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Molecular Modeling of the Amphipathic Helices of the Plasma Apolipoproteins

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ABSTRACT **In this paper we propose a classification of the amphipathic helical repeats occurring in the plasma apolipoprotein sequences. It is based upon the calculation of the molecular hydrophobicity potential around the helical segments. The repeats were identified using a new autocorrelation matrix, based upon similarities of hydrophobic and hydrophilic properties of the amino acid residues within the apolipoprotein sequences. The helices were constructed by molecular modeling, the molecular hydrophobicity potential was calculated, and isopotential contour lines drawn around the helices yielded a three-dimensional visualization of the hydrophobicity potential. Two classes of apolipoproteins could be differentiated by comparing the hydrophobic angles obtained by projection of the isopotential contour lines on a plane perpendicular to the long axis of the helix. The isopotential contour lines around apo AI, AIV, and E are more hydrophilic than hydrophobic, whereas they are of similar intensity for apo AII, CI, and CIII. In both cases discoidal lipid–protein complexes are generated, with the amphipathic helices around the edge of the lipid core. The long axis of the helices is oriented parallel to the phospholipid acyl chains and the hydrophilic side of the helix toward the aqueous phase. As a result of the differences in hydrophobicity potential, the contact between the hydrophobic side of the helices and the phospholipid acyl chains is larger for apo AII, CI, and CIII than for the other apolipoproteins. This might account for the greater stability of the discoidal complexes generated between phospholipids and these apoproteins.**

Key words: lipid–protein complexes, molecular hydrophobicity potential, protein hydrophobicity, apolipoprotein

INTRODUCTION

Since the first description of the occurrence of amphipathic helices within the sequences of the plasma apolipoproteins,[1] the significance of these particular segments toward the structural organization and the functional properties of the apolipoproteins has been well documented.[2,3] Such 22-residue helical segments contain both paralogous and orthologous homology, as they were described in most human apolipoproteins[4] and in apolipoproteins of other species.[5] The homologies were detected using either comparison matrices, such as those proposed by Dayhoff et al.[6] or correlation procedures as described by Kubota et al.[7]

The involvement of amphipathic helices in the lipid-binding properties of the apolipoproteins is supported by experimental data and by modeling of the apolipoprotein–lipid complexes.[8,9] Some helices belong to enzyme-activating domains, such as that of lecithin-cholesterol acyltransferase (LCAT) in apo AI and apo AIV[10] and to receptor–ligand domains in apo E and apo B.[11] When exposed at the surface of apolipoproteins or of lipoproteins, they belong to antigenic determinants.[12]

Although amphipathic helices were identified in all apolipoproteins, including apo AI, AII, AIV, CI, CII, CIII, and even apo B,[13] attempts to classify the amphipathic helices in order to account for the specific properties of the different apolipoproteins remained unsuccessful. Lipid binding affinity of apo AI, AIV, and E is higher than that of apo AII or apo CIII. The self-association properties,[14] the interfacial behavior,[15] and the LCAT activation[16] are also different for the two classes. As the amphipathic helices are highly homologous, a differentiation on the basis of their primary sequence could not be carried out. Differences in hydrophobicity index were not significant either,[17] so that new parameters are to be introduced to account for differences in functional properties.

Amphipathic helices are defined by a segregation of hydrophobic and hydrophilic residues on opposite sides of the helix. We therefore attempted to calculate the molecular hydrophobicity potential[24–26]

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around the helical segments, in order to achieve a differentiation between the amphipathic helices of various apolipoproteins.

In this paper, we first describe a direct autocorrelation test, based upon the hydrophobic character of the amino acids, to detect a quasi-periodicity in the apolipoprotein sequences, and thereby identify the amphipathic helices. Molecular modeling of the most stable helical repeats and the calculation of the molecular hydrophobicity potential are further carried out. From these results, two classes of amphipathic helices can be defined and further assembled with lipids. The structure of these complexes is finally compared to the available experimental data.

COMPUTATIONAL METHODS

Detection of the Quasi-Periodicity in Apolipoprotein Sequences by Autocorrelation Techniques

The internal and autologous homologies within the human apolipoproteins family had been demonstrated previously using comparison matrices based upon the rate of substitution of amino acids within protein sequences.[6] Correlation methods such as those proposed by Kubota et al.[7] or adapted from that of Levin et al.[18] by Brasseur et al.,[19] relied upon either physicochemical or secondary structure similarities.[19]

An autocorrolation matrix has been introduced[20] to find quasi-periodic patterns in the primary structure of proteins. The algorithm calculates n matrices where n is the number of amino acids in the analyzed sequence. In the rth matrix ($0 \leq r < n$) the i,j element (where the indices i and j stand for one of the 20 amino acids $1 \leq i, j \leq 20$) represents the frequency at which the ith amino acid is followed by the jth amino acid at a distance r.

The autocorrelation function is calculated as follows:

$$A_{ij}^{k} = \sum_{k=1}^{n-r} X_{ikjk+r}$$

where X_{ikjk+r} is the value of the autocorrelation matrix corresponding to the comparison between residue number k in the sequence, being an amino acid of type i, with residue number $k+r$ of type j. At residue numbers higher than r−n the method loses power and the information becomes meaningless.

The calculation of the autocorrelation function can yield two important types of information:

1. The occurrence of a given residue (i.e., Pro) along the sequence.
2. The sum of occurences of all 20 residues at a given position on the sequence.

The simplest autocorrelation matrix X_{ij} is a diagonal matrix where all elements are equal to 0 except for identical residues ($i=j$). We improved the resolution power of the autocorrelations matrix by considering the hydrophobicity of each residue. Other properties such as charge or volume, etc. would also be envisaged.

The autocorrelation matrix X_{ij} developed here is based upon the hydrophobicity properties of the 20 amino acid residues occurring in protein sequences. This matrix consists of elements x_{ij}, with i and j varying between 1 and 20. The elements x_{ij} are equal to 1 when the residues i and j are either both hydrophobic or both hydrophilic, while $x_{ij} = 0$ when they are of opposite hydrophobicity. The diagonal elements of this matrix are therefore equal to 1. For the construction of the matrix, the amino acids were classified as follows: Asp, Glu, Lys, Arg, His, Ser, Thr, Asn, Gln are considered as hydrophilic and Ala, Val, Leu, Ile, Phe, Met, Trp, Tyr as hydrophobic. Pro and Cys are considered as unique, whereas Gly was set equivalent to all other residues except for Cys.

