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Review Article

Amphipathic Helix Motif: Classes and Properties

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INTRODUCTION

The amphipathic/amphiphilic α-helix is an often-encountered secondary structural motif in biologically active peptides and proteins. For the purpose of this review, an amphipathic/amphiphilic helix will be defined as an α-helix with opposing polar and nonpolar faces oriented along the long axis of the helix. In most instances, evidence for the existence of amphipathic/amphiphilic helixes is indirect but convincing. Although the emphasis in this review is on amphipathic/amphiphilic α-helixes whose properties include interactions with lipids, other classes of amphipathic/amphiphilic helixes will be discussed.

Amphipathic helixes were first described as a unique structure/function motif involved in lipid interaction by Segrest et al. in 1974.[1] Prior to this, Perutz et al.[2] had noted that α-helixes in globular proteins often have narrow nonpolar edges along the long axis of the helix facing the nonpolar interior of the protein. More recently, amphipathic helical domains have been described for other lipid-associating proteins, including certain polypeptide hormones such as endorphins,[3,4] polypeptide venoms such as bombolitin,[5,6] polypeptide antibiotics such as the magainins,[7,8] and certain complex transmembrane proteins such as bacteriorhodopsin.[9,10] In addition, amphipathic helixes involved in both intra- and intermolecular protein-protein interactions have been described in a number of proteins, including globular α-helical proteins such as hemoglobin,[2] calmodulin-regulated protein kinases such as skeletal muscle light chain kinase,[11] and coiled-coil containing proteins such as myosin.[12,13] The functional properties suggested for amphipathic helixes include lipid association, membrane perturbation in the form of fusion or lysis, hormone-receptor catalysis, transmembrane signal transduction, regulation of kinase-calmodulin signal transduction, and transmembrane helical bundle formation.

The word *amphipathic*, introduced by Hartley in 1936,[14] literally means "passion" (*pathos*) "on both sides" (*amphi*). In this review we will use the word *amphipathic* rather than the alternate *amphiphilic*, a term coined more recently by Tanford,[15] because amphiphilic suggests that the hydrophilic and hydrophobic moieties of a given molecule have strong affinities for polar and nonpolar environments, respectively. The hydrophobic effect depends predominantly upon a like/dislike (*pathos*) of water for polar and nonpolar molecules, while the like (*philos*) of nonpolar molecules for themselves (van der Waals' forces) contributes little to the hydrophobic effect. In addition, there is precedence in the chemical literature for amphipathic versus amphiphilic[16] and the *Chambers Science and Technology Dictionary*[17] does not list the latter term.

Based upon a detailed analysis of their physical-chemical and structural properties, we have grouped amphipathic helixes into seven classes (A, H, L, G, K, C, and M; Table I). After a discussion of methods for detecting this structural motif, each class will be discussed separately. Individual examples of each class are described in Figures 1 and 2. Understanding the properties of these classes should increase our understanding of this common secondary structural motif and facilitate analysis of newly sequenced proteins.

ALGORITHMS FOR IDENTIFICATION AND ANALYSIS

Several methods exist for the detection and characterization of protein domains with putative amphipathic helical structure. Two straightforward graphical techniques, the "Schiffer-Edmundson" helical wheel diagram[18] and the helical net or grid representation by Lim,[19] are the methods of choice for starting an analysis. The helical wheel diagram consists of a projection of the side-chain orientation onto a plane perpendicular to the long axis of the helix (see Fig. 1). The helical-net diagram is a projection of the side-chain positions on a cylindrical surface wrapped around the long axis of the helix.

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TABLE I. Average Properties of Seven Different Classes of Amphipathic Helixes*

Property	Classes						
	A (Apolipoproteins)	H (Polypeptide hormones)	L ("Lytic" polypeptides)	G (Globular proteins)	K (Calmodulin-regulated protein kinases)	C (Coiled-coil proteins)	M (Transmembrane proteins)
Mean hydrophobic moment per residue	0.42	0.54	0.37	0.13	0.38	0.22	0.12
Mean hydrophobicity per residue of nonpolar face	0.73	0.57	0.74	0.64	0.55	0.80	0.74
Mean charged residue density per 11mer of helix							
Positive	1.9	2.4	1.6	1.3	3.0	2.1	0.09
Lys/Arg ratio	2.0	0.8	30	5.7	0.8	1.3	0.6
Negative	2.0	0.5	0.4	1.3	0.2	2.5	0.25
Total	3.9	2.9	2.0	2.6	3.2	4.6	0.3
Mean ± charge ratio	0.9	4.8	4.0	1.1	15	0.8	0.4
Mean localization of charged residues by quadrant							
Nonpolar face Positive							
Polar face							
Nonpolar face Negative							
Polar face							
Mean angle subtended by polar face	≥180	≤100	≤100	≥180	≥180	≥320	≤60

