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Ion Pairs in Alpha Helices

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ABSTRACT **A survey of 47 globular proteins was made to determine the probability of occurrence of ion pairs separated by 1, 2, 3, . . . and 8 residues in the alpha helices. As a control, the probability of occurrence of like charged pairs was also determined. The survey showed that ion pairs of the type i, i±3 and i, i±4 are the most predominant. Such a preference was not observed for like charged pairs. The observed frequency of ion pairs is significantly greater than their expected frequency. The normalized frequencies of occurrence of the ion pairs were also found to increase generally with the helix length. These results indicate that the ion pairs may contribute to the stability of solvent-exposed alpha helices. Since the stabilization of protein secondary structure enhances the stability of protein tertiary structure, these results may throw light on the mechanism of protein folding.**

Key words: intrahelical ion pairs, frequency of ion pairs in exposed helices, alpha-helix stabilization, protein tertiary structure stabilization, protein-folding pathway

INTRODUCTION

It is well known that alpha helices are generally highly unstable in aqueous environment.[1–3] Therefore, it was surprising that in the recently determined structure of troponin C,[4,5] which has an unusual dumbbell shape, the central alpha-helix "handle," exposed to solvent, was stable. The stability of this helix was attributed to the presence of several oppositely charged residues juxtaposed 3 or 4 residues apart (referred to here as ion pairs, which may or may not be hydrogen bonded or salt bridged) along the alpha-helix handle which have the potential for direct hydrogen bonding or attractive electrostatic interactions or water bridges.[6] The "fibroglobular" structure of troponic C was of further interest because the 9-turn long central alpha helix could be regarded as being composed of three sections, each of 3 turns, which show different amounts of exposure to the solvent. It was found that the amino third is completely buried and surrounded by the remaining 4 helices of the amino domain and is not involved in any intrahelical ion pairs but in two interhelical salt bridges. The carboxyl third is only partially buried and the solvent side of the helix contains one intrahelical salt-bridged ion pair. In sharp contrast, the central third of the long helix, which is virtually completely exposed to water, contains several sequential and overlapping intrahelical ion pairs. The above observations prompted us to study the distribution of ion pairs in the alpha helices of globular proteins and their possible implications in the formation and stabilization of alpha helices.

There have been earlier attempts to understand the role of ion pairs and salt bridges in proteins.[3,7–11] Maxfield and Scheraga[7] found that Glu^- had a high probability of being located in an alpha helix 4 residues away from Lys^+. Baldwin[2] initially hypothesized that the solution stability[1] of the C-peptide helix of RNase A was due to the ion pair Glu 9^--His 12^+. Subsequent experiments[3] showed that the helix was stable even after eliminating the ion pair by replacing Glu 9^- by Leu. This led him to conclude that this ion pair does not contribute to the helix stability. It is interesting that our analysis also shows that the ion pairs involving His^+ are not significant to the helix stability. Perutz and Raidt[8] and Perutz[9] have discussed the role of salt bridges in the stabilization of protein tertiary structures. Barlow and Thornton[10] analyzed the ion pairs/salt bridges in 38 proteins on the basis of distances and concluded that they stabilize the protein tertiary structure but not the secondary structure. In contrast, our analysis is based on the sequence correlation of ion pairs on alpha helices, which indicates that the ion pairs may have an effect on the stability of alpha helices. Rashin and Honig[11] characterized the environment of ion pairs in 36 proteins in terms of their hydrogen bonding and solvent accessibility. They found that the ion pairs were almost always exposed to solvent. McLachlan et al. analyzed the periodic occurrence of hydrophobic, positively and negatively charged residues in tropomyosin[12] and myosin rod[13] coiled-coils and suggested that interhelical salt bridges involving the charged residues contributed to the stability of their tertiary structures. Sundaralingam et al.[6] noted that, besides the interhelical salt bridges, a large number of intrahelical ion pairs of type i, i±3/4 were characteristic of these fibrous proteins which could stabilize their alpha-helical secondary structures.