Molecular Modeling of the Amphipathic Helices by Energy Minimization

After identification of the quasi-periodical elements in the apolipoprotein sequences, an helical structure was imposed upon those segments and their three-dimensional (3D) structure was calculated using the values classically attributed to the torsional angles ψ and ϕ for α-helical residues. The lowest conformational energy for these peptides was subsequently obtained by minimization using the simplex method.[22] This was considered as the most stable conformation for the peptide.

Calculation of the Molecular Hydrophobicity Potential

The hydrophobicity of a molecule is usually defined by a single parameter representing its partition coefficient between water and octanol. For the analysis of complex interactions between lipids and proteins, this parameter was extended to the most elaborate concept of "helical hydrophobic moment" by Eisenberg et al.[23]

The calculation of the "molecular hydrophobicity potential" (MHP) along the peptide sequence was based upon the concept introduced by Furet et al.[24] and by Fauchère et al.[25] for a small molecule. We extended this approach to a 3D representation of the molecular envelope with isopotential contour lines around a 18-residue helical peptide.[26] We assumed that the hydrophobic interaction between two residues decreases exponentially with the distance between the residues according to the equation

$$\mathrm{MHP} = \Sigma\, E_{\mathrm{tri}} \exp(r_i - d_i) \qquad (1)$$

where E_{tri} is the transfer energy of the atome i, r_i is the radius of the atom i, and d_i is the distance between atom i and a point M.

The transfer energy E_{tri} represents the energy for individual atoms, calculated from the transfer energies compiled by Tanford.[27] Assuming that the mo-

TABLE I. Individual Atomic Transfer Energies (E_{tr}) for the Various Hybridization States of the Atoms Existing in Peptides, as Calculated by Eq. (2)

Atoms	E_{tr} (kcal/mol)
C_{sp2}	−1.503
C_{sp3}	−2.436
$H_{(q=0)}$	−0.537
$H_{(q\backslash 0)}$	1.030
O	2.833
S	−2.751
N	3.035

lecular transfer energy is the sum of the atomic transfer energies, the E_{tri} values for seven different hybridization states existing in peptide residues were calculated, by using least-squares techniques to solve Eq. (2):

$$E_{tr}^{moll} = a_l E_{tr}C_{sp2} + b_l E_{tr}C_{sp3} + c_l E_{tr}H_{(q=0)} + d_l E_{tr}H_{(q\backslash 0)} + e_l E_{tr}O + f_l E_{tr}S + g_l E_{tr}N$$

$$E_{tr}^{molk} = a_k E_{tr}C_{sp2} + b_k E_{tr}C_{sp3} + c_k E_{tr}H_{(q=0)} + d_k E_{tr}H_{(q\backslash 0)} + e_k E_{tr}O + f_k E_{tr}S + g_k E_{tr}N \quad (2)$$

where E_{tr}^{molk} is the transfer energy measured experimentaly for the molecule k. $a_k, b_k, c_k, d_k, e_k, f_k$, and g_k are the number of atoms in the hybridization states C_{sp2}, C_{sp3}, $H_{(q=0)}$, $H_{(q\backslash 0)}$, O, S, and N, respectively. $H_{(q=0)}$ and $H_{(q\backslash 0)}$ represent noncharged and charged hydrogen atoms. $E_{tr}C_{sp2}, E_{tr}C_{sp3}, E_{tr}H_{(q=0)}, E_{tr}H_{(q\backslash 0)}, E_{tr}O, E_{tr}S, E_{tr}N$ represent the transfer energy of the atoms in the different hybridization states (Table I).

The molecular hydrophobicity potential for an 18-residue helical peptide was calculated by a cross-sectional computation method. A plane perpendicular to the long axis of the helix was moved every 2 Å along the axis, and the MHP was computed for all atoms contained in this plane, according to Eq. (1). A modification of the program developed for visualizing the electronic potential[28] yielded a 3D representation of the MHP along the axis of the helix.

The extent of the hydrophobicity and hydrophilicity isopotential contour lines surrounding the helical segments was obtained by calculating the sum of their projections on a plane perpendicular to the long axis of the helix. The hydrophobicity angle pho, measured from the axis of the helix, corresponds to the domain which is predominantly hydrophobic, while the ϕ angle represents its complement.

Molecular Modeling of the Apolipoprotein–Lipid Complexes

Molecular modeling of the apolipoprotein-lipid complexes was carried out as previously described.[9] The procedure used to assemble the amphipathic helices around a phospholipid bilayer implies a modification of the method used to surround a drug with lipid molecules.[21] In this modified procedure the peptide is oriented along a line perpendicular to the interface and connecting the hydrophilic and the hydrophobic centers.[21] The initial position of the helices is defined by taking into account the molecular hydrophobic potential (MHP) and the position of each helix is subsequently modified by steps of 0.5 Å in each direction and by rotation of 2.5° around all axis. The sum of the van der Waals, electrostatic, and hydrophobic interactions between helical segments is then calculated and minimized until the lowest energy state is reached.

The dimensions of the helical segments and the diameters of the discoidal complexes are measured from the computer-built models.

All calculations were performed on an Olivetti CP486 microcomputer equiped with an Intel 80486 arithmetic coprocessor, using the PC-PROT+ (Proteins Analysis Programs) and the PC-TAMMO+ (Theoretical Analysis of Molecular Membrane Organization) procedures. Graphs were drawn with the PC-MGM+ (Molecular Graphics Manipulation) program.

RESULTS

Localization of the Internal and Paralogous Homologies in the Human Apolipoproteins

The autocorrelation functions calculated for apo AI, AII, AIV, CI, CII, CIII, and E are plotted on Figure 1. For all apolipoproteins, maxima are located at distances corresponding to 3–4, 7, 11, 22, and 33 residues. As expected, the intensity of the maxima decreases with increasing distances. The position of the maxima at 3–4, 7 and 11 residues, corresponds, respectively, to 1, 2, and 3 turns of an α-helix, each consisting of 3.6 residues. The position of these maxima supports the amphipathic character of the repeats, since residues with the same hydrophobicity reoccur after one turn of the helix. This accounts for the segregation of the hydrophobic and the hydrophilic residues on opposite sides of the helical segments. The maxima found at 11, 22, and 33 residues correspond to the repeats identified in the apolipoprotein sequences using other computational methods.[6]

Similar results (data not shown), were obtained from the analysis of apolipoprotein sequences from other species including dog, rabbit, monkey, chicken, and pig.[4] These results support the general character of the autocorrelation algorithm and stress the high degree of homology in the basic pattern underlying the apolipoprotein sequences.

