review

Apolipoproteins and the structural organization of plasma lipoproteins: human plasma high density lipoprotein-3

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The structural properties and functions of the plasma high density lipoproteins (HDL) have recently become the center of attention, largely because of their possible participation in the control of both lipid metabolism (1, 2) and the pathogenesis of atherosclerosis (3, 4). In the past, several structural models of HDL have been proposed, each trying to encompass the rather scanty information available on the physical and chemical properties of these lipoproteins and of their constituents (5–8). With the accumulation of relevant experimental data, the theoretical models became more detailed and hence more useful for guiding the experimental work. We recently described a general model of lipoprotein structure, based largely on compositional data of the serum lipoproteins and on the molecular volumes and surface areas of their lipid and polypeptide constituents (9); at that time, we made no attempt to describe the surface organization of the apoproteins. In recent years, empirical methods have been developed for prediction of the probability of secondary structure from amino acid sequence data (10). The reasonable success of some of these methods prompted us to apply them to the prediction of the secondary structure of the apolipoproteins at the lipoprotein surface. Thus, in the present studies we addressed ourselves to the question of the relationship between the secondary structure of apoproteins and the structure of plasma lipoproteins. We focused primarily on human HDL_3, because the primary amino acid sequence has been determined for its two main apolipoproteins: apoA-I and apoA-II (8). The C-peptides, although recognized as constituents of HDL_3, occur only in substoichiometric amounts. Since they probably have no essential structural role in HDL_3, they will only be considered in the context of the discussion.

PRELIMINARY CONSIDERATIONS

The development of a structural model for a particle as complex as HDL requires identification of the noncovalent forces acting between a large number of individual molecules carrying widely differing chemical functional groups. In addition to the action of these major organizing forces, a model must also satisfy several criteria imposed by particle symmetry, thermodynamic stability, and space- and surface-filling requirements. In developing an acceptable model of HDL_3, we followed two complementary approaches: we carried out calculations based on experimental data obtained from the measurement of the surface properties of apoA-I and apoA-II, and we conducted theoretical analyses on the secondary structure of these apoproteins. In parallel, to verify the theoretical predictions, we built a space-filling model of an HDL_3 molecule, using Corey–Pauling–Koltun molecular models (Ealing Co., South Natick, MA). The structural model incorporated the following premises. *1*) The structure of HDL_3 is determined solely by the physicochemical properties of its constituents; *2*) HDL and its subclasses are spherical particles with statistical symmetry, composed of the same

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TABLE 1. Physical parameters of HDL_3 and components

Component	Number per HDL_3 Particle	Surface Area Å²/component	Molecular Volume Å³/component
Amino acid	963.0	15.6	122.86
Phospholipid	51.0	68.5[a]; 62.7[b]	1270
Cholesterol, free	13.0	39.1[a]; 0[b]	600
Cholesterol, esterified	32.0		1090
Triglycerides	9.5		1600

Molecular weight = 1.75×10^5; density = 1.15 g/ml; radius = 39.2 Å; volume = 2.5×10^5 Å³/particle; outer surface = 1.93×10^4 Å²/particle; core surface = 4.4×10^3 Å²/particle.

[a] Core surface.

[b] Outer surface.

chemical constituents but differing in size, density, and stoichiometry; *3*) the chemical composition of HDL_3 is in agreement with the data compiled previously (ref. 9; see also **Table 1**); *4*) HDL_3 is an anisotropic structure in which the individual constituents are segregated according to their chemical nature, either within the surface or within the core of the particle—in other words, the HDL particles have a well-defined spatial organization.

THE SPACE-FILLING MODEL

For practical reasons, and because of the assumed symmetry of HDL_3, we built only one-half of the particle. The scale of the model was that of the atomic models used: one Å was represented by 1.25 cm. Two concentric hemispheres, with diameters of 46.0 cm and 86.0 cm, respectively, were defined by sets of sixteen removable semicircular aluminum rods curving between a common vertex and a flat plexiglass base. The inner rods defined the surface of the core of the particle, whereas the position of the outer rods defined a sphere passing through the center of the apolipoprotein surface monolayer (**Plate 1A**).

LIPIDS

According to the chemical composition and the molecular weight (9), the hemisphere defining one-half of HDL_3 had to accommodate the following numbers of molecules: 25 phospholipids, 6 cholesterols, 16 cholesteryl esters, and 5 triglycerides. We attributed no structural role to the class heterogeneity of the phospholipids. We had concluded earlier from the space- and surface-filling requirements (9) that the neutral lipids, namely, cholesteryl esters and triglycerides, occupy the core of the particle in a sphere according to the general relation

$$1556n_{tg} + 1068n_{ce} = (4\pi/3)(r - 20.5)^3 \quad \text{Eq. 1}$$

where n_{tg} and n_{ce} are the number of triglyceride and cholesteryl ester molecules per particle and r is the radius of the particle in Å. As shown in Plate 1A, these requirements were readily met in our CPK model of $r = 39.2$Å.

