Helical amphipathic moment: application to plasma lipoproteins

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The association of apolipoproteins with surfaces occurs with the development of an α-helix in which polar and non-polar faces are formed. The helix locates at the lipid–water interface with the polar face directed toward the aqueous phase and the non-polar face penetrating into the lipid phase. The energetics of this arrangement have been quantified by vector addition of the free energies of transfer of amino acids from water to hydrocarbon to give a resultant helical amphipathic moment. It is shown that the mean residue helical amphipathic moments of the apolipoproteins are consistently higher than those of membrane spanning proteins.

Lipoprotein *α-Helix* *Thermodynamics* *Protein structure* *Membrane structure* *Primary structure*

1. INTRODUCTION

A number of laboratories have been involved in the design and synthesis of surface-associating proteins, including real and model plasma apolipoproteins [1–7]. In [8], a method of distinguishing the various helices of surface associating proteins, integral membrane proteins, and globular proteins was described. The method in fig.1, consists of 3 steps:

(1) The free energy of transfer of amino acids from water to hydrocarbon δG_i, is obtained from one [9] of a number of available hydrophobicity scales;
(2) Assuming a helical arrangement of the protein or peptide the δG_i for each of N amino acids is represented as a vector directed from the helical axis through its position on the circumference of the helix;
(3) The calculated vector sum of the δG_i, the helical amphipathic moment $<\mu_A>$, is obtained on a mean residue basis as:

$$<\mu_A> = \leftarrow {}^{N}_{i=1}\vec{\delta G}_i/N$$

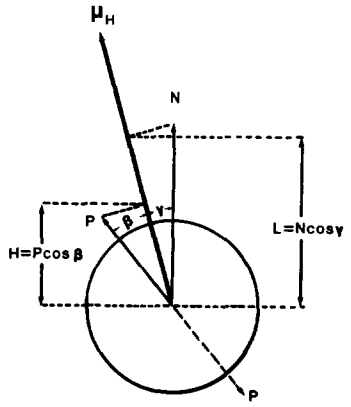

Fig.1. Schematic representation of the resolution of the polar and non-polar components of the individual δG_i into a resultant vector corresponding to the helical amphipathic moment, $<\mu_H>$. The polar vector ($---\rightarrow P$) is always a positive free energy. This is converted to a negative free energy contribution to $<\mu_H>$ which is always negative, by rotation through 180° as shown. H and L are the respective projections of the polar and non-polar vectors onto the helical amphipathic moment. The value H/L is a measure of hydrophile–lipophile balance. N and P are the respective resultants of the non-polar and polar amino acid residues.

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and the mean residue hydrophobicity is given by:

$$\langle H \rangle = \text{Å}\ {}^{N}_{i=1}\ \delta G_i/N$$

The point along the helical axis at which $\langle \mu_A \rangle$ is exerted is obtained from calculations in the same way that a center of gravity is calculated; $\langle \mu_A \rangle$ was also calculated separately for all polar and non-polar residues. The hydrophile–lipophile balance (H/L) is obtained as the ratio of the projections of the polar and non-polar vectors onto the resultant as shown in fig.1. The goodness of alignment of the polar and non-polar vectors is given as the angular defect, α_d, which is the angle between the non-polar and polar vectors ($\alpha_d = \beta + r$) and Δ[Bond], the number of bonds between the polar and non-polar vectors. F represents the fraction of the helix from the amino-terminus at which the $\langle \mu_A \rangle$ is exerted.

Sequences of apolipoproteins were selected on the basis of the following criteria:

(i) Clear evidence that a stable lipid–protein complex was formed by the peptide;
(ii) Exclusion of regions with proline residues; in proline containing peptides, the two sequence regions on each side of the proline were calculated separately;
(iii) That circular dichroism indicates a helical structure in the complexes with phospholipid.

Known integral membrane proteins and helical regions of globular proteins were included for comparison. In each case the direction of $\langle \mu_A \rangle$, which was calculated relative to amino-terminal residue being 0°, provides one with a means of predicting the orientation of single rigid helices with respect to a water–lipid interface since $\langle \mu_A \rangle$ should be orthogonal to the surface.