The helical repeats identified in the human apolipoprotein sequences using the autocorrelation matrix coincide with those previously reported using

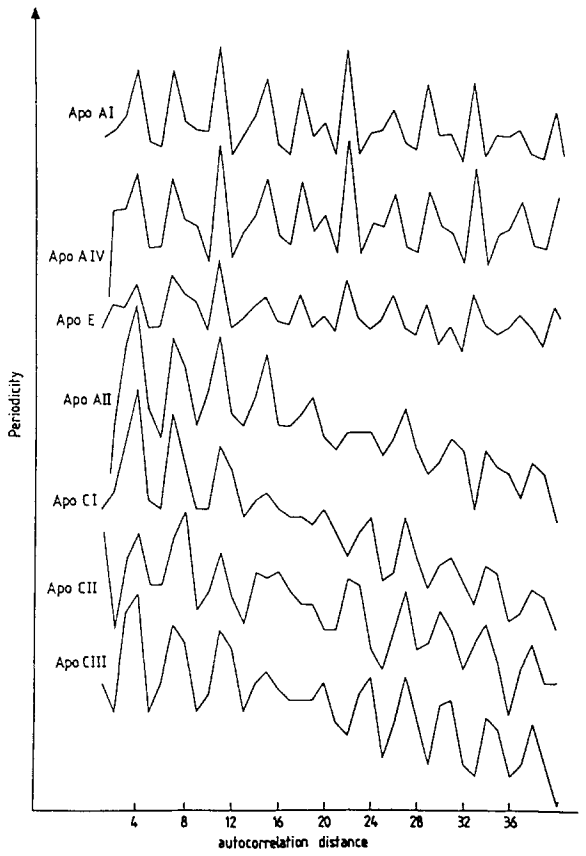


Fig. 1. Autocorrelation function representing the sum of occurrences of a residue with an index equal to 1 at a distance r of the other $n-r$ residues of the sequence, as a function of the autocorrelation distance r.

other algorithms[4] (Table II). For those apolipoproteins where the quasi-periodicity is less pronounced than for apo AI or apo AIV, the calculation of the conformational energies helped define the location of the most stable repeats. The helical conformation of segments 204–221 and 229–246 of apo E and segments 35–52 of apo CIII was more stable than that of segments 219–236 of apo E and 50–67 of apo CIII. The helical segments were further oriented at the lipid–water interface as described previously[9] and the molecular hydrophobicity potential was calculated for each helix after energy minimisation.

Calculation of the Molecular Hydrophobicity Potential for the Amphipathic Helices

The molecular hydrophobicity potential profiles of the helical segments 189–206 of human apo AI, 11–28 of human apo AII (Fig. 2), 123–140 of dog apo AI, and 52–69 of rat apo AII (Fig. 3) show that the hydrophobic and hydrophilic sides of the helices are well separated. The extent of the hydrophilic contour lines around the apo AI peptide is larger that the hydrophobic contour envelope (Figs. 2A, 3A,C). In contrast, the hydrophobic and hydrophilic envelopes were similar around the apo AII helices (Figs. 2B, 3B,D). For both helices, we observed no significant overlap between the two envelopes, but rather a uniform profile of the molecular hydrophobic potential along the long axis of the helices. From the hydrophobicity potential contours, the values of the angles pho and ϕ, representative of the mean value of the hydrophobic and hydrophilic domains, were obtained by projecting the isopotential lines on a plane perpendicular to the long axis of the helix (Fig. 2A,B). The angles pho and ϕ are listed in Table II for the helical repeats of the various apolipoproteins. For the helices of apo AI, A–IV, and E the hydrophobic angle pho has similar values, varying between 85° and 159° with mean value of 123°, 117°, and 117°, respectively (Table III). These values are lower than those calculated for the smaller apolipoproteins apo AII, CI, and CIII, with respective mean values of 201°, 177°, and 182° (Table III). Two classes of helices, differing in the magnitude of their hydrophobic contour lines, can thus be separated according to this calculation procedure. The angles pho, calculated for the amphipathic helices of apo CII (residues 17–34 and 46–63), have intermediate values of 140 and 133° (Table III).

For apo AII, CI, and CIII, the contact areas between the hydrophobic sides of the helices and the lipid phase, and the hydrophilic sides and the aqueous phase are similar as the angles pho and ϕ are equivalent. The contact area between the hydrophobic sides of the helices and the lipid phase is smaller for apo AI, A-IV, and E. For all apoproteins, the formation of discoidal lipid–protein complexes with the amphipathic helices around the edge of the lipid bilayer would be predicted from the molecular hydrophobicity potential calculations.[26] In these complexes, the amphipathic helical segments shield the phospholipid acyl chains from the aqueous phase. Due to the smaller hydrophobic side, the lipid–protein interactions and the stability of the complexes should be lower in the first class of apolipoproteins, including apo AI, AIV, and E, in agreement with the experimental data.[2]

Assembly of Apolipoprotein and Lipids Within the Protein–Lipid Complexes

Electron microscopic data have shown that discoidal particles are generated upon apolipoprotein binding to phospholipids.[2,3,8] Infrared measurements carried out on apo AI, AII, and on the CNBr fragments of apo AI have moreover demonstrated that the amphipathic helices are oriented parallel to the acyl chains of dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC) within the isolated complexes.[9,29,30]

Based upon these experimental data, the helical segments of each apolipoprotein were assembled together, as described above, with the hydrophilic

TABLE II. Location of the Helical Segments Identified in Human Apolipoproteins Using the Autocorrelation Matrix*