*Database for classification: **Class A.** A total of 28 amphipathic helical sequences were analyzed at the following residue positions: Apolipoproteins A-I [44–65, 66–87, 99–120, 121–142, 143–164, 165–186, 187–208, 220–241], A-II [18–30, 39–47, 52–66], A-IV [40–61, 62–83, 95–116, 117–138, 139–160, 161–182, 183–204, 205–226, 227–248, 249–269, 270–288, 289–310, 311–332], C-I [7–14, 18–29, 33–53], and C-III [40–67]. **Class H.** A total of 12 amphipathic helical sequences, representing the 11mer window with the highest hydrophobic moment, were analyzed at the following residue positions for the following polypeptide hormones: Calcitonin [14–24], corticotropin releasing factor [13–23], β-endorphin [20–30], glucagon [17–27], secretin [14–24], vasoactive intestinal peptide [18–28], neuropeptide Y [23–33], growth hormone releasing factor [19–29], parathyroid hormone 1–34 [10–20], adrenocorticotropin hormone [5–15], pancreatic polypeptide [24–34], calcitonin gene related peptide [10–20]. **Class L.** A total of 13 amphipathic helical sequences were analyzed full length as amphipathic helixes from the following lytic peptides: Magainin 1 and 2; bombolitin I, III, IV, V; crabrolin; mastoparan M, X, A, II, C; and *Polistes* mastopraran. **Class G.** A total of 21 amphipathic helical sequences were analyzed at the following residue positions: Worm myohemerythrin [18–38, 40–62, 69–87, 93–110], bacterial cytochrome b562 [2–19, 24–45, 62–86, 88–108], worm hemerythrin [21–37, 41–64, 69–86, 90–103], bacterial cytochrome c3 [5–23, 42–54, 79–100, 106–117], and sperm whale myoglobin [6–17, 21–35, 59–77, 100–117, 126–147]. **Class K.** A total of 6 amphipathic helical sequences were analyzed at the following residue positions: rabbit smooth muscle myosin light chain kinase [2–17], rabbit skeletal muscle myosin light chain kinase [343–358], chick smooth muscle light chain kinase [9–25], phosphorylase b kinase [244–261], rabbit muscle phosphofructose kinase [13–26 in M10 + 11], and rabbit fast muscle troponin I [96–109]. **Class C.** A total of 5 amphipathic helical sequences from coiled-coils were analyzed at the following residue positions: Myosin c-β [448–483, 484–519, 520–555] and myosin C-α [446–481, 482–517]; and a total of 4 sequences from putative leucine zipper amphipathic helical motifs were analyzed at the following residue positions: v-fos [165–192], v-jun [465–492], c-erb [309–344], and I-myc [333–360]. **Class M.** A total of 59 transmembrane amphipathic helical sequences were analyzed. In order to exclude the possibility of charged residues at either end of each transmembrane sequence "snorkeling" out of the hydrophobic interior of the membrane, the N- and C-terminal four residues were omitted from each putative transmembrane sequence analyzed. Transmembrane helixes analyzed were as follows: β-adrenergic receptor (helixes A–G), bacteriorhodopsin (helixes A–G), Band III (helixes 1–10), λ-adrenergic receptor A (helixes 1–4), λ-adrenergic receptor B (helixes 1–4), glucose transporter (helixes 1–12), sodium transporter (helixes 1–8), and rhodopsin (helixes A–G).

Both methods are easy to perform and have been constantly utilized.

There are, however, some drawbacks to these methods. As noted by Flinta et al.,[20] the graphical representation of the helical wheel can lead to overestimation of the amphipathicity. Furthermore, both methods are tedious and impractical for the analysis of long sequences such as apolipoprotein B.[21]

There is therefore clearly a need for more quantitative methods. An excellent example of such a method is the "helical hydrophobic moment" introduced by Eisenberg et al.,[22] a numerical way of expressing the helical amphipathicity of a protein seg-

Fig. 1. "Schiffer-Edmundson" wheel diagrams of examples representing the different classes of amphipathic helixes. Wheels are oriented with hydrophobic moment (approximately representing center of nonpolar face) vertically upward. **Class A,** apolipoprotein C-III [40–67]; **class H,** pancreatic polypeptide [24–34]; **class L,** bombolitin I; **class M,** bacteriorhodopsin helix C (N and C terminal 4 residues omitted); **class G,** myohemerythrin helix [18–38]; **class K,** rabbit smooth muscle myosin light chain kinase [2–17]; and **class C,** myosin c-β [448–483].

Fig. 1.

ment. This method consists of the vectorsum of the hydrophobicity values of the amino acids, taking into account their specific periodic orientation in the α-helix, i.e., one residue every 100° or 3.6 residues in a turn. This analysis, usually performed in a windowing fashion, is most often combined with the calculation of average hydrophobicities. A plot of both values for every n-residue-long segment in a protein, often referred to as an "Eisenberg plot," enables the detection of different kinds of helixes as they cluster into specific regions of the plot. Transmembrane helixes have a low helical hydrophobic moment and high hydrophobicity, surface seeking helixes have an average hydrophobicity and high helical hydrophobic moment, and the helixes of most globular proteins have both average hydrophobicity and helical hydrophobic moment characteristics.[22,23] Lipid-associating amphipathic helixes of the apolipoproteins mostly are located between the "surface seeking" and globular helixes.[23–25] Moreover, this type of analysis also enables the detection, due to their clustering in a specific region, of amphipathic helixes with receptor-binding characteristics.[26–28]

An extension of the above method consists of calculating hydrophobic moments considering all possible side-chain orientations, i.e., hydrophobic moments, using periodicity angles between 0° and 180°; this is equivalent to performing a Fourier analysis.[29,30] This is often the method of choice for a good localization of an amphipathic helix.[30,31] Additional information on other periodic structures in a protein is also obtained[29] and in some cases further characteristics of the amphipathic helixes are revealed.[24] This method recently has been used in an extensive characterization of the helixes in globular proteins with known 3D structure.[32]

The quantitative nature of the moment calculations allows an estimation of the probability that a set of residues would form an amphipathic structure by chance. A simple Monte-Carlo-type approach gives us that answer: scramble a certain sequence, while conserving the relative frequencies of its residues, and compare the results of the native sequence with the distribution of values obtained by the randomizations.[20,33]

Still other computer methods have been used for "detecting" domains with amphipathic helical characteristics in newly sequenced proteins. In general, methods that detect homology between sequences can be useful. The "comparison matrix methods,"[34] and especially its graphical implementations (e.g., reference 35), have been useful for comparing the amphipathic domains of apo A-I, A-IV, and E[36] or localizing the helical amphipathic domains within apo B.[21] Another algorithm for the detection of specific sequence patterns: the calculation of cross-correlation coefficients between an n-residue-long segment, with known characteristics, and all n-residue-long segments of a protein, as first described by Kubota et al.,[37] has also been used successfully for the same purposes.[21,36]

While there is now a full range of methods available to detect domains with amphipathic helical characteristics at the residue level, new methods for describing the amphipathic nature of protein segments at the atomic level are still under development. A program currently under development named HAL, describing the α-helix at an intermediate level using certain general assumptions of the geometry of the α-helix and using residue hydrophobicity scales, allows an estimate of the partition properties of an amphipathic helix at an interphase (Dr. John Weinstein, personal communication). Attempts incorporating the atomic hydrophobicity values[38,39] in describing the amphipathic nature of peptides or other molecules have been described.[40] In the future, however, side-chain flexibility and effective solvent accessible surfaces will have to be included in order to obtain an accurate description of the amphipathic nature of these protein fragments at an atomic level.