We have also shown that only phospholipids and cholesterol are in direct contact with the surface of the hydrophobic core; in consequence, the content of these amphiphilic lipids obeys the following general relation:

$$68.5n_{pl} + 39.1n_c = 4\pi(r - 20.2)^2, \quad \text{Eq. 2}$$

where n_{pl} and n_c are the number of phospholipid and cholesterol molecules per particle, respectively. In arranging the space-filling models of phospholipid molecules, we had to take into account the chain fluidity of the phospholipids, which results in total molecular volumes larger than the collision volumes represented by the CPK models. For this reason, the limiting volumes were represented by lucite cylinders of elliptical cross-section ($15 \times 8 \times 42$ cm), obtained by thermal deformation of round cylinders of appropriate diameter. In the actual construction of the model, these cylinders were truncated to a height of 30 cm, which permitted their fitting between the inner and outer rods, leaving the polar head group of phospholipids unshielded (**Plates 1B** and **2A**). Contrary to the case of phospholipids, the plastic shell defining the molecular volume of cholesterol corresponded closely to that of the space-filling model ($10 \times 8 \times 30$ cm).

As predicted by Eq. 2, the surface of the core accommodated exactly the bases of the cylinders enclosing the phospholipid and cholesterol molecules which radiate toward the surface of the particle (Plates 1B and 2A). In agreement with the predictions, the polar head groups of the phospholipids are located on a spherical surface different from that defined by the hydroxyl functions of the cholesterol molecules (Plates 1B and 2A; see arrows showing the position of the hydroxyl group of cholesterol).

APOPROTEINS

Space- and surface-filling considerations showed that apoproteins are located at the surface of the particle between the areas occupied by phospholipid head groups. The number of molecules on the surface is defined by the following general relation:

$$4\pi r^2 = 15.6n_{aa} + 62.7n_{pl}, \quad \text{Eq. 3}$$

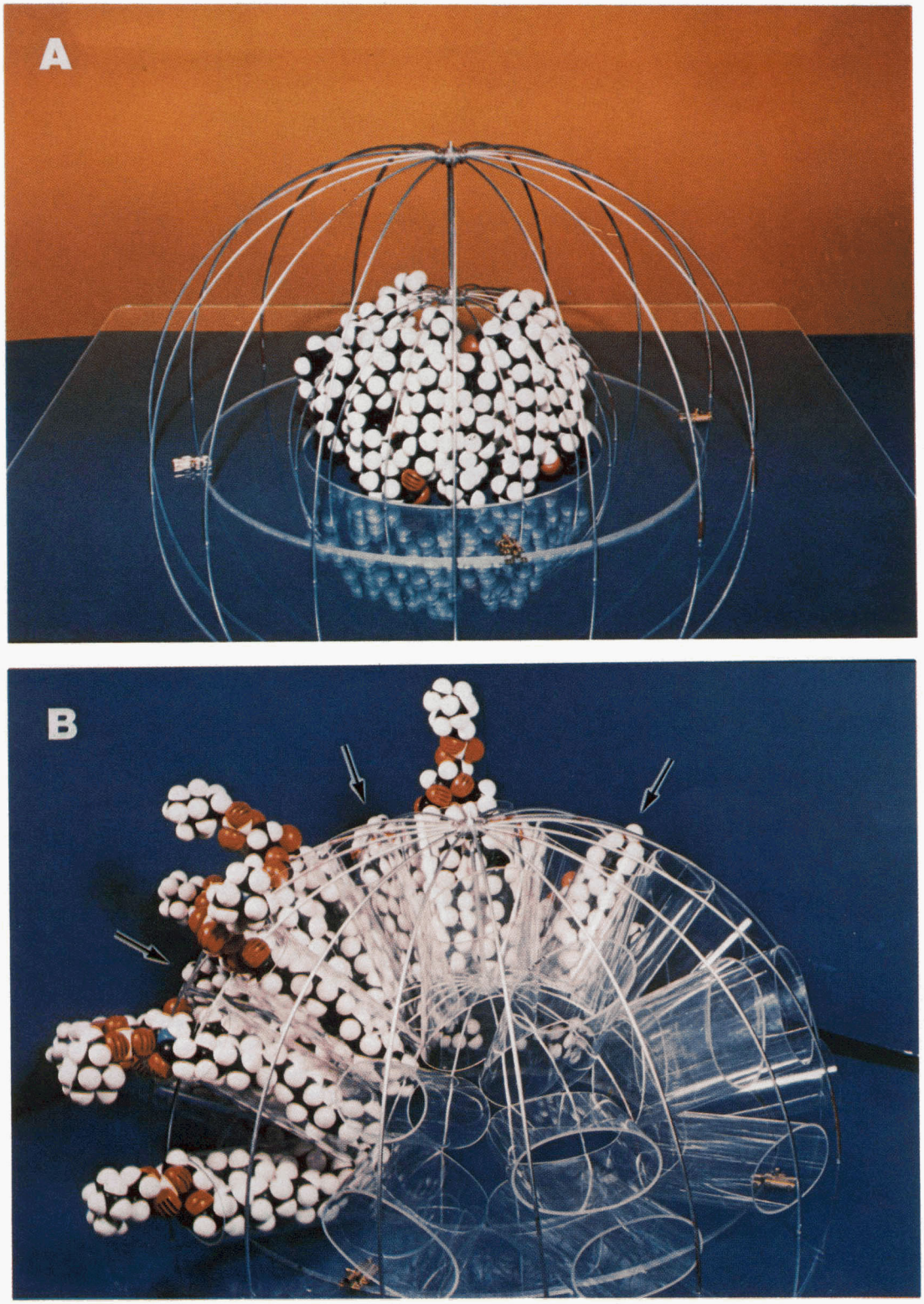

Plate 1. *A*. Model frame comprising two concentric hemispheres defined by outer and inner rods curving from a common vertex to a flat plexiglass base (for details, see text). The volume of the core, defined by the inner rods, is occupied by neutral lipids, cholesteryl esters, and triglycerides. *B*. Spatial arrangement of phospholipids and free cholesterol as a monolayer at the surface of the unfilled core. Note their radial distribution and the difference in position of the polar head group of phospholipid and hydroxyl function of cholesterol. The position of the latter is indicated by arrows. Both filled and unfilled cylinders are shown.