Apoprotein	Helical segment	Pho (°)	ϕ (°)	D_1 (Å)	D_2 (Å)	d (Å)
Apo AI	68–85	116	244	17.1	14.1	
	101–118	114	246	18.9	16.1	17.5
	123–140	121	239	17.2	12.8	19.9
	145–162	121	239	15.3	11.4	16.7
	167–184	125	235	15.0	10.4	18.8
	189–206	123	237	15.3	13.0	16.1
	222–239	134	226	16.0	13.4	16.8
Apo AIV	64–81	88	272	17.0	14.9	
	97–114	109	251	19.4	15.4	17.2
	119–136	104	256	15.9	15.6	18.8
	141–158	111	249	16.7	14.5	17.8
	163–180	159	201	17.4	11.5	18.8
	187–204	122	238	16.1	11.4	18.4
	207–224	124	236	17.0	13.1	17.9
	229–246	100	260	16.9	11.0	17.4
	251–268	119	241	15.9	13.0	18.0
	291–308	113	247	14.7	12.7	16.6
	324–341	133	227	15.3	13.6	13.6
Apo E	86–103	85	275	17.0	12.5	
	108–125	104	256	17.9	12.3	19.0
	141–158	96	264	17.8	14.0	18.2
	165–182	108	252	17.1	13.0	18.3
	204–221	127	233	16.5	13.6	18.8
	229–246	112	248	16.0	14.7	18.2
	269–286	150	210	15.6	13.2	15.8
Apo AII	11–28	179	181	16.8	13.1	
	52–69	224	136	15.9	14.0	16.9
Apo CI	7–24	177	183	15.1	14.4	15.5
APO CII	17–34	140	220	14.8	11.7	
	46–63	133	227	13.4	13.6	15.2
						15.6
Apo CIII	11–28	195	165	15.6	13.7	
	35–52	169	191	14.4	11.3	16.0

*The first and last residue of each helix are given together with the angles pho and ϕ characterizing the hydrophobic and hydrophilic isopotential contours. D_1 and D_2 represent the cross-sectional diameters of the helices measured tangentially and perpendicular to the edge of the discs, d is the distance between the centers of adjacent helices.

sides facing the aqueous phase and the hydrophobic faces interacting with the acyl chains of the phospholipid bilayer. The most stable conformation for the discoidal complexes is obtained by minimization of the energy of assembly. Examples of half of such discoidal complexes, comprising 6, 11, and 6 helices for apo AII, AIV, and CI, respectively, are drawn on Figure 4A–C. The molecular parameters characteristic of the dimensions of the complexes are schematically represented on Figure 4D. These parameters correspond to the internal (D_{in}) and external (D_{out}) diameters of the disc, the average diameters of the helices, measured both tangentially (D_1) and perpendicularly (D_2) to the edge of the disc, and the distance (d) between the centers of adjacent helices. The dimensions of the various helices associated to phospholipids in the complexes are listed in Table II. The average values for each apolipoprotein are summarized in Table III and the dimensions of the complexes in Table IV. These data show that the helical segments have a slightly ellipsoidal shape with an axial ratio of about 1.2 (Fig. 5). The mean distance between the outer sides of adjacent helices in the complexes is around 1 Å indicating a close contact between helices.

Table IV compares the composition and dimensions obtained by computer assembly of the helical segments of apo AI, AIV, E, AII, CI, and CIII, with the experimental data available from compositional analysis and from size measurement by gradient gel electrophoresis and electron microscopy.[9,29–32] The experimental number of helices per complex was calculated from the secondary structure data obtained by infrared spectroscopy.[9,30]

The diameter of the complexes generated with the largest apolipoproteins, apo AI, AIV, and E, was