3. RESULTS AND DISCUSSION

3.1. *Distinction of types of helical proteins*

We evaluated the helical amphipathic moment of: (1) globular proteins; (2) integral membrane proteins; and (3) surface-associating proteins, including real and model plasma apolipoproteins. The latter group contained mellitin and glucagon whose structure and affinity for lecithins in vitro mimics that of the apolipoproteins.

We have correlated the helical amphipathic moment and the mean residue hydrophobicity of the 3 groups of proteins and peptides in fig.2; table 1 gives the identity of these peptides. Integral membrane proteins formed a small cluster of peptides having a high hydrophobicity (−0.5 to −1.1 kcal/residue) and a low helical amphipathic moment (0 to 0.4 kcal/residue). This finding is similar to that in [8]. The surface associating peptides formed a larger cluster that included peptides with a lower mean residue hydrophobicity (−0.2 to 1.0 kcal/residue) and a much higher helical amphipathic moment (0.5–1.25 kcal/residue). There was no overlap of regions for the integral membrane proteins and the surface-associating proteins. Globular proteins appeared within or below the lower half of the region for surface-associating proteins.

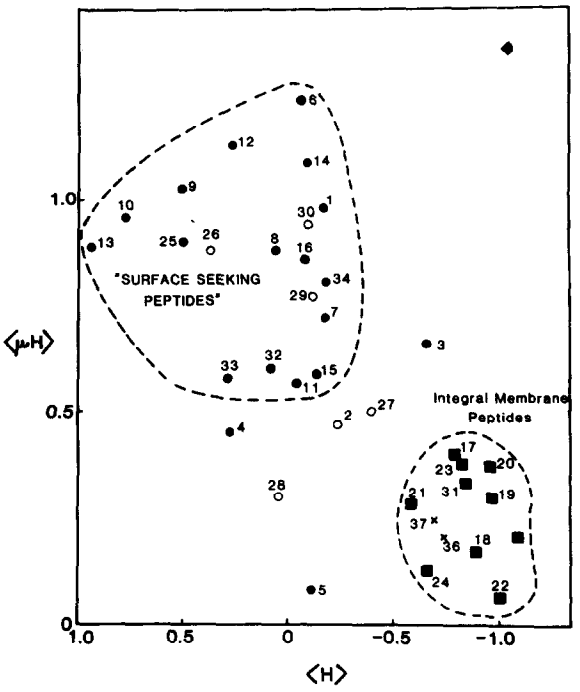

Fig.2. Correlation of the helical amphipathic moment with: the mean residue hydrophobicity of real and model apolipoproteins (●); integral membrane proteins (■); globular proteins (○). Table 2 contains the identity and reference for the sequence of these.

3.2. *Mellitin and glucagon: peptides with two independent helices*

Glucagon, which is known to bind to gel phase lipid, is located far outside of the region of the

Table 1

	Peptide	Sequence Length	First Residue	Protein Type	Ref
1.	LAP-20	20	1	model apolipoprotein	7,11
2.	Mellitin	26	1		
3.	" "	13	1		29,30
4.	" "	12	15		
5.	Porcine glucagon	29	1	peptide hormone	12,13
6.	" " "	10	6		
7.	" " "	13	17		
8.	apoA-II	25	7	apolipoprotein	14,15
9.	" "	15	17	apolipoprotein	14,15
10.	" "	8	39	apolipoprotein	14,15
11.	" "	12	51	apolipoprotein	14,15
12.	Peptide I	18	4	model apolipoprotein	3
13.	" "	23	1	model apolipoprotein	4
14.	apoC-III	21	47	apolipoprotein	16,17
15.	apoC-III	39	41	apolipoprotein	16,17
16.	apoC-III	28	41	apolipoprotein	16,17
17.	glycophorin	26	72	integral membrane	18,19
18.	Bacteriorhodopsin	26	7	integral membrane	20,21
19.	" " "	28	40	integral membrane	
20.	" " "	28	76	integral membrane	
21.	" " "	27	105	integral membrane	
22.	" " "	27	132	integral membrane	
23.	" " "	28	165	integral membrane	
24.	" " "	29	196	integral membrane	
25.	ApoC-I	21	33	apolipoprotein	22
26.	myoglobin H-helix	17		globular	23
27.	myoglobin-G-Helix	24		globular	23
28.	bovine serum albumin	24	314	globular	24
29.	" " "	21	339		
30.	" " "	26	367		
31.	cytochrome b_5	40	91	integral membrane	25
32.	apoC-II	36	43	apolipoprotein	26
33.	apoA-I	19	204	apolipoprotein	27,28
34.	" "	20	224	apolipoprotein	
35.	Bacteriophage fd coat protein			integral membrane	31,32
36.	Pre apoA-I human			Membrane penetrating proteins	33 33
37.	Pre pro apoA-I human				