LIPID-ASSOCIATING AMPHIPATHIC HELIXES

Class A: Apolipoproteins

Background

Apolipoproteins are the protein components of plasma lipoproteins and through their lipid-associating characteristics these proteins allow the transport of the otherwise water-insoluble lipids in plasma. Certain apolipoproteins have an additional metabolic function through their interaction with cell-surface receptors or activation of lipolytic enzymes.

There are two subclasses of apolipoproteins, those capable of moving from one lipoprotein particle to another (apolipoproteins A-I, A-II, A-IV, C-I, C-II, C-III, and E), and those that remain with one lipoprotein particle from biosynthesis to catabolism (apolipoproteins B-100 and B-48). Numerous experiments have shown the amphipathic α-helixes to be responsible for the lipid-associating properties of the exchangeable apolipoproteins. The acute phase reactant protein serum amyloid A (SAA) is known to contain class A amphipathic helixes through which it associates with HDL during the acute phase response.[41] In contrast, other structures in addition to the amphipathic helixes were proposed to cause the different lipid-associating properties of apolipoprotein B.[21] These structures need further experimental characterization and will not be discussed in more detail in the present review.

Properties

Physical-chemical. All the major human apolipoprotein genes have been cloned and sequenced.

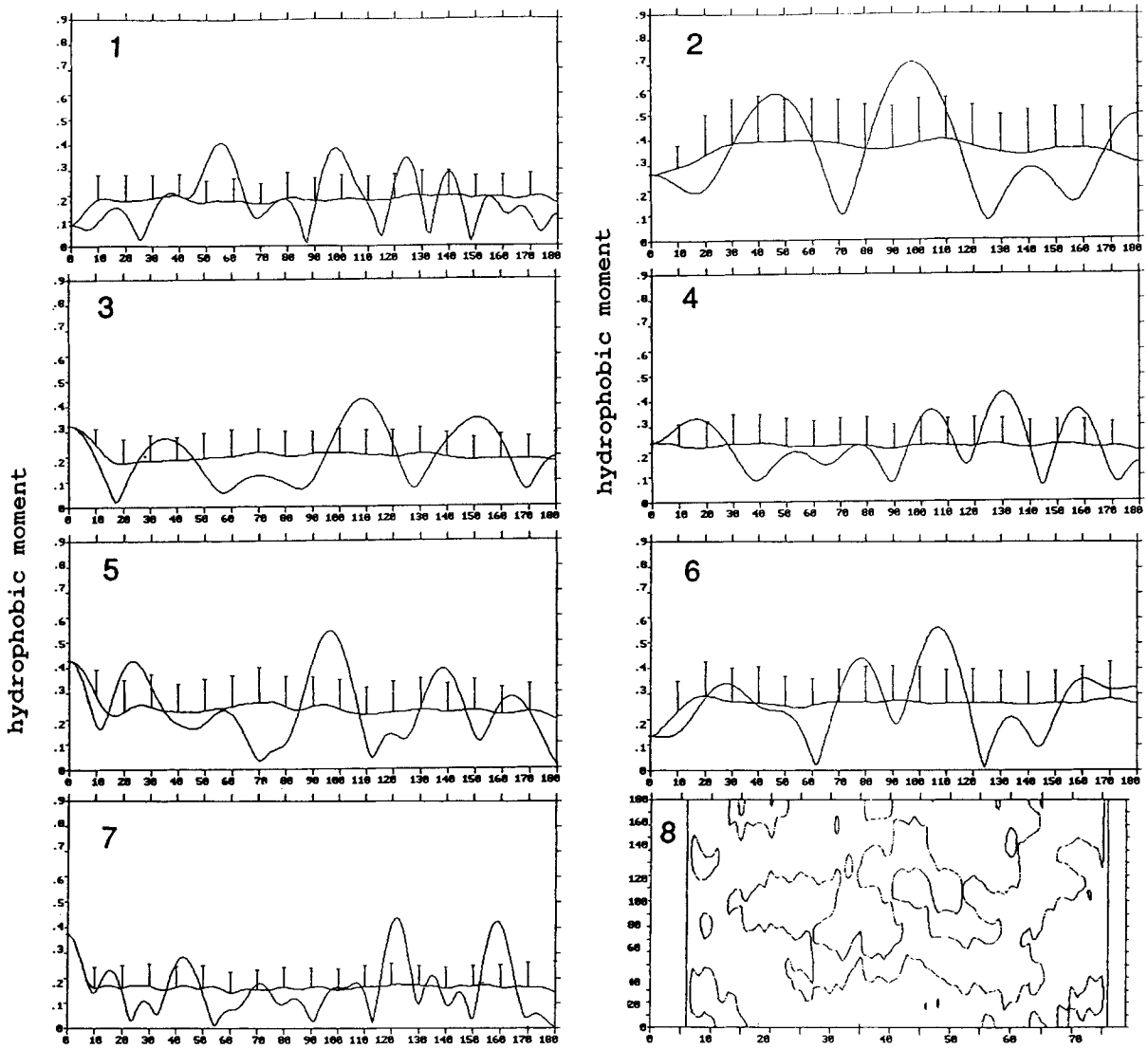

Fig. 2. Hydrophobic moments of the example helixes of Figure 1: **1)** class A; **2)** class H; **3)** class L; **4)** class M; **5)** class G; **6)** class K; **7)** class C. Moments (y-axis), using a per residue helical angle between 0 and 180° (x-axis), were calculated with a normalized version of the hydrophobicity scale developed by Engelman et al.[9,10] Normalization was accomplished by setting the average hydrophobicity of the complete scale equal to 0 and one standard deviation equal to 1. For each example the hydrophobic moments of the real sequence (curved line) were compared to the average and standard deviation (T-bars) of 100 randomizations of the same sequence. An angle of 100°, or a periodicity of 3.6 residues per helical turn, corresponds to the periodicity of an α-helix, and all examples show a maximum at or close to that value. This result indicates that from all possible periodic structures, α-helixes produce the best separation of hydrophilic/hydrophobic residues on the opposing faces. Note that due to the 20° twist of the hydrophobic surface of the coiled-coil helixes (panel 7) the maximum appears at 120°. With the exception of bacteriorhodopsin[20], the magnitude of the α-helical moment is about 2 standard deviations higher than the average randomized helix with the same amino acid composition. This corresponds to a probability of approximately 0.046 (based on a normal distribution). **8:** Contour plot of the hydrophobic moment: i.e., for all 11 residue long segments of the mature apo C-III sequence (x-axis), hydrophobic moments were calculated between 0 and 180° (y-axis). The contour lines correspond to a moment of 0.3. Note the maxima at about 100°.