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TABLE I. Proteins Considered in the Analysis

Actinidin
(sulfhydryl proteinase)
Adenylate kinase
(porcine muscle)
L-arabinose-binding protein
(*E. coli*)
Asparatate trans-carbamylase
(*E. coli*)
Avian pancreatic polypeptide
(turkey pancreas)
Azurin
(*Alcaligenes dentrificans*)
Calcium-binding parvalbumin B
(carp)
Calcium-binding protein
(vitamin D–dependent minor
A form)
Carbonic anhydrase B
(human)
Carbonic anhydrase C
(human)
Carboxypeptidase A
(bovine)
Catalase
(beef liver)
Citrate synthase
(pig heart)
Alpha-chymotrypsin
(*Bos taurus*)
Crambin
(*Crambe abyssinica*)
Cyan methemoglobulin
(Lamprey)
Cytochrome b5, ferri
(bovine; oxidized)
Cytochrome C
(albacore tuna heart)
Cyatochrome C2, ferri
(*Rhodospirillum*)
Cytosolic aspartate
aminotransferase
complex with 2-oxo-
glutaric acid*
(chicken heart)
Dihydrofolate reductase
(*Lactobacillus cassi*)
Endothiapepsin
(acid proteinase)
Flavodoxin
(*Clostridium* MP; oxidized)
Glucagon
(porcine; pH 6.7 form)
Glutathione reductase
(human erythrocyte)
D-glyceraldehyde-3-phosphate
dehydrogenase
(lobster)
GPD
(human muscle)
Hemerythrin
(sipunculid worm)
Hemoglobin
(Erythrocruorin Cyan Met)
Inorganic pyrophosphatase
(baker's yeast)
Insulinlike growth factor
(*Homo sapiens*)
Leghemoglobin
(yellow lipin; deoxy)
Lysozyme
(human)
Myoglobin
(sperm whale)
Myohemerythrin
(marine worm retractor muscle)
Papain (sulfhydryl proteinase)
(*Papaya carcica*)
Pencillopepsin
(acid proteinase)
Phosphoglycerate mutase
(dried baker's yeast)
Phospholipase A-2
(bovine pancreas)
Prealbumin
(human plasma)
Ribonuclease A
(bovine pancreas)
Rhodanese
(bovine liver)
Taka-amylase A
(*Aspergillus cryzae*)
Thermolysin
(*Bacillus thermoprotes lyticus*)
Thioredoxin reductase*
(*E. coli* B; oxidized form)
Triose phosphate isomerase
(chicken)
Trypsin inhibitor
(bovine pancrease)

*These proteins were not included in distance calculations because alpha-carbon atoms only were available.

MATERIALS AND METHODS

In this paper the structures of 47 proteins have been examined. The atomic coordinates of these proteins were obtained from the Brookhaven data bank. The sample contains proteins (Table I) of diverse tertiary structure and function. The alpha-helical segments in these proteins vary in number, length, and exposure to solvent. In these alpha helices, besides the occurrence of ion pairs, there are a number of cases with contiguous ion pairs where a central charged residue is flanked by oppositely charged residues i+3/4 and i−3/4 residues apart. There are also cases with overlapping ion pairs. Some examples of these in globular proteins are shown in Table II.

TABLE II. Ion Pairs and Triplets in Alpha-Helices of Some Proteins

Glucagon
9D→12K→15D→18R→21D
17R→21D
Adenylate kinase (porcine muscle)
100K→103E→107R
100K→104E→107R
144E→147K→151E
144E→148K→151E→155K→158E
Phosphoglycerate kinase (horse muscle)
259E→263K→267D→271K
318E→321K→325D
318E→322K→325D
Myoglobin (sperm whale)
27D→31R
34K→38E→42K
52E→56K→59E→62K
56K→60D→63K
133K→136E→139R
136E→140K
141D→145K
Troponin-C (contiguous or relay of salt bridges)
84R→88E→91K→95E
89D→93K→96E
93K→97E
100D→103R→106D
135E→139K
136D→139K→142D
153E→156K→159E
156K→160D
Calmodulin (overlapping salt bridge)
74R→78D
75K→78D
77K→80D
82E→86R
83E→86R
87E→90R→93D

