-Ile
Shark (*Squalus acanthias*)	(124)	Ac-Met-Asp-Ile-Ala-Ile-Gln-His-Pro-Trp-Leu
β-Crystallin		
βBp-chain		
Ox (*Bos taurus*)	(130, 131)	Ac-Ala-Ser-Asn-His-Glu-Thr-Gln-Ala-Gly-Lys
Ribosomal proteins		
30S Ribosomal protein S5		
Escherichia coli	(132)	Ac-Ala-His-Ile-Glu-Lys-Gln-Ala-Gly-Glu-Leu

30S Ribosomal protein S18		
E. coli		Ac-Ala-Arg-Tyr-Phe-Arg-Arg-Arg-Lys-Phe-Cys
50S Ribosomal protein L7		
E. coli		Ac-Ser-Ile-Thr-Lys-Asp-Gln-Ile-Ile-Glu-Ala[n]
Elongation factor Tu		
E. coli	(133—136)	Ac-Ser-Lys-Glu-Lys-Phe-Glu-Arg-Thr-Lys-Pro
Chromosomal proteins		
Histone H1		
Thymus Lys-rich histone fr. 1 (CTL-1), ox	(1, 137, 138)	Ac-Ser-Glu-Thr-Ala-Pro-Ala-Ala-Pro-Ala-Ala
Thymus Lys-rich histone fr. 2 (RTL-2), rabbit	(139)	Ac-Ser-Glu-Thr-Ala-Pro-Val-Ala-Pro-Ala-Ala
Thymus Lys-rich histone fr. 3 (RTL-3), rabbit	(1, 137)	Ac-Ser-Glu-Ala-Pro-Ala-Glu-Thr-Ala-Ala-Pro
Thymus Lys-rich histone fr. 4 (RTL-4), rabbit	(1, 137)	Ac-Ser-Glu-Ala-Pro-Ala-Glu-Thr-Ala-Ala-Pro
Erythrocyte (CEL-5), chicken	(139)	Ac-Ser-Glu-Ala-Pro-Thr-Val-Ala-Ala-Pro-Ala
Testis, trout	(140)	Ac-Ala-Glu-Val-Ala-Pro-Ala-Pro-Ala-Ala-Ala[o]
Maize (*Zea mays*)	(141)	Ac-S=A-Thr-Asp-Val-S=A-Glu-Thr-Pro-S=A-Pro[p]
Maize (*Z. mays*)	(141)	Ac-S=A-Thr-Glu-Val-S=A-Glu-Thr-Pro-S=A-Pro[p]
Histone H2A		
Spleen H2A(1), two variants, man	(142)	Ac-Ser~Gly~Arg.Gly~Lys.Gln~Gly~Gly~Lys.Ala
Spleen, H2A(2), two variants, man	(142)	Ac-Ser~Gly~Arg.Gly~Lys.Gln~Gly~Gly~Lys.Ala
Thymus, two variants, ox	(1, 143)	Ac-Ser-Gly-Arg-Gly-Lys-Gln-Gly-Gly-Lys-Ala
Chloroleukemia cells, three variants, rat		Ac-Ser-Gly-Arg-Gly-Lys-Gln-Gly-Gly-Lys-Ala
Friend leukemia cells, H2A(2), mouse	(144)	Ac-Ser~Gly~Arg.Gly~Lys.Glu~Gly~Gly~Lys.Ala[q]
Rainbow trout (*Salmo gairdnerii*)		Ac-Ser-Gly-Arg.Gly-Lys-Thr-Gly-Gly-Lys-Ala
Sea urchin (*Parechinus angulosus*)	(145)	Ac-Ser-Gly-Arg-Gly-Lys-Gly-Ala-Lys-Ala-Lys
Sea urchin (*Psammechinus miliaris*)	(146)	Ac-Ser-Gly-Arg-Gly-Lys-Gly-Ala-Lys-Gly-Lys
H2A(2), wheat germ (*Triticum aestivum*)	(147)	Ac-Met-Asp-Gly-Ser-Lys-Leu-Lys-Lys-Val-Ala
H2A(3), wheat germ (*T. aestivum*)	(147)	Ac-Met-Asp-Ala-Ser-Lys-Ala-Lys-Lys-Val-Ala
Histone H4		
Ox		Ac-Ser-Gly-Arg-Gly-Lys-Gly-Gly-Lys-Gly-Leu
Pig		Ac-Ser-Gly-Arg-Gly-Lys-Gly-Gly-Lys-Gly-Leu
Rat		Ac-Ser-Gly-Arg-Gly-Lys-Gly-Gly-Lys-Gly-Leu
Rainbow trout (*S. gairdnerii*)		Ac-Ser-Gly-Arg-Gly-Lys-Gly-Gly-Lys-Gly-Leu
Sea urchin (*P. miliaris*)		Ac-Ser-Gly-Arg-Gly-Lys-Gly-Gly-Lys-Gly-Leu
Pea		Ac-Ser-Gly-Arg-Gly-Lys-Gly-Gly-Lys-Gly-Leu
Hormones and active peptides		