Five genes, apo A-I, apo E, apo A-II, apo C-II, and apo C-III, show a remarkable similarity, by having four exons and three introns. In addition, several of these genes are closely located to each other on the genome.[42] The most striking feature of these exchangeable apolipoproteins is the presence of internal 22-residue-long repeats. Most importantly, this repeat unit has the periodicity of an amphipathic α-helix. Alignment of apolipoprotein sequences using these segments as the basic unit shows prolines consistently appear at the first and only the first position, punctuating the different helical repeats. These 22mer repeats appear predominantly in exon 4 and their number ranges from 13 in apo A-IV to one in apo C-III. Based on their degree of homology and pattern of internal repeats, an evolutionary tree

has been proposed[42] for the exchangeable apolipoprotein in which, through gene duplications, a single gene has evolved to the current multigene family.

De novo design and synthesis of peptides containing the sequence of an amphipathic helix and subsequent studies of the physical-chemical properties of these peptides has provided strong support for the amphipathic helix hypothesis. With the aid of this powerful technique, synthetic peptides have been designed having no homology to the sequence of naturally occurring apolipoproteins or peptide hormones[43–52] but containing the potential to form an amphipathic helix. Studies of these peptides have demonstrated that 1) the degree of amphipathicity correlates with the affinity of the peptides for phospholipid[43–52]; 2) an increase in the hydrophobicity of the hydrophobic face increases the lipid affinity[46]; 3) inclusion of a prolyl residue within the sequence of the putative helix decreases the lipid affinity of the peptide[46,48]; 4) lipid association increases the α-helicity of the peptides, as measured by circular dichroism spectroscopy.[46–52]

A number of experimental approaches have been used to study the binding of amphipathic peptides and proteins to lipid. These include ultracentrifugation,[53] electron microscopy,[54] electrophoresis,[55] CD spectroscopy,[44] IR spectroscopy,[56] fluorescence polarization and life-time measurements,[57] isothermal calorimetry,[58] low angle X-ray diffraction,[59] NMR spectroscopy,[60] differential scanning calorimetry,[44] determination of the relative ability of peptides to displace native apolipoproteins from intact lipoproteins,[44,46] surface pressure measurements,[61] and neutron scattering.[62]

Structural. The class A amphipathic helixes, like two other classes of lipid-associating amphipathic helixes, classes H and L (polypeptide hormones and the "lytic" peptides), have a high mean hydrophobic moment, although there is a considerable degree of variation between the helical domains of the different apolipoproteins. Class A differs, however, from classes H and L in three significant ways in the structure of the polar face. The most distinctive feature of the class A amphipathic helix is the unique clustering of positively charged residues at the polar-nonpolar interface and of negatively charged amino acid residues at the center of the polar face.[1] Arginyl and lysyl residues cluster within 20° of either side of the 180° polar-nonpolar plane; negatively charged residues subtend a radial angle of 100° centered on the polar face (see Table I). Class A helixes share two of the distinctive features of class G helixes (globular proteins): a zwitterionic polar face that subtends a mean radial angle, perpendicular to the long axis of the helix, of 180° or slightly greater. In addition, the polar face of the class A amphipathic helix has the second highest mean charge density of the seven classes; in an average 22mer amphipathic helix of class A there are 4 lysyl/arginyl and 4 glutamyl/aspartyl residues.

There are two interesting, and probably related, features of the tandem repeating 22mer domains in apolipoproteins A-I and A-IV: 1) the polar/nonpolar faces of adjacent domains remain approximately in register and 2) essentially all of the 22mer junctions are punctuated by prolyl residues. These two structural features would seem well suited to allow the association of a long, continuous amphipathic helix to the surface of the high density lipoprotein particle with its small radius of curvature (approximately 40 Å). In this model the prolyls would produce a bend and thus curvature to the continuous helix. An alternate model suggests that the prolyls induce α-α hairpin bends.[44,46]

Structure/function. Amphipathic helixes have a number of putative functions in the plasma apolipoproteins: 1) They serve in general as the lipid-associating domains for the exchangeable apolipoproteins[1] and therefore act as protein detergents to emulsify the lipid components of HDL to approximately 80 Å diameter particles.[63] 2) They play a role in the activation of the enzyme lecithin:cholesterol acyl transferase (LCAT).[48,64] 3) They may act to regulate the size of lipoprotein particles through putative hinged domain regions[65,66]: regions of amphipathic helix that have alternate lipoprotein bound or unbound conformations. 4) An amphipathic helix enhances the lipoprotein lipase activation capacity of apo C-II.[67] 5) In apo E and apo B, they are responsible for the interaction with cell surface receptors, and probably also with heparin.[21,68] 6) They stimulate the release of human placental lactogen from trophoblast cells in culture,[69] probably due to a direct interaction with the plasma membrane.[70] 7) Apo A-I, perhaps while associated with HDL in one of at least two alternate conformations, has been hypothesized to play a role in in vivo regulation of inflammation through inhibition of neutrophil activation probably via association with the cell surface membrane.[71]

Class H: Polypeptide Hormones

Background

Amphipathic α-helical structures with measurable lipid affinity are found in several polypeptide hormones. Early studies by Bodansky et al.[72] appreciated the fact that [5–27] secretin, and the homologous portion of glucagon, could be modelled into α-helixes containing segregated hydrophilic and hydrophobic residues similar to those described by Perutz et al.[2] The discovery that lipids can induce α-helical conformation in β-endorphin and ACTH led to the idea that this may be physiologically important.[73–75] Physicochemical assessment of secondary structures of native and analog β-endorphins then established the principle that the surface-seeking properties of the amphipathic helix may

be of fundamental biological importance for polypeptide hormones.[4,76,77] Approximately 11 other peptide hormones now have been ascribed similar amphipathic α-helical properties. These include calcitonin,[78] growth hormone releasing factor,[79] and corticotropin releasing factor.[80]

Properties

Physical-chemical. While these peptides have little ordered structure in aqueous solutions, they adopt the amphipathic α-helical conformation at hydrophobic-hydrophilic interfaces such as might occur at cell surfaces. An ability to increase surface tension at the air-water interface and to adopt an α-helical conformation when mixed with lipid, as measured by circular dichroism, are two of the common physical-chemical methods upon which this conclusion is based. These approaches, coupled with helical wheel projections, have been used to guide the design and study of analogs of this class of amphipathic helix. A correlation between modeled amphipathicity, physicochemical measurements, and biological activity has confirmed a role for this structure in peptide hormones. The crystal structure of two amphipathic helical peptide hormones, avian pancreatic polypeptide[81] and glucagon,[82] have been determined, confirming the more indirect approaches. No direct quantitative comparisons of lipid affinity between the peptide hormones of this class and other amphipathic helical classes have been performed.