To determine the preference for ion pairs at i, i±3/4, a computer search was performed on the alpha helices of 47 proteins for both oppositely as well as like charged residues separated by 1, 2, 3, . . . 8 amino acid residues and the results are given in Table III. The charged residues constitute Glu^-, Asp^-, Lys^+, Arg^+, and His^+. The distribution of like charged pairs provided a control for the analysis. Since hydrophobic triplets at i, i±3 and i±4 contribute to the stability of buried alpha helices,[14] ion triplets were also searched for. Possible correlations between the ion-pair type (i, i±n where n=1, 2, 3, . . . 8) and the helix length and the order of the charged residues in the ion pair (+ − / − +) were also studied. The distances between the charged groups both in ion pairs and like charged pairs were computed to obtain the frequency of hydrogen bonding and to estimate the cutoff distance for the effective electrostatic interactions. In the calculation of the distances between the charged groups the following charge-carrying atoms—$O^{\epsilon 1}$ and $O^{\epsilon 2}$ in Glu^-, $O^{\delta 1}$ and $O^{\delta 2}$ in Asp^-, N^{ζ} in Lys^+ and N^{ϵ} and $N^{\eta 1}$ and $N^{\eta 2}$ in Arg^+—were considered.

To evaluate the statistical significance of the observed distribution of ion-ion pairs (ion pairs and like charged pairs), their expected numbers were computed by using the following formula, which is similar to the one used by Ptitsyn et al.[15] to compute the expected numbers of amino-acid residue pairs in proteins. The expected number ($\eta_{i,xy}$) of an x-y residue pair separated by i number of residues in an alpha helix is given by

$$\eta_{i,xy} = N_i \cdot P_{x,i} \cdot P_{y,i} \quad (1)$$

where $P_{x,i}$ and $P_{y,i}$ are the correlated probabilities for the amino acids x and y to occur on alpha helices and are given by the expression $P_{x,i} = n_{x,i}/n_{t,i}$ where $n_{x,i}$ is the number of x-z (where z is any amino acid) pairs on the alpha helices with i number of intervening residues and $n_{t,i}=\sum_A n_{A,i}$, where A represents the 20 amino acids. Each term in the summation is defined by $n_{x,i}$. The value of N_i in equation 1 is given by $N_i = N_a - (i + 1)N_h$ where N_a is the total number of amino acids in the sampled helices and $(i + 1)N_h$ is the helix end-effect correction term. N_h is the total number of helical segments in the sample. The calculated probabilities and expected numbers are given in Tables IV and V, respectively.

RESULTS AND DISCUSSION

The following is a summary of the survey in the globular proteins: The total number of alpha-helical segments present in the proteins is 299. The lengths of the helical segments vary from 2 to 9 turns and the mean length is about 3 turns.[16]

The frequency of occurrence of the eight types of ion pairs separated by 1, 2, 3, . . . 8 amino acid residues is shown in Figure 1. It is seen that the i±3 and i±4 types of ion pairs are the most predominant followed by the i±1 type and then the i±2. There appears to be a slight preference for the i±4 type of pair over the i±3 (Fig. 1). If, however, aspartate aminotransferase containing 7 i±3 type pairs and 14 i±4 type pairs, and flavodoxin containing 3 i±3 type pairs and 6 i±4 type pairs are removed from the survey, the order is reversed. The ion-pair distribution is rationalized on the basis of the alpha-helix geometry, where, in 1 turn of a helix, the i±4 type of ion pair is favorably juxtaposed on the same side of the helix followed by i±3 and i±1. Ion pairs of the type i±2 show the least-favored distribution; this is to be expected because the residues are on opposite sides of the helix. It was found that there were very few ion triplets.