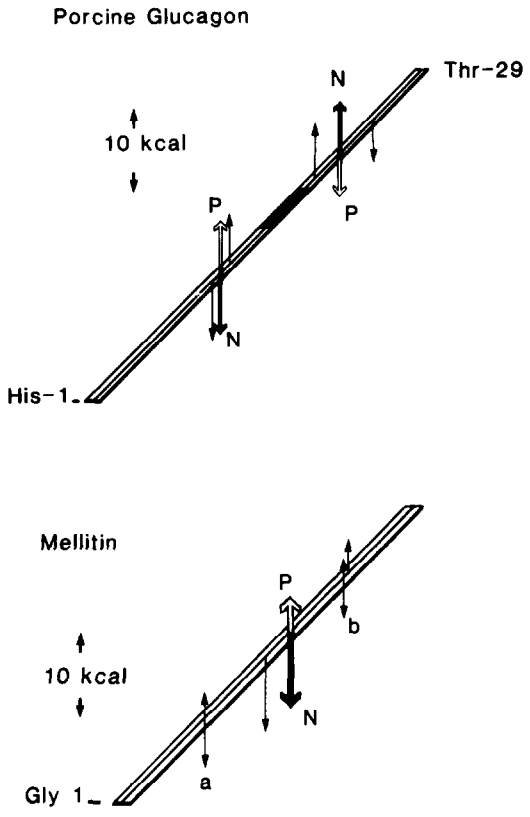

Fig.3. Schematic representation of the location of the helical amphipathic moment along the helical axis of porcine glucagon and bee venom mellitin. In glucagon a pair of helices are separated by 3 consecutive polar amino acid residues which are represented by the solid bar in the middle of the helical axis. The small arrows represent the location of the polar and non-polar components whereas the large arrows locate $\Pi\mu_H>$. The lengths of the unfilled (polar component) and filled arrows (non-polar component) represent their relative contributions to $<\mu_H>$.

other surface-associating proteins (fig.2). One can rationalize this by inspecting molecular models of glucagon in a helical conformation or by locating the regions of glucagon that have the maximum helical amphipathic moment. This exercise led to the identification of two separate helices in glucagon that are separated by 4 consecutive polar residues, Asp–Ser–Arg–Arg, beginning at residue 15. If the entire peptide is helical, the orientation of the helical amphipathic moment of the second helix does not reinforce that of the first helix so that Arg-17 is projecting into the bilayer and the moment of the one helix largely cancels the contribution of the other. The location of these two moments along the helical axis is shown in fig.3. It is probable that the helices do not include all of the charged residues from residues 15–19 and that these residues are arranged in a bend that will permit orientation of the hydrophobic face of both helical segments toward the bilayer. On the basis of this finding, we suggest that 3 or more polar (or non-polar) residues will terminate helix development in a surface associating peptide.

Mellitin, a lytic factor from bee venom (table 2) represents another case in which evidence suggests the presence of two independent helices [8,34]. Proline-14 a proline divides the peptide into two halves, both of which have properties of surface associating helices. The polar and non-polar components of the $<\mu_A>$ of the total peptide are separated by more than 7 bonds (Δ[Bond] = 7.3) further suggesting that 2 domains of helices are present. We calculated each region, residues 1–13 and 15–22, and observed that the first segment contained 1 polar residue and 6 hydrophobic residues that are on the opposite side of the helix so that their moments reinforce each other as well as that of the single lysine at residue 7. The second helical segment is poorly balanced; the 5 terminal polar residues [21–26] have almost no net moment and are probably not surface associating. The region 15–22 has a large amphipathic moment but is poorly balanced; the moment in this segment has a large Δ[Bond], a high H/L-value, and an F-value that locates the moment far from the center of this segment. It is likely that this portion of the structure does not spontaneously form a surface associating helix.