Structural. With the exception of parathyroid hormone at 84 residues, most of the peptides of this structural class are 27–44 residues in length. The helical domains make up only a portion of the peptide, averaging approximately 18–20 residues, and often are inset several residues from the N-terminus.[4,26,72] Each of these peptides is encoded by a single exon. In some cases, it appears that a peptide evolved through gene duplication and that, although little primary sequence homology remains, secondary amphipathic helical structures do.[77,83,84]

This class of amphipathic helixes have similar structural properties to those of the lytic polypeptides, class L (see below). Both have mean hydrophobic moments >0.3 and are highly positively charged. In addition, both have intermediate charge densities and have polar faces that subtend an average angle of 100° or less perpendicular to the long axis of the helix.

There are two significant differences between the two classes. Most strikingly, the peptide hormones have a mean lysyl/arginyl ratio of 0.81, while the class L have a mean lysyl/arginyl ratio of 30. Second, the class H have nonpolar faces with a mean hydrophobicity per residue of 0.57, considerably lower than the mean hydrophobicity per residue of 0.74 for the nonpolar faces of the class L helixes. In spite of these differences in hydrophobicities, the hormones have a higher mean hydrophobic moment than the lytic peptides, due in part to the higher polar face charge density for the former (Table I).

Structure/function. Because of the lipid-associating properties of these polypeptide hormones, it has been proposed that binding to the cell membrane lipids facilitate subsequent interaction with specific receptors in the resulting two-dimensional plane.[85] Consistent with this relatively nonspecific role of the amphipathic domain is the finding that extensively substituted helixes, with preserved amphipathicity, maintain biologic activity and receptor affinity.[77] However, the fact that truncated peptides consisting largely of the amphipathic helix alone still bind their receptors, indicates that this domain carries some specificity.[86,89]

The existence of two distinct functional domains within this group of peptides has been proposed. An N-terminal domain contains the specific "active site" where each residue is critical for biological activity. This isolated domain, however, lacks membrane affinity and is therefore only a weak agonist.[3,77] An amphipathic helix portion, usually located C-terminal to the active site, is thought to enhance receptor binding in a relatively nonspecific manner by increasing affinity for the hydrophobic membrane environment (and possibly for the receptor). Peptide hormone fragments containing the amphipathic domain alone can bind to their specific receptors but they have little agonist activity. Interestingly, some of these peptides are able to inhibit specifically the activity of the parent hormone.[86–89] Inherent in this two-domain principle is the possibility of developing peptide analogs with either inhibitory or increased pharmacological activity compared to the native hormone.

An additional implication of the presence of amphipathic helical domains in peptide hormones is that this class of hormones may bind to a common type of receptor. Because these amphipathic helical domains bind receptors and lipid, a plausible possibility is that these peptides bind to hydrophobic receptor sites. As an example, a portion of the ligand binding site of the β-adrenergic receptor, and possibly many other integral membrane receptors, appears to be located in a largely hydrophobic transmembrane domain.[90–92]

Class L: Lytic Polypeptides

Background

The venom of several hymenopterae species, such as bees and wasps, contains large amounts of small cationic amphipathic α-helical peptides responsible for some of the toxic effects of these insect bites.[5,6,93,94] Key physiologic properties include mast cell degranulation and phospholipase A_2 activation. A family of structurally similar peptides, the magainins, have more recently been described in the skin of frogs from several species. The magainins

are thought to act as antibiotics by disrupting the membranes of prokaryotes.[95,96] Melittin, whose amphipathic domain most closely resembles that of the transmembrane proteins (class M), has not been included as a member of class L.

Properties

Physical-chemical. Although not all of these peptides have been subjected to the same physical-chemical and functional measurements, they appear to share many properties. In the presence of lipids and trifluroethanol they have a high α-helical content and they interact with membrane bilayers to produce dramatic structural changes.[94–97]

Structural. All of the peptides of class L consist entirely of an amphipathic helix. As noted above, the class L helixes are similar to those of class H: both have high helical hydrophobic moments and are highly positively charged, both have intermediate charge densities, and both have polar faces that are narrower than those of most other classes. The major difference is that, in 13 class L examples, lysyl residues occur 30 times for only one arginyl residue. Additionally, class L helixes have a more hydrophobic nonpolar face than do class H.

Structure/function. The antibiotic effects of the magainins and other frog skin peptides have been attributed to their lipid affinity and may be due to disruption of prokaryotic cell membranes followed by osmotic lysis.[7,95,96] Some of the toxic effects of the insect peptides also may be due to a lipid effect. The activity of phospholipase A_2, for example, is facilitated by mastoparan and bombolitin, probably via increased substrate availability.[6,98] Interestingly, mast cell degranulation does not appear to be a result of a toxic effect. Mastoparan has been shown to activate phospholipase C[99] and recently it was demonstrated that this peptide may directly and specifically activate mast cell G-proteins without damaging the cell.[100] The other mast cell activating venom peptides are sufficiently similar to suggest a similar mode of action. These peptides may provide interesting tools with which to study cellular activation pathways.

Class M: Complex Transmembrane Proteins

Background

Based upon the first low-resolution electron diffraction image of an integral membrane protein, the purple membrane protein bacteriorhodopsin appears something like a sandwich of three helixes packed against four, all roughly perpendicular to the membrane plane.[101] With another first for integral membrane proteins, the sequence of bacteriorhodopsin[102,103] added to our maturing view of the amphipathic helix as a structural building block. Engelman and colleagues[9,10] examined the charged and polar group positions relative to the hydrophobic residues and helped solidify the current thinking of membrane protein as "inside-out" proteins. Subsequent to the sequencing of bacteriorhodopsin by conventional protein methods, advances in recombinant DNA technology have allowed the sequencing of many proteins. Some of the algorithms discussed earlier for the detection of amphipathic and hydrophobic domains have been the principle or only methods used for analyzing the structures of these proteins. Exceptions will be discussed below.