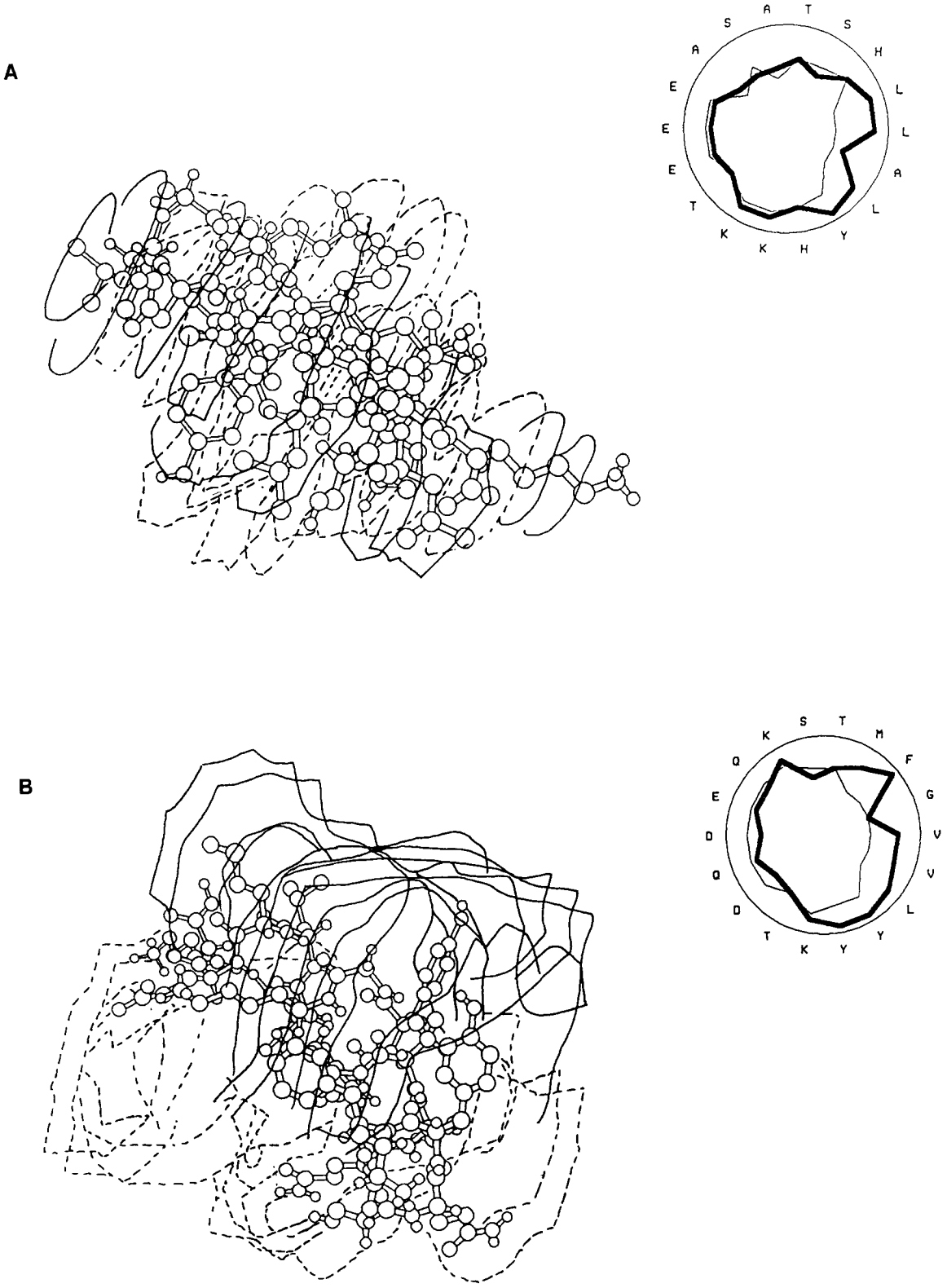


Fig. 2. Representation of the hydrophobic (——) and hydrophilic (---) isopotential lines, corresponding to a transfer energy of 2 kcal/mol per amino acid residue, along the axis of the helical segment 189–206 of human apo AI **(A)**, and segment 11–28 of human apo AII **(B)**. A projection of these contour lines on a plane perpendicular to the long axis of the helical segment is shown on the right-hand side of the figure. The amino acid residues are indicated outside the circle; the bold contour corresponds to the hydrophobic isopotential lines and the lighter line to the hydrophilic potential. The angle pho, measured from the center of the circle, characterizes the domain which is predominantly hydrophobic.

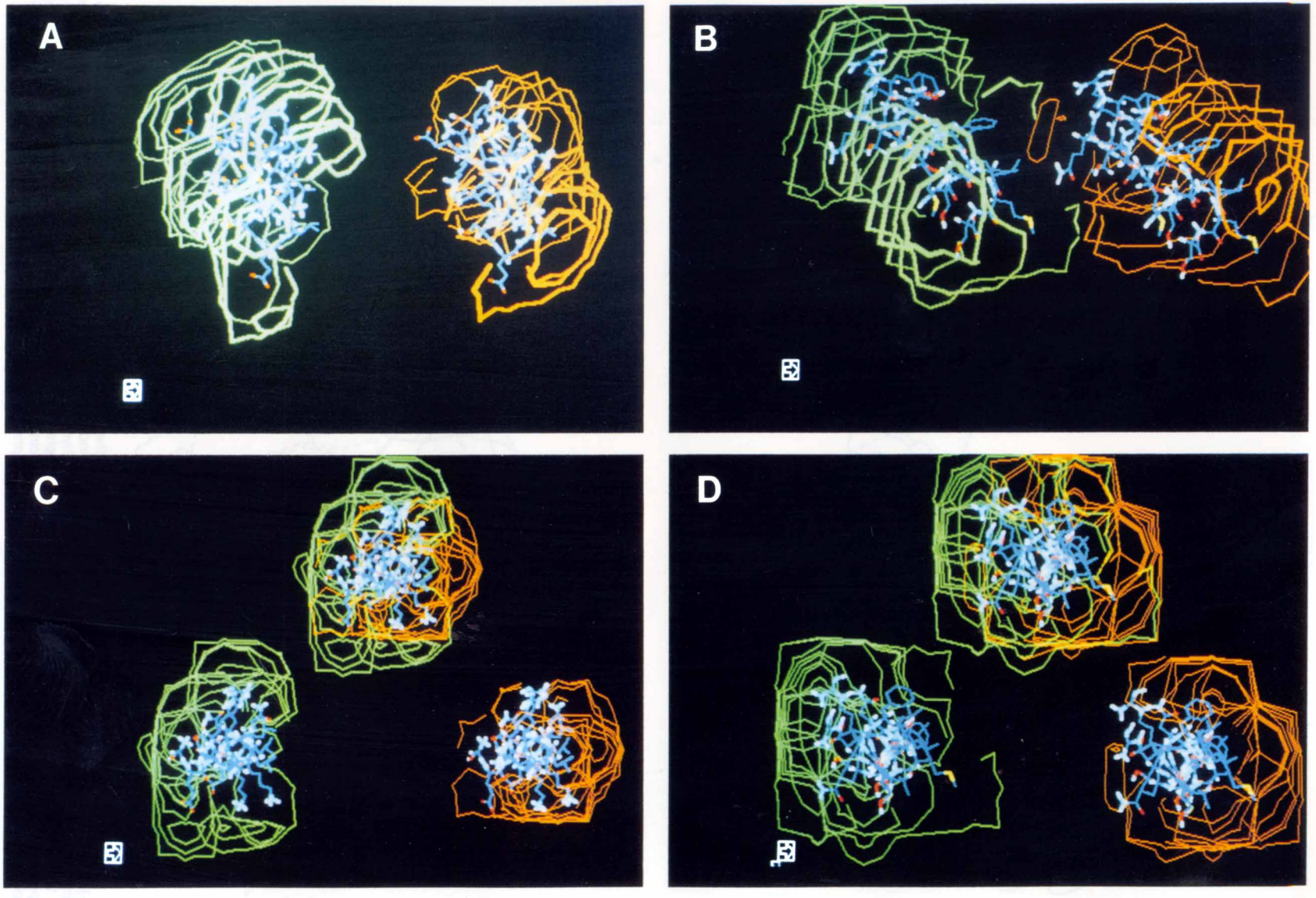


Fig. 3. Computer images of perspective **(A,B)** and axial **(C,D)** views of the molecular hydrophobicity potential. Hydrophilic envelopes are drawn in green, hydrophobic envelopes in red-brown. In the axial view **(C,D)** the upper drawing represents the sum of the hydrophobic (red-brown) and hydrophilic (green) envelopes shown below. **A,C** represent the helical segment 123–140 of the dog apo AI sequence and **B,D** correspond to the helical segment 52–69 of the rat apo AII sequence.