3.3. *Apolipoproteins*

Fig.2 shows that the apolipoproteins have unusually high helical amphipathic moments but hydrophobicities that lie between −0.2 and 1.0 kcal/residue. We will now compare in detail the helical structures of 2 proposed lipid-binding regions of native apolipoproteins and 3 model apolipoproteins whose designs were based on the model in [1]. The region 7–31 of apoA-II was identified as a lipid-binding region [10] and the 47–67 region of apoC-III was identified in [1]. We have evaluated the quality of these lipid-associating peptides by looking for the following properties:

Table 2

Helical hydrophobic moment analysis of surface-associating polypeptides

Peptide	Polar/(bond)	Nonpolar/Bond)	Resultant/(bond)	Angle	Summary
					$<\mu_A>$=0.88
ApoA-II,7-31	10.0(16.8)			14	H/L=0.83
		12.1(11.4)		11	Δ[Bond]=5.4
			22.1(13.8)	12	F=0.55, α_d=3°
					$<\mu_A>$=1.09
ApoC-III,47-67	9.4(12)			290	H/L=0.68
		13.8(11.7)		308	Δ[Bond]=0.3
			23. (11.8)	300	F=0.56, α_d=18°
Kaiser-Kezdy					$<\mu_A>$=0.89
Peptide I	11.8(11.3)			183	H/L=1.3
		8.9(11.3)		176	Δ[Bond]=0
			20.6(11.3)	0	F=0.5, α_d=7°
Kanellis-Segrest					$<\mu_A>$=1.13
Peptide I	11.7(9.8)			195	H/L=1.3
		8.8(8.4)		188	Δ[Bond]=1.4
			20.4(9.2)	192	F=0.54, α_d=7°
Sparrow-Gotto					$<\mu_A>$=0.98
LAP-20	7.7(12.5)			301	H/L=0.43
		13.4(16.2)		345	Δ[Bond]=2.3
			19.6(11.1)	329	F=0.58, α_d=44°
					$<\mu_A>$=0.47
Mellitin	4.0(19.5)			46	H/L=0.43
		8.8(12.2)		78	Δ[Bond]=7.3
			12.4(14.5)	68	F=0.56, α_d=32°
					$<\mu_A>$=0.66
1-13	3.0(7.0)			60	H/L=0.49
		5.9(7.3)		88	Δ[Bond]=0.3
			8.7(7.2)	78	F=0.6, α_d=28°
					$<\mu_A>$=1.07
15-22	4.2(21.3)			70	H/L=0.92
		4.5(18.)		53	Δ[Bond]=3.3
			8.6(19.6)	44	F =0.8, α_d, 17°
Porcine Glucagon					
1-29	0.8(15.1			36	$<\mu_A>$=0.08
		3.3(17.4)		207	H/L=0.24
			2.5(16.9)	204	Δ[Bond]=2.3
					F=0.59, α_d=171°
					$<\mu_A>$=1.24
6-15	5.7(11.8)			195	H/L=0.77
		7.3(10.3)		163	Δ[Bond]=1.5
			12.5(11.)	177	F=0.6, α_d=32°
					$<\mu_A>$=0.72
17-29	6.6(18.9)			22	H/L=1.5
		5.0(24.3)		309	Δ[Bond]=5.4
			9.4(21.3)	351	F=0.39, α_d=73°