The distribution of i±3/4 type ion pairs and like pairs as a function of the helix length is shown in Table VI. It is seen that the total number of helices (135) with ion pairs (248) is much more than the total

TABLE III. Sequence Preference of Ion Pairs in Helices*

	n							
i i+n	1	2	3	4	5	6	7	8
Glu Lys	25	17	22	31	9	16	19	13
Glu Arg	11	10	14	14	6	8	4	7
Lys Glu	22	10	18	23	9	5	11	10
Arg Glu	4	8	7	11	5	3	4	2
Asp Lys	8	6	33	17	11	15	11	6
Asp Arg	6	6	12	10	7	5	4	7
Lys Asp	11	8	9	20	8	6	3	9
Arg Asp	8	5	5	2	1	4	3	1
His Glu	6	7	3	5	4	5	0	1
His Asp	4	6	3	1	3	4	0	3
Glu His	4	4	9	3	8	2	2	6
Asp His	2	7	4	10	1	3	2	4
Like pairs								
Glu Glu	20	14	15	12	6	8	9	9
Glu Asp	13	6	16	8	9	7	7	4
Asp Asp	11	8	8	6	6	8	8	3
Asp Glu	20	12	7	9	8	2	7	8
Arg Arg	9	7	1	6	7	6	4	1
Arg Lys	8	5	5	4	4	6	5	8
Lys Lys	26	13	16	20	11	11	15	11
Lys Arg	11	10	8	8	7	5	7	2
His His	0	3	3	3	3	0	0	0
His Lys	11	10	6	3	6	5	1	3
His Arg	7	1	6	2	4	2	2	0
Lys His	7	6	9	2	3	5	2	4
Arg His	2	2	3	1	3	4	2	5

*Since His^+ shows a stronger preference for being buried in the protein interior rather than being involved in an ion pair, it was not used in the computation of normalized frequencies (Figs. 2, 3). It may be pointed out that the inclusion of His^+ would increase the density of ion pairs.

TABLE IV. Correlated Probabilities of the Amino Acids in Alpha Helices*

Amino acid	i ± 1	i ± 2	i ± 3	i ± 4	i ± 5	i ± 6	i ± 7	i ± 8
Leu	0.100	0.099	0.099	0.094	0.090	0.089	0.089	0.092
Ile	0.050	0.052	0.054	0.052	0.050	0.052	0.052	0.050
Val	0.064	0.064	0.064	0.065	0.063	0.063	0.065	0.065
Ala	0.114	0.116	0.112	0.109	0.110	0.105	0.103	0.100
Phe	0.046	0.046	0.046	0.045	0.043	0.039	0.035	0.027
Tyr	0.026	0.026	0.027	0.026	0.026	0.023	0.022	0.023
Thr	0.055	0.055	0.055	0.057	0.059	0.061	0.058	0.059
Met	0.021	0.022	0.022	0.021	0.020	0.019	0.017	0.017
Ser	0.058	0.054	0.054	0.055	0.058	0.061	0.061	0.061
His	0.027	0.026	0.025	0.025	0.025	0.024	0.025	0.025
Trp	0.021	0.021	0.022	0.023	0.022	0.021	0.022	0.022
Asp	0.060	0.063	0.064	0.066	0.071	0.071	0.071	0.074
Glu	0.073	0.074	0.077	0.077	0.078	0.077	0.080	0.082
Asn	0.042	0.042	0.042	0.045	0.047	0.048	0.051	0.052
Gln	0.044	0.043	0.044	0.044	0.045	0.044	0.040	0.043
Arg	0.039	0.039	0.039	0.039	0.038	0.038	0.041	0.042
Lys	0.080	0.076	0.069	0.067	0.064	0.068	0.063	0.060
Gly	0.040	0.041	0.044	0.045	0.047	0.051	0.053	0.053
Cys	0.016	0.016	0.016	0.017	0.016	0.016	0.018	0.019
Pro	0.022	0.024	0.026	0.029	0.028	0.030	0.033	0.034

*No. of helices = 299; No. of residues = 3,666.