Proopiomelanocortin-related polypeptides		
α-MSH		
Monkey		Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly
Sheep		Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly
Ox		Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly
Pig		Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly
Arabian camel (*Camelus dromedarius*)		Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly[r]
Horse		Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly
Leu[5]-enkephalin		
Rat	(148)	Ac-[s]
β-Endorphin		
β-Endorphin-(1-27), ox	(149)	Ac-Tyr~Gly~Gly~Phe~Met~Thr~Ser~Glu~Lys~Ser[t]
β-Endorphin, pig	(150)	Ac-Tyr~Gly~Gly~Phe~Met~Thr~Ser~Glu~Lys~Ser[u]
β-Endorphin-(1-27), pig	(150, 151)	Ac-Tyr~Gly~Gly~Phe~Met~Thr~Ser~Glu~Lys~Ser[u]
β-Endorphin, horse	(152)	Ac-Tyr~Gly~Gly~Phe~Met~Ser~Ser~Glu~Lys~Ser[u]
β-Endorphin, rat	(153—155)	Ac-[u]
β-Endorphin-(1-27), rat	(153, 155)	Ac-[u]
β-Endorphin -(1-26), rat	(155, 156)	Ac-Tyr~Gly~Gly~Phe~Met~Thr~Ser~Glu~Lys~Ser[u]
β-Endorphin-I, salmon (*Oncorhynchus keta*)	(157)	Ac-Tyr-Gly-Gly-Phe-Met-Lys-Pro-Tyr-Thr-Lys
β-Endorphin-II, salmon (*O. keta*)	(158)	Ac-Tyr-Gly-Gly-Phe-Met-Lys-Ser-Trp-Asn-Glu
Nervous system proteins		
Myelin basic protein		
Man		Ac-Ala-Ser-Gln-Lys-Arg-Pro-Ser-Gln-Arg-His
Chimpanzee		Ac-Ala-Ser-Gln-Lys-Arg-Pro-Ser-Gln-Arg-His
Ox		Ac-Ala-Ala-Gln-Lys-Arg-Pro-Ser-Gln-Arg-Ser
S(mall) variant, rat		Ac-Ala-Ser-Gln-Lys-Arg-Pro-Ser-Gln-Arg-His
Myelin P2 protein		
Peripheral nerve myelin, ox	(159, 160)	Ac-Ser-Asn-Lys-Phe-Leu-Gly-Thr-Trp-Lys-Leu
Sciatic nerve myelin, rabbit	(161, 162)	Ac-Ser-Asn-Lys-Phe-Leu-Gly-Thr-Trp-Lys-Leu
S-100b Protein		
Brain, β-subunit, ox	(163, 164)	Ac-Ser-Glu-Leu-Glu-Lys-Ala-Val-Val-Ala-Leu
Metal-binding proteins		
Metallothionein		
Liver, MT-2, containing Zn, man	(165)	Ac-Met-Asp-Pro-Asn-Cys-Ser-Cys-Ala-Ala-Gly
Liver, MT-1A, containing Zn, horse	(166)	Ac-Met-Asp-Pro-Asn-Cys-Ser-Cys-Pro-Thr-Gly
Kidney, MT-1A, containing Cd + Zn, horse	(166)	Ac-Met-Asp-Pro-Asn-Cys-Ser-Cys-Pro-Thr-Gly

