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THE HELICAL HYDROPHOBIC MOMENT AVOIDS PROLINES IN PHOSPHOLIPID-BINDING PROTEINS

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SUMMARY Proline residues appear to punctuate the distribution of amphiphilic phospholipid-binding regions of apolipoproteins. We have applied a quantitative test to this hypothesis and shown that the magnitude of the helical hydrophobic moment around proline residues is reduced in lipid-binding proteins and peptides.

INTRODUCTION Segrest et al.[1] first suggested that plasma apolipoproteins form amphiphilic helices in which the axis of the helix is parallel to the lipid-water interface. The helical hydrophobic moment described by Eisenberg et al.[2] represents one quantitative expression of the amphiphilicity of a peptide. One of the hallmarks of the Chou-Fasman predictive method[3] is the low probability of a proline appearing in a helix. By contrast, the calculation of the helical hydrophobic moment contains no such restriction. In this report we tested the hypothesis that the proline-containing regions of lipid-associating proteins have a low hydrophobic moment.

METHODS We have used the hydrophobicity scale of Levitt[4] which is based, in part, upon the partitioning of amino acids between water and an organic phase[5] because the polarity of the interfacial region of phospholipids is similar to that of ethanol. Fluorescence probes that localize at the lipid-water interface have emission spectra that are characteristic of their fluorescence in ethanol[6]. Moreover, the intrinsic tryptophan fluorescence of native lipoproteins is characteristic of tryptophan in an environment that probably contains some water[7] Selection of other scales did not affect our conclusions.

The helical hydrophobic moment of the ith residue of a protein composed of n residues is given by

$$\langle \mu H \rangle_i = \sum_{i-5}^{i+5} \overrightarrow{\delta G_i}$$

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The average helical hydrophobic moment for all residues in the protein, $\langle \mu_H \rangle_{\bar{T}}$, and for all prolines, $\langle \mu_H \rangle_{\bar{P}}$, was also calculated. We correlated the helical hydrophobic moment with the location of prolines by determining the fraction, F_{50}, of the total number of prolines in a given protein that have a hydrophobic moment greater than the average for the whole protein and by calculating the ratio, R_p, of the average helical hydrophobic moment in the segments around prolines to the average of the (n-10) segments for the entire protein according to

$$R_p = \frac{\langle \mu_H \rangle_{\bar{P}}}{\langle \mu_H \rangle_{\bar{T}}}$$

We included all known sequences of the apolipoproteins and several phospholipid-binding proteins for which there is evidence that the protein or peptide binds to phospholipid surfaces. The remainder of our data base is not exhaustive; however, we included representative proteins from the major protein groups.

RESULTS

Our survey (Table I) of proteins, exclusive of phospholipid-binding proteins, revealed that out of a total of 24 proteins containing 347 prolines between 5 and n-5, the average helical hydrophobic moment on a mean residue basis was 0.50; the value for the 11-residue segments centered around proline residues was 0.45. This gave a value of R_p = 0.90, which was less than the average for all sequences. The fraction, F_{50}, for this group of proteins indicated that 62% of the prolines resided in regions where the helical hydrophobic moment was less than the average for all nonsurface associating proteins studied.

Figure 1 contains plots of $\langle \mu_H \rangle$ vs sequence for the 6 plasma apolipoproteins whose primary structures are known from protein sequencing (Table II). The magnitude of $\langle \mu_H \rangle$ is unevenly distributed along the sequence with values extending from nil to nearly 1.5 kcal at Gln-105 of apoA-I. The mean residue value of $\langle \mu_H \rangle$ for all 6 apolipoproteins is similar to that of a number of other lipid-associating peptides; however, the magnitude of the helical hydrophobic moment for all of the lipid-associating proteins is 40% greater than that of other proteins. In contrast, the mean residue helical hydrophobic moment of the regions of peptides surrounding prolines is similar for both surface and nonsurface associating proteins. Thus, the higher value of R_p for lipid-associating peptides compared to other proteins is due to the higher hydrophobic moment in the regions devoid of protein.