(i) That the polar and non-polar components of the $<\mu_A>$ is colinear; i.e., $\alpha_d = 0$ is perfect alignment;
(ii) That the helical amphipathic moment be exerted at the midpoint of the helical axis; $F = 0.5$ is perfect;
(iii) That the polar and non-polar components of the helical amphipathic moment appear at the same point along the helical axis; Δ[Bond] = 0 is perfect;
(iv) The hydrophile–lipophile balance on the basis of H/L: the selected segments of apoA-II and apoC-III conform fairly well to our model of a good surface-associating helix (table 2). For apoA-II [7–31] the polar and non-polar components of $<\mu_A>$ are well-aligned ($\alpha_d = 3°$) and $<\mu_A>$ is close to the middle of the helical axis, $F = 0.55$. However, the polar and non-polar components are fairly far apart (Δ[Bond] = 5.4). For apoC-III (47–67) the polar and non-polar components of $<\mu_A>$ are close (Δ[Bond] = 0.3). The $<\mu_A>$ is at the midpoint of the helical axis ($F = 0.5$), but the polar and non-polar components are slightly misaligned ($\alpha_d = 18°$).

3.4. *Model apolipoproteins*

The 3 synthetic peptides, as one would expect, have good amphipathic characteristics. The Kaiser–Kezdy peptide I is excellent; although its $<\mu_A>$ (0.89) is average for a surface-associating helix, the $<\mu_A>$ is in the middle of the peptide ($F = 0.5$), the polar and non-polar components of $<\mu_A>$ are at the same position on the helix (Δ[Bond] = 0) and nearly perfectly aligned ($\alpha_d = 7°$). The Kanellis-Segrest peptide I is similar except that it has a higher $<\mu_A>$, the polar and non-polar vectors are not at the helical midpoint ($F = 0.54$), and they intersect at different points on the helical axis (Δ[Bond] = 1.4). The Sparrow-Gotto LAP-20 gives a somewhat less satisfactory analysis; the polar and non-polar vectors are far apart (Δ[Bond] = 2.3) and misaligned ($\alpha_d = 44°$) and $<\mu_A>$ is not at the midpoint of the helix ($F = 0.58$).

An interesting and important point is the large range in $<H>$ and H/L among the surface associating peptides (fig.2). Of these, the greatest difference is between Kaiser-Kezdy peptide I ($<H> = 0.93$) and the Sparrow-Gotto LAP-20 ($<H> = -0.18$). Both peptides associate with lecithin with free energies of nearly -10 kcal/mol. The main difference between these two peptides is that the polar face of the Kaiser-Kezdy peptide contains only charged residues ($\delta G_i = 2.5–3.0$) whereas that of the Sparrow-Gotto peptide contains 8 serines; the serines ($\delta G_i = 0.3$) satisfy the requirement for a polar face without contributing much to the $<\mu_A>$. We suggest that this difference is irrelevant with respect to formation of a stable helix at a lipid–water interface. The hydrophobic face and the hydrophobicity thereof is important since the energetics of transfer from water to an interface involves the transfer of these residues from water to an apolar environment. This is not true for the polar residues; after transfer from water to the surface they still remain in contact with the aqueous phase. Therefore, it is probable that the polar face does not contribute much to the free energy of transfer of apolipoproteins from water to a phospholipid surface. It is more likely that their role is to provide a surface anchor that prevents the helix from penetrating very far into the hydrocarbon region of the lipid; without the polar residues, the surface associating peptides would probably be similar to integral membrane proteins.

We conclude that amphipathic moment plots and the amphipathic parameters, α_d, F, Δ[Bond] and H/L are useful for identifying correlations between primary and secondary structure and might be applicable to the design of new surface-associating peptides.

REFERENCES

[1] Segrest, J.P., Jackson, R.L., Morrisett, J.D. and Gotto, A.M. jr (1974) FEBS Lett. 38, 247–253.
[2] Segrest, J.P. (1977) Chem. Phys. Lipids 18, 7–22.
[3] Kanellis, P., Romans, A.Y., Johnson, B.J., Kercret, H., Chiovetti, R., Allen, T.M. and Segrest, J.P. (1980) J. Biol. Chem. 255, 11464–11472.
[4] Fukushim, D., Kupferberg, J.P., Yokoyama, S., Kroon, D.J., Kaiser, E.T. and Kezdy, F.J. (1979) J. Am. Chem. Soc. 3703–3704.
[5] Kaiser, E.T. and Kezdy, F.J. (1983) Proc. Natl. Acad. Sci. USA 80, 1137–1143.
[6] Sparrow, J.T. and Gotto, A.M. jr (1980) Ann. NY Acad. Sci. 348, 187–208.
[7] Pownall, H.J., Hu, A., Albers, J.J., Gotto, A.M. and Sparrow, J.T. (1980) Proc. Natl. Acad. Sci. USA 77, 3154–3158.