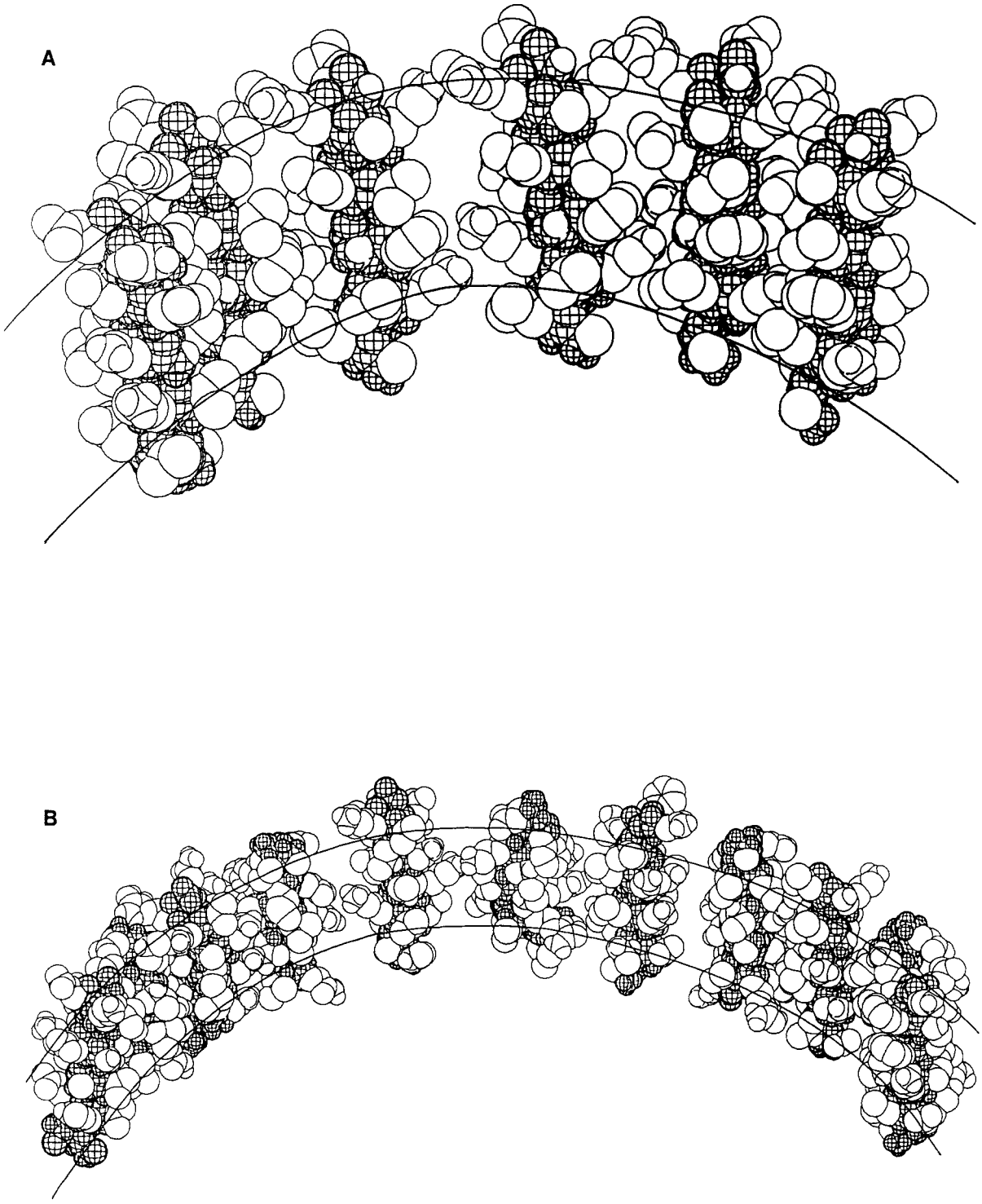
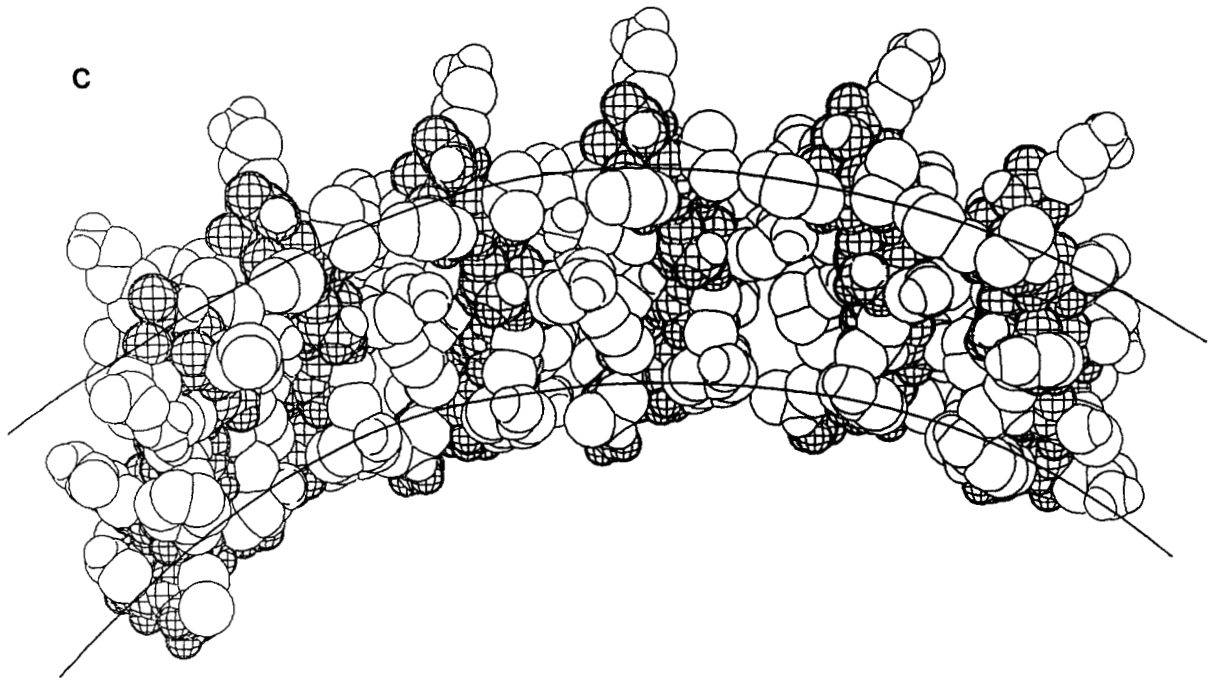


Fig. 4. Structure of half a discoidal apoprotein–phospholipid complex, built by molecular modeling for **(A)** monomeric human apo AII; **(B)** human apo AIV; **(C)** human apo CI. Definition of the molecular parameters characteristic of the complexes: D_{in}, D_{out}: internal and external diameters; D_1, D_2, diameters of the helices are shown in **(D)**. Parts C and D appear on page 254.