Properties

Physical-chemical. What may have been a surprise when first observed for bacteriorhodopsin has now become the rule rather than exception for integral membrane proteins: the placement of polar as well as charged groups within the hydrophobic membrane-embedded sequences. Amphipathic alignment of residues give rise to helical interfaces that have been proposed to be *more* polar than the surface residues, rather than less polar, as they are for the packing interfaces of water-soluble helix bundles.[10,104]

The only atomic resolution structure for an integral membrane protein at present is that of the bacterial photosynthetic reaction center from *Phodopseudomonas viridis*[105] and *Rhodobacter sphaeroides*.[106,107] This structure has allowed a closer inspection of the distribution of residues between the interior and exterior of a membrane protein. In this structure the apolar residues of leucyl, isoleucyl, phenylalanyl, and valyl are located on the surface of the helixes, in contact with the fatty acyl chains of the membrane. In contrast to bacteriorhodopsin, there is no preference for the hydroxyl-containing residues to be in the interior of the protein and there are no buried ionizable residues in the reaction center.[106]

Unlike the helixes so far discussed, the term *amphipathic* carries a slightly different meaning for membrane proteins where charged residues are scarce and the role of polar uncharged groups in the amphipathic nature of transmembrane helixes is still unclear. Although the interior residues of membrane proteins are more polar than the surface residues, a simple reversal of the polarities found for the interior and surface residues, respectively, of water-soluble proteins does not exist. A sequence analysis of the amphipathic alignment of transmembrane helixes has shown that the polarity of interior residues is comparable for integral membrane and water-soluble proteins.[104] It is the polarity of the surface residues that differ between the two classes. In several recent examples,[108–115] site-directed mutagenesis has shown that the location of membrane-embedded charged groups is important to membrane protein function, which implicates their location in the interior of the protein.

Structural. In the selection of the database for analysis, the first four amino acid residues at either

end of each purported transmembrane helix were excluded. This exclusion was to minimize a) uncertainty as to the ends of the transmembrane domain and b) possible misleading results due to the "snorkel" effect (see below).

Class M amphipathic helixes differ the most in their properties from the other classes of amphipathic helixes. They have a very low charge density and are the only class with a net negative charge. Class M has the narrowest polar face, subtending a mean radial angle, perpendicular to the long axis of the helix, of less than 60°. Class M, like class G helixes, have a low mean helical hydrophobic moment (0.12 per residue). Finally, class M have a high mean nonpolar face hydrophobicity of 0.74 per residue. However, this is not as high as class C with a hydrophobicity of 0.80 per residue, and is no higher than either class L ("lytic" polypeptides) or class A (apolipoproteins), with hydrophobicities of 0.74 and 0.73 per residue, respectively.

Structure/function. Two channels were used in the database for analysis of the class M amphipathic helixes, one anion channel (band III) and one cation channel (sodium transporter). The cation channel helixes differ little from the remainder of the transmembrane database in regard to charge distribution on their polar faces. However, band III amphipathic helixes contain 4 of the 8 positively-charged residues found in the database of 59 transmembrane helixes. Thus the presence of positively-charged residues in class M would appear to bias the general database toward a slightly higher +/− charge ratio. Excluding anion channels this ratio might be even lower than is indicated in Table I.

PROTEIN-PROTEIN INTERACTIONS

Class G: Globular α-Helical Proteins

The first high resolution structures of soluble proteins were obtained on members of the globin family, hemoglobin[2] and myoglobin.[116] It was from this class of proteins that the amphipathic nature of helixes was identified[2] as "a regular periodicity of invariant non-polar sites." Furthermore, Perutz et al. realized this periodicity would be a potential tool "to recognize helical regions" in proteins of unknown structure. It is not surprising that about 50% of all α-helixes in soluble globular proteins are amphipathic,[32] because soluble proteins, by definition, have come to terms with an aqueous environment.

Class G amphipathic helixes from multiple α-helix-containing globular proteins, such as myoglobin and hemoerythrin, have several similar properties to the class A helixes from plasma apolipoproteins. Both, on average, have zwitterionic polar faces with moderately high to high mean charge densities and both have wide polar faces that subtend an angle, perpendicular to the long axis of the helix, of 180° or greater. The major physical difference, and part of the reason why most globular proteins generally do not interact with lipids, is their low mean hydrophobic moment (0.13 per residue) and their lower mean hydrophobicity of the nonpolar face (0.64 versus 0.73 per residue). In addition, class G helixes have a random distribution of negative and positive charges around the perimeter of the polar face, in contrast to the highly typical clustering seen in class A. Several laboratories have made use of these features of class G amphipathic helixes to design ab initio four helical bundle proteins.[117,118]

Class K: Calmodulin-Binding Domains of Calmodulin-Regulated Protein Kinases

Calmodulin is a ubiquitous intracellular protein that, in the presence of calcium, binds to and regulates the enzymatic activity of a number of enzymes.[11] Many of these enzymes are protein kinases that are targeted to and result in the phosphorylation of specific proteins. Experimental data suggests that the specificity for the binding of calmodulin to its target proteins is regulated in part by amphipathic helical domains of the protein kinases.[119] Many of the class L amphipathic peptides, such as mastoparan, bind to calmodulin with affinities similar to that of the calmodulin-regulated kinases.[120] With these initial observations as a guide, binding studies of synthetic model peptides and proteolytic fragments of calmodulin-regulated kinases by DeGrado et al.[121] have led to the identification of a number of putative calmodulin-binding amphipathic helical domains in the sequences of these kinases.

These putative amphipathic helixes have been analyzed together as one group, class K. Amphipathic helixes in this class have similarities to class H and L: 1) Class K has a high mean hydrophobic moment; 2) class K amphipathic helixes are highly positively charged; and 3) class K has an intermediate mean charge density.