TABLE V. A Comparison of Observed and Expected Frequencies of Ion-Ion Pairs*

	1 ± 1	i ± 2	i ± 3	i ± 4	i ± 5	i ± 6	i ± 7	i ± 8
Ion pairs								
Glu-Lys/Lys-Glu	47 (36)	27 (32)	40 (26)	54 (22)	18 (18)	21 (16)	30 (12)	23 (10)
Glu-Arg/Arg-Glu	15 (18)	18 (16)	21 (14)	25 (13)	11 (11)	11 (10)	8 (8)	9 (6)
Asp-Lys/Lys-Asp	19 (30)	14 (26)	42 (22)	37 (20)	19 (17)	21 (16)	14 (12)	15 (8)
Asp-Arg/Arg-Asp	14 (16)	11 (14)	17 (12)	12 (11)	8 (10)	9 (8)	7 (8)	8 (6)
Total	95 (100)	70 (88)	*120 (74)*	*128 (66)*	56 (54)	62 (50)	59 (40)	55 (30)
Like pairs								
Glu-Glu	20 (16)	14 (15)	15 (15)	12 (13)	6 (11)	8 (9)	9 (8)	9 (7)
Glu-Asp/Asp-Glu	33 (28)	18 (23)	23 (24)	17 (22)	17 (20)	9 (18)	14 (14)	12 (12)
Asp-Asp	11 (11)	8 (11)	8 (10)	6 (9)	6 (9)	8 (8)	8 (7)	3 (5)
Lys-Lys	26 (20)	13 (16)	16 (12)	20 (10)	11 (8)	11 (7)	15 (5)	11 (4)
Lys-Arg/Arg-Lys	19 (20)	15 (16)	13 (14)	12 (20)	11 (10)	11 (8)	12 (6)	10 (4)
Arg-Arg	9 (5)	7 (4)	1 (4)	6 (3)	7 (3)	6 (2)	4 (2)	1 (2)
Total	118 (100)	75 (85)	76 (79)	73 (77)	58 (61)	53 (52)	62 (42)	46 (34)

*Expected numbers are given in parentheses. The observed (expected) numbers of i±3/4 type of Glu-His/His-Glu ion pairs are 12 (10) and 8 (8), respectively. These numbers for Asp-His/His-Asp are 7 (8) and 11 (8), respectively. Note that the total observed (expected) number of i ± 3/4 type of ion pairs (in italics) show the greatest difference.

TABLE VI. The Distribution of Ion Pairs and Like Pairs as a Function of Helix Length

Helix length in turns	Total No. of helices	N* vs. (ion pairs)		N vs. (like pairs)		Helices with both ion pairs (1) and like pairs (2)		
		N (i ± 3)	N (i ± 4)	N (i ± 3)	N (i ± 4)	N	(1)	(2)
<2	62	7 (8)	4 (4)	2 (2)	1 (1)	2	2	2
2–3	62	18 (19)	17 (23)	11 (17)	11 (15)	11	22	15
3–4	78	17 (20)	16 (22)	13 (18)	12 (16)	14	21	32
4–5	50	25 (38)	26 (41)	12 (16)	17 (19)	16	34	23
5–6	32	14 (22)	15 (25)	7 (13)	9 (12)	10	29	24
6–7	8	5 (7)	2 (2)	2 (2)	3 (5)	4	7	7
7–8	5	1 (4)	4 (7)	3 (5)	0 (0)	3	6	5
8 and above	2	2 (2)	2 (4)	2 (3)	2 (5)	2	6	8
Total		(120)	(128)	(76)	(73)	62	127	116
		(248)		(149)				

*N refers to number of helices.

number of helices (90) with like pairs (149). There are 62 helices that contain both ion pairs and like pairs. It is interesting that the shorter helices, 2–4 turns, contain as many like charged pairs as ion pairs, while the longer helices, 4–6 turns, contain more ion pairs than like pairs. About 85% of the like pairs occur together with the ion pairs, while about 50% of the ion pairs occur without like pairs. Thus, the like pairs show a low probability of occurence on alpha helices without ion pairs.