TABLE I

Summary of Helical Hydrophobic Moments of Proline-Containing Undecapeptides in Representative Globular and Integral/Peripheral Membrane Proteins

Protein (residues)	F_{50}	$\langle\mu_H\rangle_T$	$\langle\mu_H\rangle_p$	R_p	P_T	Reference
bovine serum albumin (582)	22/28	0.64	0.46	0.72	0	15
human hemoglobin α (141)	4/7	0.53	0.37	0.70	1	15
sperm whale myoglobin (147)	2/4	0.62	0.76	1.22	0	15
human myelin basic protein (170)	7/11	0.49	0.42	0.85	1	15
phospholipase A_2, N. melanoleuca (130)	2/4	0.51	0.51	1.00	0	14
phospholipase A_2, Sus domesticus (122)	4/5	0.52	0.30	0.57	0	14
small subunit of D-ribulose-1,5-bisphosphate carboxylate/oxygenase, Nicotiana tabacum (123)	3/7	0.51	0.54	1.05	2	16
porcine heart citrate synthase (437)	10/22	0.52	0.53	1.01	0	15
diphtheria toxin (536)	15/21	0.49	0.44	0.91	0	15
bovine superoxide dismutase (Cu-Zn) (152)	4/6	0.47	0.51	1.09	0	15
bovine ribonuclease (124)	2/4	0.40	0.45	1.16	0	15
human pancreatic trypsin inhibitor (57)	2/2	0.49	0.38	0.76	1	15
human cytochrome C (105)	2/4	0.48	0.61	1.27	0	15
bovine cardiac troponin C (162)	2/2	0.75	0.50	0.67	0	15
human glycophorin A (130)	4/10	0.42	0.45	1.06	1	15
bacteriorhodopsin (247)	6/11	0.33	0.32	0.96	0	15
human liver collagen α (1-229)	33/53	0.31	0.29	0.94	0	15
chicken apovitellenin I (95)	0/2	0.54	0.56	1.04	0	15
human low density lipoprotein receptor (839)	22/38	0.45	0.43	0.97	0	17
yeast alcohol dehydrogenase (347)	9/12	0.70	0.51	0.72	1	15
T. californica, acetylcholine receptor α subunit (437)	14/21	0.48	0.43	0.90	1	15
spiny dogfish trypsin (222)	6/10	0.38	0.31	0.82	0	16
E. coli β-galactosidase (1021)	41/63	0.47	0.43	0.91	0	15
chachalaca lysozyme (129)	2/2	0.47	0.35	0.74	0	15
mean residue value	216/347	0.50	0.45	0.90		

DISCUSSION

Amphiphilic helices play an important role in the structure and stability of proteins and lipoproteins[8-10]. The helical hydrophobic moment[2] provided the first quantitative model that could be subjected to additional testing with apolipoproteins[11,12]. Stoffel[13] has noted that the amphiphilic helices that form the putative lipid-binding regions of apolipoproteins are separated by the insertion of prolines. Although α-helices are an important part of