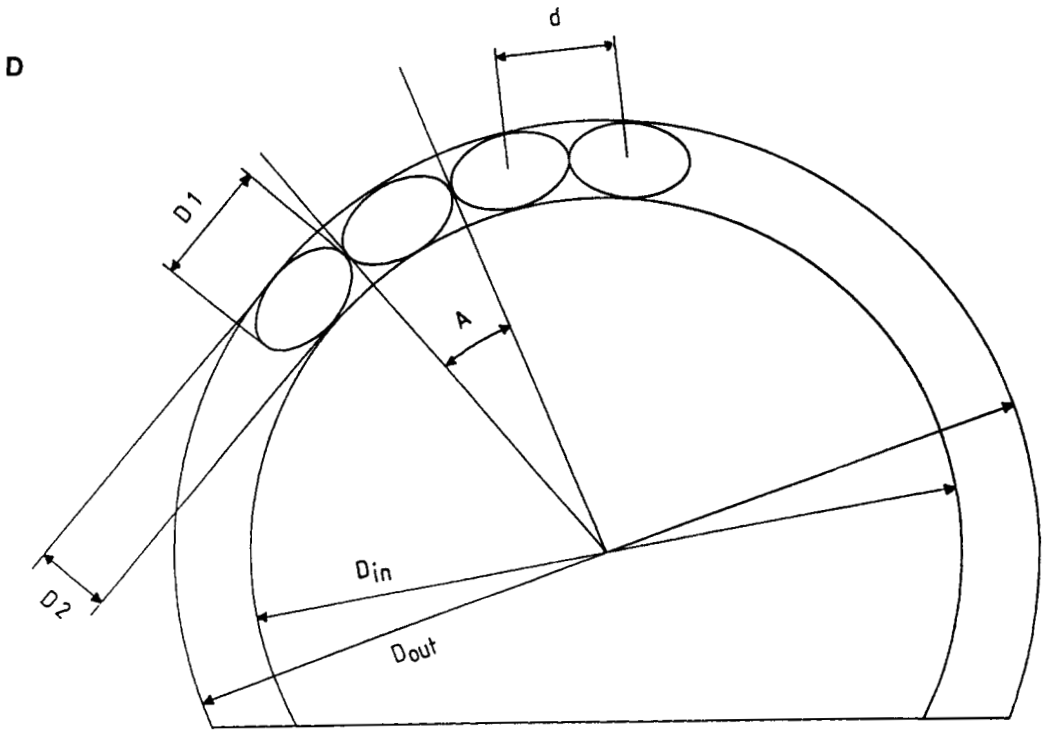


Fig. 4C,D. Legend appears on page 253.

TABLE III. Mean Values per Apoprotein, of the Parameters Described in Table II, for the Different Helices*

Apoprotein	Pho (°)	ϕ (Å)	D_1 (Å)	D_2 (Å)	d (Å)
Apo AI	123	237	16.4	13.0	17.6
Apo AIV	117	243	16.6	13.3	17.7
Apo E	117	243	16.8	13.3	18.1
Apo AII	201	159	16.4	13.6	16.9
Apo CI	177	183	15.1	14.4	15.5
Apo CII	137	223	14.1	12.7	15.4
Apo CIII	182	178	15.0	12.5	15.4

*The angles pho and ϕ define the extent of the hydrophobic and hydrophilic isopotential contours. The diameters D_1 and D_2 correspond to the cross-sectional diameters of the helices measured tangentially and perpendicular to the edge of the discs and d is the distance between the centers of adjacent helices.

TABLE IV. Dimensions of the Discoidal Apoprotein–Phospholipid Complexes

Apoprotein	Apo AI	Apo AIV	Apo E	Apo AII	Apo CI	Apo CIII
D_{out} (Å)						
Theoretical	89	131	110	77	84	78
Experimental*	104 ± 3	140 ± 3	140 ± 3 (32)††	94 ± 3	—	—
D_{int} (Å)	62	107	84	54	65	54
Number apo/complex						
Theoretical	2	2	2	6	12	6
Experimental†	2	2	2 (32)	6‡	—	—
Number hel/complex						
Theoretical	12	22	16	12	12	12
Experimental§	12–14	22	—	12	—	—
Number lipid/complex						
Theoretical	224	556	368	167	218	171
Experimental**	200	520	320 (32)	207	—	—

*Determined by gradient gel electrophoresis.
†Determined by cross-linking experiments.[29]
‡Number of Apo AII molecules: expressed as monomers.
§Determined by infrared spectroscopy.
**Determined by phospholipid and apolipoprotein quantitation on isolated complexes.
††(32): ref. 32.

around 100 Å, while the complexes with the smaller apolipoproteins had diameters around 80 Å. The complexes generated with apo AI, AIV, and E contain 2 mol of protein each; for the smaller apolipoproteins, 6 mol of monomeric apo AII, 6 mol of apo CIII, and 12 mol apo CI are required to cover the edge of the disc. For the smaller apolipoproteins, this figure corresponds to 12 helical segments of 18 residues each, per complex, compared to 22 and 16 for apo AIV and E, respectively. The number of lipids per complex was also higher for the apo AI, AIV, and E compared to apo AII, CI, and CIII (Table IV). When expressed as number of lipids per helix, this figure increases from 14 for apo AII and CIII up to 19, 24, and 25 for apo AI, E, and AIV, respectively.

Whenever available, the experimental composition and dimensions were in good agreement with the calculated values (Table IV), thus supporting the theoretical approach.