The normalized frequencies for ion pairs on alpha helices are displayed in Figure 2. It is seen that the helices with about 2 turns contain 0.12 ion pairs of types i±3/4 per turn, while the helices with 4, 5 and 6 turns contain 0.15, 0.30, and 0.39 ion pairs per turn, respectively. The helices with 3 turns contain a relatively larger number (0.27 pairs/turn) of i±3/4 type pairs. Thus, in general, longer alpha helices tend to contain more than their proportionate number of i±3/4 type ion pairs (Fig. 2).

The distribution of like charged pairs showed no preference for the i, i±3/4 type (Fig. 1). It is seen from the difference profile of ion pairs and like pairs in alpha helices (Fig. 1) that the 1±3/4 type of like charged pairs is only 63% and 57% of the corresponding ion pairs. In contrast, the frequency of occurrence

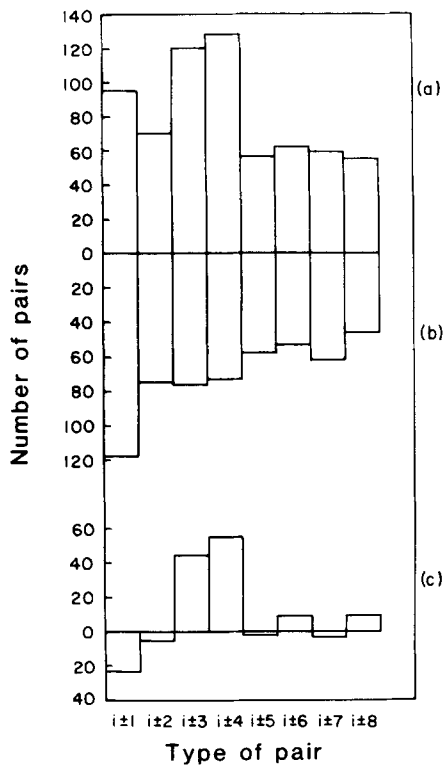

Fig. 1. Frequency of occurrence of charged pairs in alpha helices. **a:** The frequency of occurrence of ion pairs with 1, 2, 3, . . . 8 intervening residues. **b:** The frequency of occurrence of like charged pairs. **c:** The difference profile for ion pairs and like pairs.

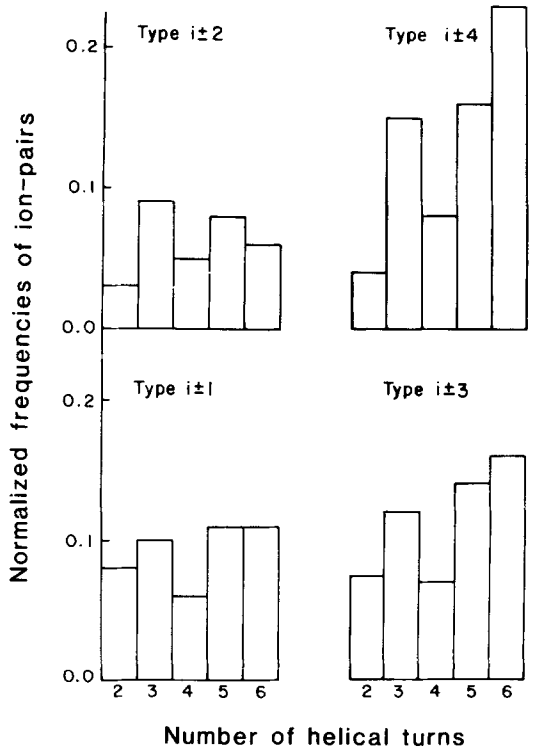

Fig. 2. The normalized frequencies of the ion pairs in alpha helices. The normalized frequencies represent the density of ion pairs per turn. To determine the normalized frequencies all the alpha helices in the sample are used.