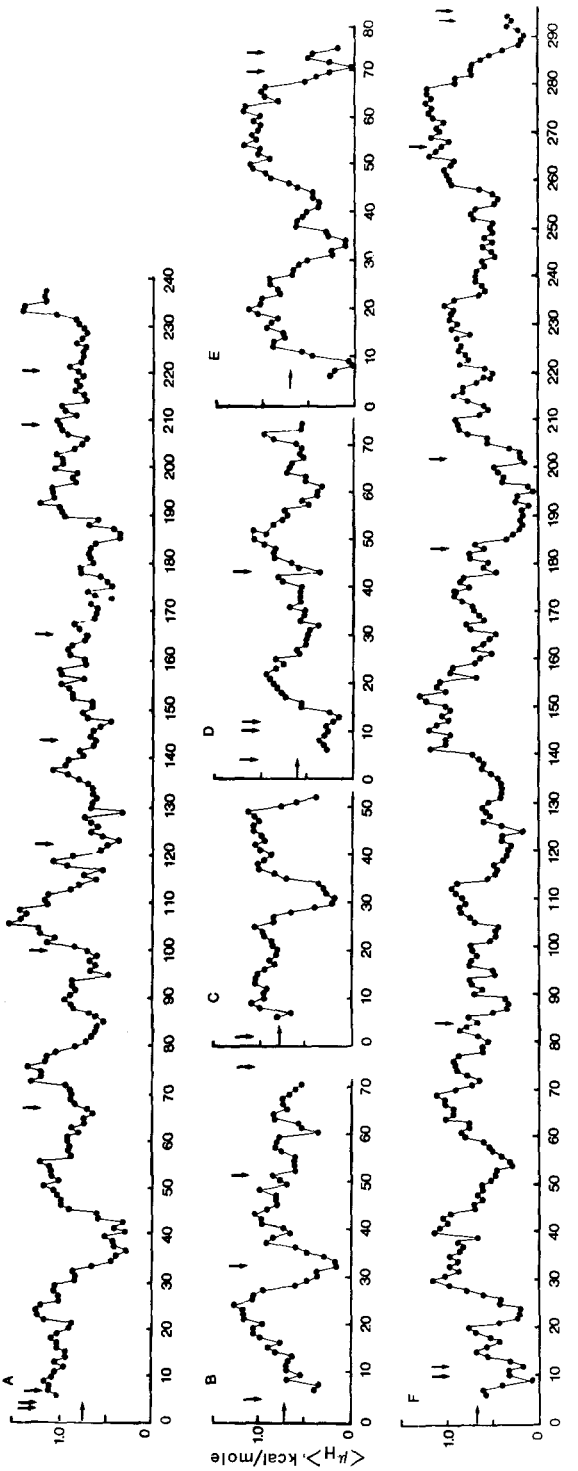

Figure 1: Helical hydrophobic moment plots of apolipoproteins as a function of their primary structure. The vertical arrows locate the proline residues. The horizontal arrow adjacent to the ordinate in each plot locates at the mean helical hydrophobic moment ($<\mu_H>$) for each protein. A) apoA-I; B) apoA-II; C) apoC-I; D) apo C-II, E apo C-III; F) apoE.

TABLE II

Summary of Helical Hydrophobic Moments of Proline Containing Undecapeptides in Plasma Apolipoproteins

Apoprotein (residues)	F_{50}	$\langle\mu_H\rangle_T$	$\langle\mu_H\rangle_p$	R_p	P_T	References
apo A-I (243)	6/8	0.74	0.67	0.90	2	15
apo A-II (77)	1/2	0.72	0.57	0.79	2	15
apo C-I (57)		0.80	--	--	1	15
apo C-II (79)	3/3	0.61	0.28	0.46	1	15
apo C-III (79)	2/2	0.69	0.36	0.52	0	15
apo E (299)	6/7	0.69	0.46	0.67	1	15
apo A-IV (391)	8/13	0.64	0.41	0.64	2	18
mean residue value (apoLP)	26/35	0.70	0.48	0.68	1.3	
bovine glucagon (29)	--	0.62	--	--	--	15
D. hemolysin (26)	--	0.95	--	--	--	15
Cecropin B (37)	1/1	0.69	0.36	0.59	0	15
Melittin (26)	1/1	0.51	0.39	0.77	0	15
Amyloid A Fragment (45)	--	0.74	--	--	-	15
mean residue value	28/37	0.70	0.47	0.67	0.82	

apoprotein structure, there is no clearly defined relationship between the location of prolines and the hydrophobic moment. Our results demonstrate that the regions of high helical hydrophobic moment have a low proline content. This is confirmed by the fraction (F_{50}) of prolines occurring in segments with $\langle\mu_H\rangle_p < \langle\mu_H\rangle_T$ (26/35 = 0.74), which is greater than a random distribution of prolines (F_{50} = 0.50), and by the average magnitude of the helical moment at prolines in all apolipoproteins, which is 68% of the total for that group. Both observations suggest that the helical hydrophobic moment "recognizes" prolines in apolipoproteins. The available data base for other lipid-associating proteins is limited but supportive; in melittin and cecropin B,

the hydrophobic moments around prolines are relatively low. Although the incidence of a high helical hydrophobic moment through regions devoid of prolines is also observed in our representative group of proteins that do not associate with surfaces, the correlation is poorer than that observed for apolipoproteins.

The lipid-associating peptides are more helical when bound to lipid surfaces than when in water[7]. This observation suggests that the helix → random coil → helix transitions are important steps in the transfer of these proteins between lipid surfaces. If one of these steps involves the slow cis-trans isomerization of the prolines which join the helical lipid-associating regions, then the rate of protein transfer may depend upon the location of prolines with respect to the helical regions[13].

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