[8] Eisenberg, D., Weiss, R. and Terwilliger, T.C. (1982) Nature 299, 371.
[9] Levitt, M. (1976) J. Mol. Biol. 104, 59–107.
[10] Chen, T.C., Sparrow, J.T., Gotto, A.M. jr and Morrisett, J.D. (1979) Biochemistry 18, 1617–1622.
[11] Pownall, H.J. and Massey, J.B. (1982) Biophys. J. 37, 177–178.
[12] Bromer, W.W., Sinn, L.G. and Behrens, O.K. (1957) J. Amer. Chem. Soc. 792, 2807–2810.
[13] Epand, R.M., Jones, A.J.S. and Sayer, B. (1977) Biochemistry 17, 4360–4368.
[14] Lux, S.E., John, K.M., Ronan, R. and Brewer, H.B. (1972) J. Biol. Chem. 247, 7519–7527.
[15] Massey, J.B., Rohde, M.F., VanWinkle, W.B., Gotto, A.M. jr and Pownall, H.J. (1981) Biochemistry 20, 1569–1574.
[16] Brewer, H.B., Shulman, R., Herbert, P., Ronan, R. and Wehrly, K. (1974) J. Biol. Chem. 249, 4975–4984.
[17] Sparrow, J.T., Gotto, A.M. and Morrisett, J.D. (1973) Proc. Natl. Acad. Sci. USA 70, 2124.
[18] Tomita, M. and Marchesi, V.T. (1975) Proc. Natl. Acad. Sci. USA 72, 2964–2968.
[19] McDonald, R.I. and MacDonald, R.C. (1975) J. Biol. Chem. 250, 9206.
[20] Ovchinnikov, Y.A., Abdulaev, N.G., Feigina, M.Y., Kiselev, A.V. and Lobanov, N.A. (1979) FEBS Lett. 100, 219–224.
[21] Marsh, D. and Watts, A. (1982) in: Lipid–Protein Interactions, vol.2 (Jost, P. and Griffith, O.H. eds) pp.53–126, Wiley Interscience, New York.
[22] Chen, T.C., Knapp, R.D., Rohde, M.F., Brainard, J.R., Gotto, A.M., Sparrow, J.T. and Morrisett, J.D. (1980) Biochemistry 19, 5140–5146.
[23] Edmundson, A.B. (1965) Nature 205, 883–887.
[24] Brown, J.R. and Shockley, P. (1982) in: Lipid–Protein Interactions, vol.1 (Jost, P. and Griffith, O.H. eds) pp.25–68, Wiley Interscience, New York.
[25] Ozols, J. and Gerard, C. (1977) Proc. Natl. Acad. Sci. USA 74, 3725–3729.
[26] Jackson, R.L., Baker, H.N., Gilliam, E.B. and Gotto, A.M. (1977) Proc. Natl. Acad. Sci. USA 74, 1942–1945.
[27] Baker, H.N., Delahunty, T., Gotto, A.M. and Jackson, R.L. (1974) Proc. Natl. Acad. Sci. USA 71, 3131–3134.
[28] Pownall, H.J., Massey, J.B., Kusserow, S.K. and Gotto, A.M. (1978) Biochemistry 17, 1183–1188.
[29] Haberman, E. and Jentsch, J. (1967) Hoppe-Seyler's Z. Physiol. Chem. 348, 37–50.
[30] Podo, F., Strom, R., Crifo, C. and Zulauf, M. (1982) Int. J. Peptide Protein Res. 19, 514–527.
[31] Chamberlain, B.K., Nozaki, Y., Tanford, C. and Webster, R.E. (1978) Biochim. Biophys. Acta 510, 18–37.
[32] Nakashima, Y. and Konigsberg, W. (1974) J. Mol. Biol. 88, 598–600.
[33] Law, S.W., Gray, G. and Brewer, H.B. (1983) Biochem. Biophys. Res. Commun. 112, 257–264.