of the i±1 type of like charged pairs is higher than the corresponding ion pairs by 19.5%. It is interesting that a total of 37% of these like charged pairs are situated at the appropriate ends of the helix. Thus, just as Chou and Fassman[17] showed that there is a preference for one charged residue at each of the helix termini, we find that there is also a preference for adjacent like charged residues to be located at helix termini. This is probably due to the favorable interactions of the charged residues at ends of the helix with the helix dipole, as suggested by Blagdon and Goodman.[18] The normalized frequencies of i±1, 2, 3, and 4 type like charged pairs were also determined (Fig. 3) and they showed no dependence on helix length except for the i±1 type pairs.

The Observed and Expected Frequencies of Ion Pairs

The expected frequencies of the various types of ion-ion pairs (ion pairs and like charged pairs) computed by the method described above are compared with the corresponding observed frequencies in Table V. It is striking that the observed frequencies of the i±3/4 type of ion pairs involving Glu^-, Asp^-, Lys^+, and Arg^+ are significantly higher than the expected frequencies. On the other hand, the observed and expected frequencies of the like pairs are comparable.

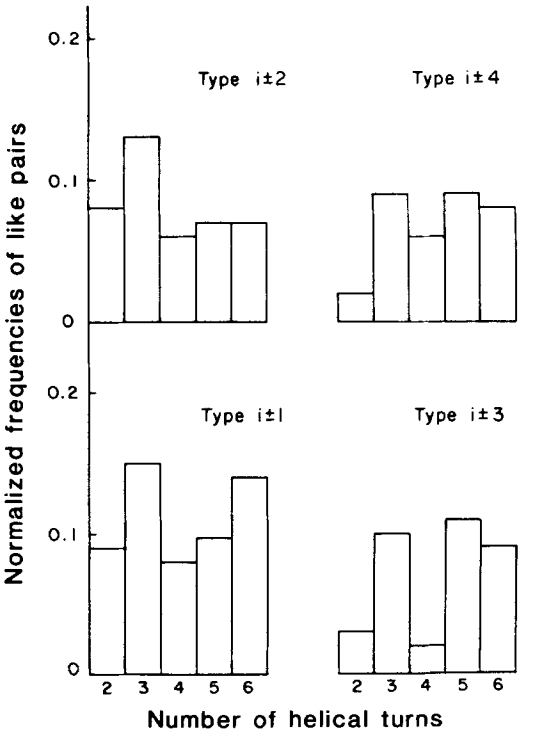

Fig. 3. The normalized frequencies of like charged pairs in alpha helices.

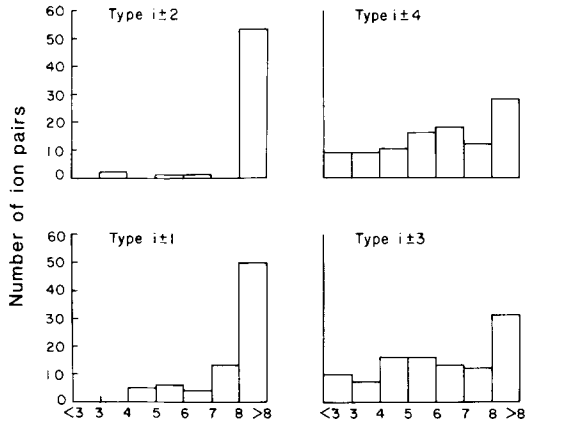

Fig. 4. The distance distribution of ion pairs in alpha helices.