Class K is like class H and unlike class L in two respects: classes K and H have similar mean lysyl/arginyl ratios of 0.77 and 0.81, both considerably lower than that of L (30.0), and similar nonpolar faces with mean hydrophobicities per residue of 0.55 and 0.57, lower than that of class L (0.74) (Table I).

The major difference in class K versus class H is in the width of the polar face. Like classes A and G, class K amphipathic helixes have wide polar faces that subtend an average angle of 180° or greater perpendicular to the long axis of the helix (Table I); the polar faces for class H (and L) average 100° or less in width. Classes K and G differ in that class K has a considerably higher hydrophobic moment (0.38 versus 0.13, respectively) and has a highly positively-charged polar face (that of class G is zwitterionic).

Class C: Coiled-Coil Proteins

Long before the α-helix was observed at atomic resolution, and only 2 years after it was proposed as a possible regular structural unit for proteins by Pauling and Corey,[122] Crick[12] proposed a bundle of two parallel intertwining α-helixes as the structure for the class of fibrous proteins, members of which include keratin, myosin, and tropomyosin. The supercoiling of right-handed helixes gives rise to a left-handed twist and maximizes the surface contact area at the packing interface between the two helixes. A heptad repeat with hydrophobic residues at positions 1 and 4 results in a hydrophobic packing interface where the hydrophobic side chains pack by way of the now classical "knobs-into-holes" arrangement—for a recent review, see Cohen and Parry.[13] A characteristic of the fibrous protein helix is its unusually long length (> 200 aa) which is stabilized by the hydrophobic interactions of the coiled-coil. A potential new member of this class, a DNA-binding protein motif termed the "leucine zipper," has been identified[123,124] and is distinguished by its much shorter length of only about 30 residues. An identifiable heptad repeat and parallel dimeric structure make it a likely candidate for the coiled-coil structure. When analyzed separately, the properties of these two types of amphipathic helixes are essentially indistinguishable at the secondary structural level; thus we have included them in a single class of amphipathic helix.

Not surprisingly, class C helixes have some similarities to class G (globular α-helical proteins). Both have approximately zwitterionic polar faces that are wider than 180° on average and have high to intermediate mean charge densities. In fact, class C has the highest charge density of all seven classes of amphipathic helixes (4.6 charged residues per 11mer of helix). Class C has one feature in common with both classes G and H and that is a moderate mean hydrophobicity per residue of nonpolar face (0.55 versus 0.64 and 0.57, respectively).

Class C amphipathic helixes have three distinctive features: 1) they have an intermediate mean hydrophobic moment per residue (0.22); 2) they have the widest polar face of all seven classes of amphipathic helixes (> 320°); and 3) the nonpolar face has a left-handed twist of 20° every two turns of the helix.

FUTURE PERSPECTIVES

Snorkel Model

By using peptide analogs of amphipathic helixes of the A class it has been shown that localization of positively charged residues at the polar-nonpolar interface and negatively charged residues at the center of the polar face are important for lipid affinity.[44-46,50,51] Recent studies indicate that the positively charged amino acid residues, because of their marked amphipathic nature, can increase the lipid affinity of the class A amphipathic helix.[125] Specifically, amphipathic lysyl and arginyl side chains, when at or close to the polar-nonpolar interface of a class A amphipathic helix, provide a significant increase in lipid affinity due to the contribution of its hydrocarbon moiety to the overall hydrophobicity of the nonpolar face.

Based on these results, the model shown in Figure 3, termed the "snorkel" model, has been proposed for the lipid association of the class A amphipathic helixes. The bulk of the van der Waals surface areas of the positively charged residues is hydrophobic. It is proposed that these amphipathic basic residues, when associated with phospholipid, extend toward the polar face of the helix to insert their charged moieties into the aqueous milieu (for aqueous solvation). Thus, essentially the entirety of the uncharged van der Walls' surface of the amphipathic helixes of the apolipoproteins can be buried within the hydrophobic interior of a phospholipid monolayer.

The snorkel model may be relevant to some of the differences between the three classes of surface-active amphipathic helixes (classes A, H and L—Table I). Amphipathic helixes of classes H and L have narrow positively-charged amino acid residues clustered toward the center of the polar face. The snorkel model predicts that this particular amphipathic helical organization would allow deeper penetration into a membrane bilayer than an amphipathic helix with either a broader face or a zwitterionic face.

Deep membrane penetration might be important in the biological effects of classes H and L. The interaction of polypeptide hormones with receptors may involve a deep penetration of their amphipathic helical domains to produce a contact with an intramembranous portion of the receptor or to induce some coordinate alteration in the properties of the inner half of the membrane bilayer. Lysyl residues have a longer hydrophobic side chain than arginyl residues; the restriction of the positive residues in the "lytic" class of amphipathic helixes to lysyl residues may allow deeper membrane penetration and, therefore, in some way, the destabilization of membrane integrity. It thus may be that certain classes of amphipathic helixes can produce signal transduction via direct interaction with the cell membrane-bilayer, bypassing cell surface receptor interactions.[126]

HIV GP41

Recently, David Eisenberg and colleagues[127,128] reported that the carboxy terminal end of the membrane spanning glycoprotein, gp41, from the HIV virus contains two domains with very high hydrophobic moments. This has been confirmed by two other laboratories.[129,130] We recently have compared the features of these putative amphipathic he-