CONCLUSIONS

In this paper we describe a new approach for the characterization of the amphipathic helices of apolipoproteins. This procedure first involves the identification of a quasi-periodicity within apolipoprotein sequences using an autocorrelation matrix. The elements of this matrix reflect similarities in the hydrophobicity and hydrophilicity properties of the amino acids of the apolipoprotein sequences. This autocorrelation algorithm can further be used to detect the proline periodicity within the apolipoprotein sequences (data not shown). We suggested previously that Pro residues located between two adjacent helices reverse the orientation of two consecu-

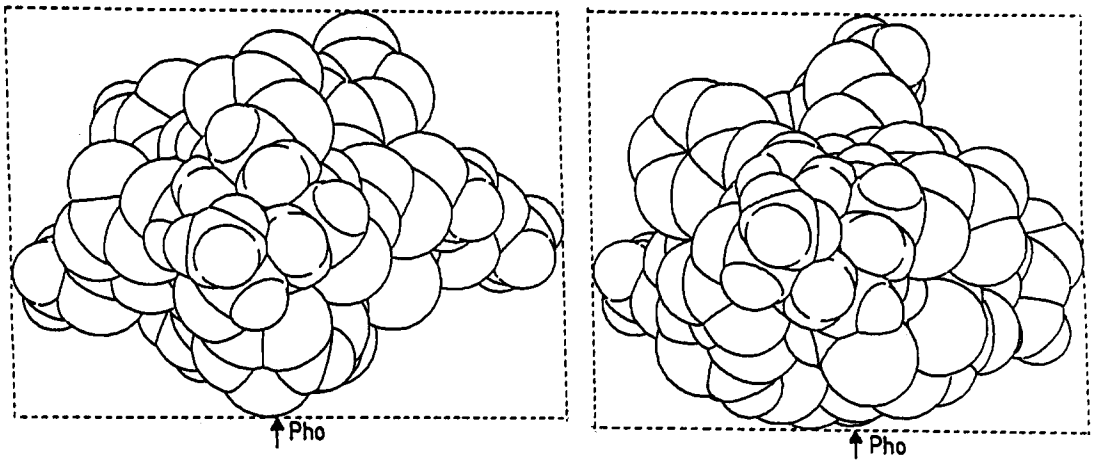


Fig. 5. Computer graphics representation of the top view of two helices of human apo AIV (left: residues 163–180; right: residues 324–341), demonstrating the ellipsoidal shape of the cross section of the helices. In both cases the diameter D_1, measured along the edge of the complex, with respective values of 17.5 and 15.3 Å is larger than the corresponding values of 11.5 and 13.6 Å for D_2. The helices are therefore oriented tangentially along the edges of the discoidal particle.

tive helical segments by forming a β-turn.[9] The autocorrelation matrix correctly identified the Pro residues located at the beginning of the 22-residue helical repeats in apo AI and AIV (data not shown).

The computational methodology presented here is more elaborated than the conventional search for internal homology based upon sequence similarities.[5–7] Its major advantage is to provide a better insight in the conformation and hydrophobic properties of the apolipoprotein helical segments. The structural calculations based upon energy minimization procedures enable the selection of the helical segments which can form stable complexes with phospholipids and the identification of common features for all apolipoproteins. The size and shape of the apolipoprotein helical segments were very similar, as they appear as oblate ellipsoids, with an axial ratio of around 1.2, oriented tangentially to the edge of the discoidal complexes. The ellipsoidal shape ensures an optimal coverage of the edge of the disc and shielding of the lipids from the aqueous phase in the discoidal lipid–protein complexes. The diameters of the helices, D_1 and D_2, are similar for all apoproteins, as D_1 ranges from 14.1 Å for apo CII, up to 16.6 Å for apo AIV and apo E, while D_2 varies from 11.3 Å for apo CIII to 14.4 Å for apo CI.

Although the apolipoprotein helical segments share common shape and dimensions, the extent of their hydrophobic and hydrophilic contour envelopes is different. The apo AI, AIV, and E proteins are surrounded by a more hydrophilic than hydrophobic contour envelope, whereas the hydrophobicity and hydrophilicity contours around the helical segments of apo AII, CI, and CIII are similar. The apolipoproteins can therefore be classified into two classes according to the value of the pho angle, representing the extent of the hydrophobic contour lines, around the helical segments. The differences between the properties of these helices were not appreciable by hydrophobicity or hydrophobic moment calculations[33] alone, and require a three-dimensional representation of the helices and of their hydrophobic profiles.

Previous estimations of the number of helices surrounding the discoidal complexes relied upon the assumption that helices have a spherical cross section, with a mean diameter of 15 Å.[31,33] This hypothesis leads to an overestimation of the number of helices surrounding the lipid core of the disc and to an underestimation of the number of lipids per helix.[31] According to our results, the lipid–protein complexes generated with apolipoproteins belonging to the first class contain 12, 16, and 22 helices for apo AI, AIV, and E, respectively, compared to 12 helices for apo AII, CI, and CIII. From the hydrophobic areas of the two classes of helices we estimate that in apo AI, AIV, and E, each turn of the helix contains an average of 1.2 hydrophobic residue, compared to 1.8 in apo AII, CI, and CIII. These residues are in contact with 3–4 phospholipid molecules located in the outer phospholipid layer of the lipid core of the disc.

The model described in this paper proposes a general mode of organization for the apolipoprotein–phospholipid complexes, and can be further extended to the structure of spherical HDL particles. After generation of a cholesteryl ester core and an increase of the radius of curvature of HDL, we propose that, in analogy with the model derived for apo AI,[9] the protein–protein interactions between the apoprotein helical segments are retained. The helices would lie on the surface of the HDL as patches of protein, and be oriented perpendicular to the phospholipid acyl chains of the lipid monolayer, in agree-

ment with the results from monolayer studies.[15] The concept of the molecular hydrophobicity potential around helical segments was first introduced to classify the lipid-associating helices.[26] It applies not only to the helical segments of apolipoproteins but can also account for a differentiation between receptor-anchoring helices, channel-forming helices, and fusogenic helical peptides of membrane proteins.[26] In this respect the water-soluble plasma apolipoproteins can be considered as the most hydrophilic components of the family of lipid-binding proteins.

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