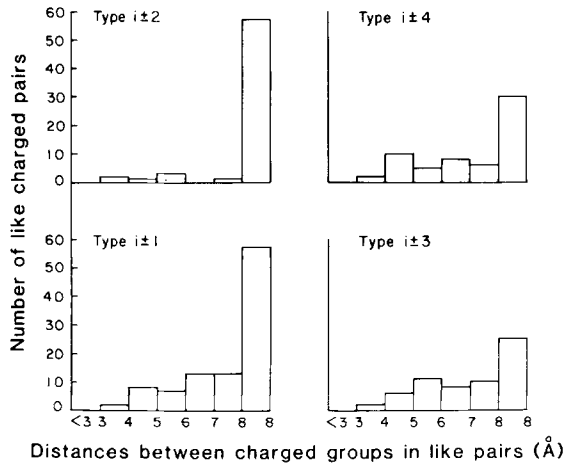

Fig. 5. The distance distribution of like pairs in alpha helices.

This indicates that the preponderance of the i±3/4 ion pairs is probably not due to the intrinsic frequencies of the charged residues in the alpha helices. It may be noted that the calculation also shows that the i±4 type of ion pairs has a slightly greater preference to occur than the i±3 type of ion pairs.

In the case of His^+, the observed and expected numbers of ion pairs are comparable, suggesting that the occurrence of ion pairs involving $His^{\pm}$ are not significant. This observation is of interest in the light of the result of Baldwin[3]—that the Glu 9^-–His 12^+ ion pair does not contribute to the stability of the C-peptide helix of RNase A.

Ion-Pair Distances and Energetics

The nonbonded distances between the atoms of the charged groups in the ion pairs are summarized in Figures 4 and 5. It is found that about 17% of the distances in 1±3/4 type of ion pairs are less than 4 Å (Fig. 4), which may be involved in direct hydrogen bonds or strong electrostatic interactions. About 43% of the ion pairs are at distances ranging from 4 to 7 Å. These ion pairs may be bridged through water molecules. The remaining 40% of the ion pairs are separated by more than 7 Å. These may be hydrogen bonded to solvent molecules which may form plumes around the charged groups. The ion-pair hydrogen bonding, electrostatic interactions, and water bridges can screen the helix backbone hydrogen bonds from solvent "erosion." It may be noted that the i±/1, 2, 5, 6, 7, 8, types of pairs do not favor hydrogen bonding since a large percentage of these are separated by more than 7 Å. In contrast to the ion pairs, the like charged pairs are rarely found at distances less than 4 Å (Fig. 5). About 45% of the distances of like charged pairs lie between 4 and 7 Å. This suggests an approximate cutoff value of 4–5 Å (Figs. 4, 5) for the effective electrostatic interactions, which is similar to the value of 4 Å deduced by Barlow and Thornton.[10] The ion-pair distances indicate that only a few of them are salt bridged or hydrogen bonded. Since there is uncertainty in the atomic positions of the solvent-exposed charged residues, it is not possible to draw any firm conclusion regarding the forces in ion pairs at this stage.

It is known that the free energy of formation of a salt bridge in protein varies from −3 kcal/mol to −1 kcal/mol depending on its exposure to solvent.[8,19] It is also known that the charged residues exposed to solvent contribute to the stability of proteins by entropy gain in the form of hydration potentials.[20] The hydration potentials of charged residues are comparable to the energies obtained by burying hydrophobic residues. When ion pairs and like pairs are exposed to the solvent, the hydration potentials are expected to be higher. Thus the charged pairs, particularly ion pairs, may contribute to the stability of solvent-exposed helices.

CONCLUSIONS

This analysis has shown that the observed frequency of i, i±3/4 ion pairs involving the charged residues Glu^-, Asp^-, Lys^+, and Arg^+ on the solvent side of the alpha helices is greater than their expected frequencies, unlike the like charged pairs. It has also shown that the longer alpha helices tend to contain more than their proportionate number of ion pairs. These results suggest that the ion pairs may have a stabilizing effect on solvent-exposed alpha helices. This is reminiscent of the stabilization of the buried alpha helices by hydrophobic triplets.[14] Thus alpha helices may be stabilized by hydrophobic triplets, by favorable interaction of charged residues with the helix dipole, as well as by ion pairs.

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