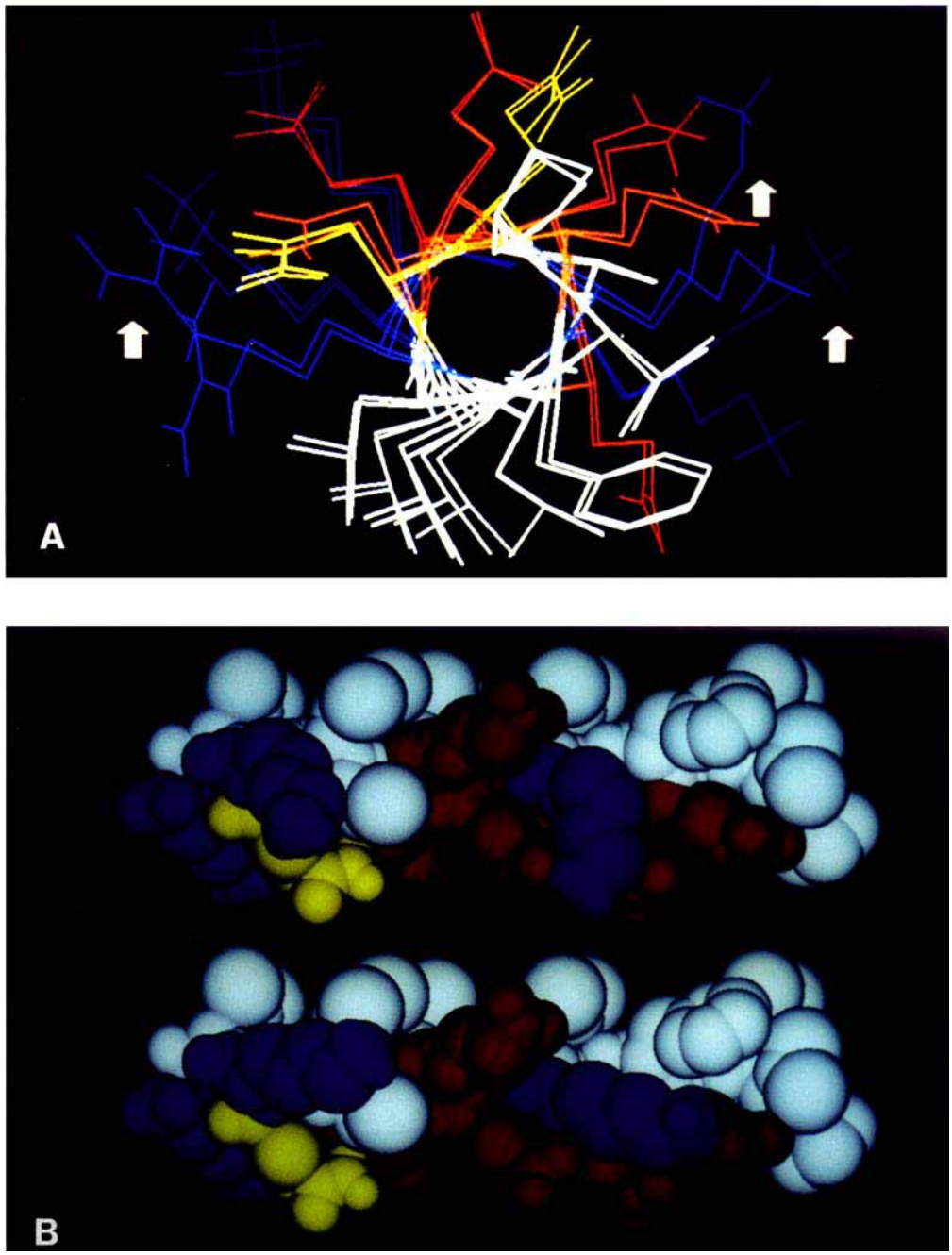

Fig. 3. Computer model of a model amphipathic peptide indicating features of the "snorkel model". The consensus class A apolipoprotein amphipathic helical peptide with the sequence: pro val leu asp glu phe arg glu lys leu asn glu glu leu glu ala leu lys gln lys leu lys (apo A-I consensus) was modeled using the SYBYL program-package (TRIPOS Associates, Inc.). An idealized α-helix was built with backbone dihedral angles $\psi = -58°$ and $\phi = -47°$. One side chain dihedral angle of each positively charged residues was changed from the trans to a cis conformation so as to bring the end of the side-chains closer to the hydrophilic side of the helix. Both the initial and the "snorkel" structure were subsequently minimized with SYBYL using the AMBER force field parameters. The energy difference between them was very small showing that the "snorkel-configuration" is possible without unfavorable intramolecular steric contacts. Graphics were made with the program Quanta (Polygen Corporation) on a silicon graphics 4D220/GTX. Hydrophobic residues are shown in white, acid residues in red, positively charged residues in blue while all other residues are shown in yellow. **A:** Superposition of the two helixes in a view along the long axis. **B:** Space-filling lateral view of the two helixes with the snorkel configuration on the top half of the figure.

lical regions from both HIV-1 (residues 768–788 and 826–854) and HIV-2 (residues 757–779 and 828–854) with the seven classes discussed in the present review. Because each gp41 amphipathic helix has similar features, the four sequences were analyzed as a single group. The HIV amphipathic helixes most closely resemble class K, the calmodulin-binding domains of the calmodulin-regulated protein kinases. Both have high mean hydrophobic moments (0.62 and 0.38, respectively), both are highly charged (mean charged residue densities of 3.7 and 3.2 per 11mer of helix), both are positively charged (mean ± charge ratios of 3.1 and 15.0), and both have wide polar faces that subtend angles of 180° or greater. Following HIV fusion with target cells, the amphipathic helical domains of gp41 presumably are exposed to the cytoplasmic interior of the cell. Because of the similarities of these domains to class K amphipathic helixes, one is tempted to speculate that gp41, as part of its biological role, may interact with or inhibit calmodulin.

There have been two recent suggestions as to the role of the two amphipathic helixes from gp41: 1) They play a role in virus-cell fusion.[127] 2) They form a transmembrane hairpin loop via ion-pairing between the polar faces,[129] thus creating membrane defects that lead to cytotoxicity. Of possible relevance to these hypotheses, the HIV amphipathic helixes have several unusual features that distinguish them from class M transmembrane and the majority of the other classes of amphipathic helixes. The HIV amphipathic helixes have an uniquely high mean hydrophobic moment and a mean hydrophobicity per residue of nonpolar face of 0.80 that is matched only by the class C coiled-coils. Finally, the HIV amphipathic helixes have a lysyl/arginyl ratio (0.04) that is far lower than any of the seven classes analyzed here.

CONCLUSIONS

In this review we have analyzed the database of proteins reported to have amphipathic helical domains. On the basis of this analysis we have classified amphipathic helixes into seven distinct classes. In devising this classification we have considered the charged, but not the uncharged, polar residues of the polar face. Most of the differential between the classes is in their polar faces: charge, charge density, distribution, and angle subtended. We think the classification presented for the first time here will prove useful in analysis of protein sequences. Further, the striking differences between several of the classes of amphipathic helixes may provide clues that will a) aid in a future understanding of the different biological roles assumed by these important structural motifs and b) help to identify new classes as new protein sequences become available.

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