Kidney, MT-1B, containing Cd + Zn, horse		Ac-Met-Asp-Pro-Asn-Cys-Ser-Cys-Val-Ala-Gly
Liver, MT-I, containing Cd + Zn, mouse	(167)	Ac-Met-Asp-Pro-Asn-Cys-Ser-Cys-Ser-Thr-Gly
Liver, MT-II, containing Cd + Zn, mouse	(168)	Ac-Met-Asp-Pro-Asn-Cys-Ser-Cys-Ala-Ser-Asp
Ferritin		
Spleen, apoferritin, horse	(1, 169)	Ac-Ser-Ser-Gln-Ile-Arg-Gln-Asn-Tyr-Ser-Thr
Liver, apoferritin, rat	(170)	Ac-Ser-Ser-Gln
Miscellaneous proteins		
ADP/ATP carrier		
Mitochondrion, heart, ox	(171)	Ac-Ser-Asp-Gln-Ala-Leu-Ser-Phe-Leu-Lys-Asp
Band 3 polypeptide from erythrocyte membranes		
Man	(172, 173)	Ac-Met-Glu-Glu/
Ovalbumin		
Chicken	(1, 174—177)	Ac-Gly-Ser-Ile-Gly-Ala-Ala-Ser-Met-Glu-Phe
Phosphatidylcholine exchange protein		
Liver, ox	(178, 179)	Ac-Met-Asp-Pro-Gly-Ala-Gly-Ala-Phe-Ser-Glu
Rhodopsin		
Ox	(180—182)	Ac-Met-Asn-Gly-Thr-Glu-Gly-Pro-Asn-Phe-Tyr
Structural protein from mitochondria		
Heart, ox	(183)	Ac-Ser
Thymosin α1		
Thymus, ox	(1, 184)	Ac-Ser-Asp-Ala-Ala-Val-Asp-Thr-Ser-Ser-Glu
Z-protein		
Liver, rat	(185)	Ac-Met-Asn-Phe-Ser-Gly-Lys-Tyr-Gln-Val-Gln[v]

[a] Isozyme-I is acetylated for 90 to 95%.
[b] Isozyme-II is acetylated for 60 to 80%.
[c] Asx-2 is probably Asn.
[d] δ-Actin is a nonacetylated form of β-actin, which is present in small amounts in cultured cells. It probably is the precursor.
[e] ϵ-Actin is a nonacetylated form of γ-actin, which is present in small amounts in cultured cells. It probably is the precursor.
[f] A component, behaving as the cytoplasmic type II on IEF, is present in small amounts in cultured cells. It probably is a nonacetylated precursor.
[g] Type III is a nonacetylated form of type II, which is present in small amounts in cultured cells. It probably is the precursor.
[h] A nonacetylated form is present in small amounts in cultured cells. It probably is the precursor. In vitro translation in a recticulocyte lysate system gives processing of the N-terminus with two N^{α}-acetylation steps: Met-Asp-Gly-...→Ac-Met-Asp-Gly-...→Asp-Gly-...→Ac-Asp-Gly-...
[i] A nonacetylated form is present in small amounts in cultured cells. It probably is the precursor.
[j] This section contains calcium-binding proteins, which do not form part of CONTRACTILE SYSTEM AND CYTOSKELETON PROTEINS.

[k] The minor component is present for 10.6%.
[l] The minor component is present for 30%.
[m] The fetal γ-chain is only partly acetylated.
[n] This protein is also present in a nonacetylated form, which is called L12.
[o] This protein is acetylated for 90 to 95%.
[p] Positions 1, 5, and 9 have not been determined with certainty; either serine (S) or alanine (A) is present. This protein is acetylated for 94%.
[q] H2A(1) probably has the same N-terminal sequence.
[r] This polypeptide is acetylated for about 50%.
[s] Leu^5-enkephalin is acetylated for 20 to 25%. This acetylated form is found only in *pars intermedia* of the pituitary.
[t] This polypeptide is partly acetylated.
[u] This polypeptide is partly acetylated. The acetylated form is found only in pituitary.
[v] This protein is acetylated for about 84%.

APPENDIX
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