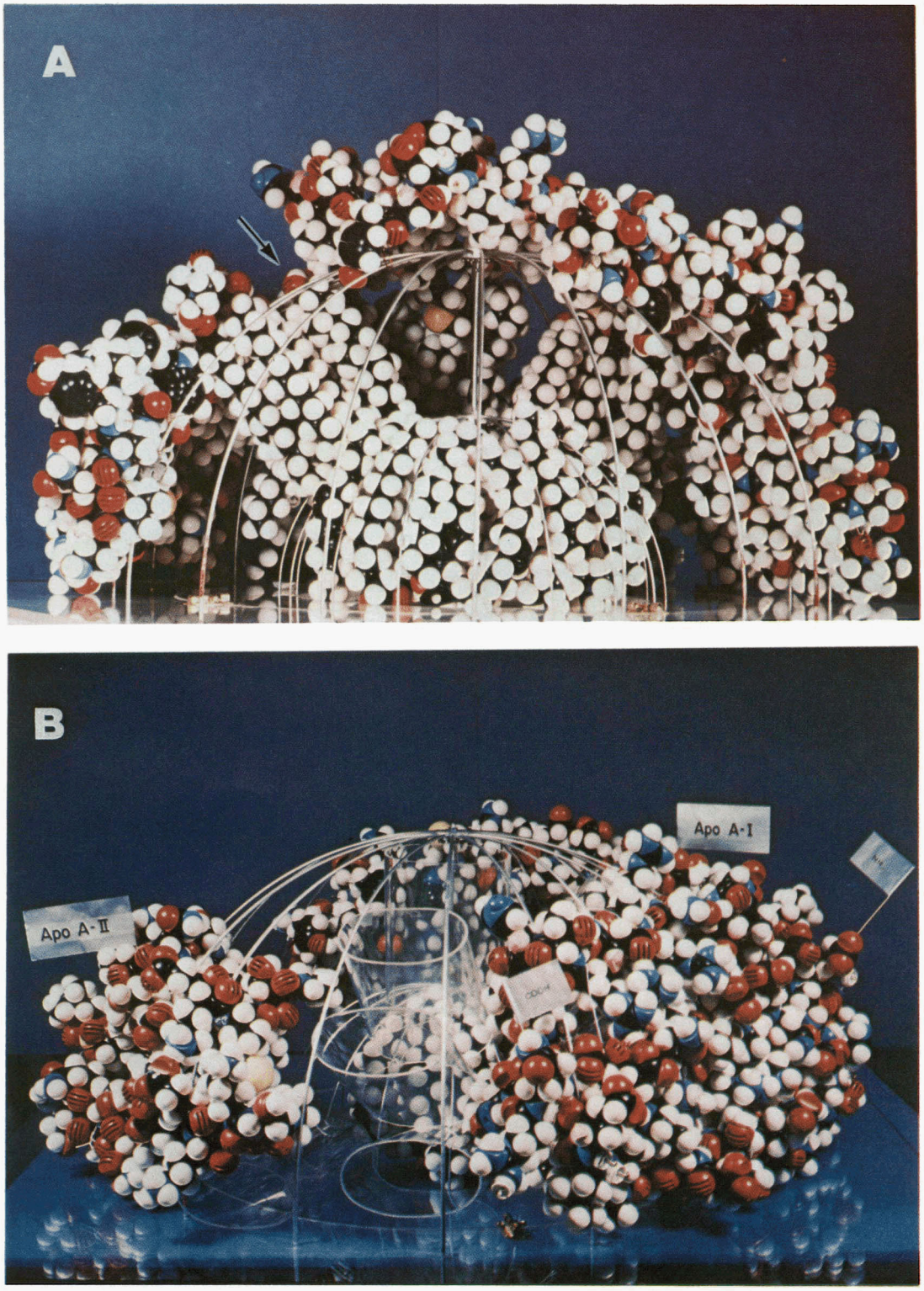

Plate 2. *A*. Cross-sectional view of the core and of the phospholipids and cholesterol radiating from the core. The protein forms a monolayer at the surface. Note the compactness of both core and apoprotein surface. *B*. Side view of the HDL_3 model with the protein monolayer constructed to cover the surface only partially. Segments of apoA-I are on the right, and those of apoA-II are on the left of the viewer. Between these two segments the surface permits a view of the truncated phospholipid cylinders and of the core. Note again the compactness of the protein monolayer.

where n_{aa} is the number of amino acids per HDL particle (9).

In treating the problem of the surface organization of apoA-I and apoA-II, we based our reasoning on the surface properties of these apoproteins as derived from the studies at the air–water interface. An important conclusion from these studies was that the A-apolipoproteins have only a two-dimensional structure at the air–water interface. Indeed, apoA-I migrates preferentially to the interface, where it forms stable monolayers (11). These protein monolayers are highly compressible and have a limiting area, extrapolated to zero pressure, of 22 Å²/amino acid.[3] This value is much smaller than that observed for two-dimensional random-coil structures (46 Å²/amino acid), but yet significantly larger than that displayed by the majority of partially denatured globular proteins (15 Å²/amino acid at $\pi = 0$ dyne/cm) (12).

A priori, the preferential location of the apoproteins at the lipoprotein surface could be due either to their interaction with phospholipids, or solely to their intrinsic properties. We discarded the first possibility because *1*) experimental results showed that the apolipoproteins compete against phospholipids for the surface rather than being stabilized by them (13); *2*) HDL_3 digested by phospholipase A_2 retains its structural stability (14); and *3*) NMR studies show that the choline group of phospholipids in HDL has a high mobility (6–8). From these considerations, we concluded that the affinity of the apoproteins for the interface and their propensity to assume a two-dimensional structure are the result of their intrinsic properties alone.

If both apoA-I and apoA-II are thermodynamically more stable at the interface than their respective monomers in solution, these molecules at the interface should be asymmetric. In other words, at the inner side of the lipoprotein surface there will be interactions between the side chains of the apoproteins and hydrocarbons. According to the statistical analysis of the primary amino acid sequence based on genetic considerations, a large portion of apoA-I has repeating sequences of 11 or 22 amino acid units (15, 16). When represented in an Edmondson "helical wheel" (15), the amino acid side chains segregate between hydrophobic and hydrophilic regions, which cover the surface of the helix in a 4:7 ratio. The prediction of the repeating α-helical segments by this method was corroborated by an analysis of the helix-forming potentials carried out according to the method of Chou and Fasman (17). However, in spite of the fact that there was fairly good agreement between the two predictions, a large portion of the apoprotein sequence remained without assigned conformation.

Because the protein has to occupy the interface, and hence at least part of the stabilizing force has to be derived from amphiphilicity, we proposed to analyze the two-dimensional structure of the A-apoproteins with the assumption that the only stabilizing principle was the hydrophobic–hydrophilic distribution in the polypeptide chain, while the secondary structure of the latter respected the predictive rules by Chou and Fasman. To calculate the thermodynamic stability of the different possible conformations of the peptide chains at the interface, we used the method of the solvation energies of the side chains, proposed earlier by Segrest and Feldmann (18). For our calculations, we assumed that the relative solvation energies of the amino acid side chains of the protein in the hydrophilic and hydrophobic environments of the lipoprotein are in the same ratio as those measured in an ethanol–water system (19, 20). The free energies of transfer of amino acid side chains from a polar to a nonpolar environment which were used in these calculations are summarized in **Table 2.** With the help of these values and with the ratio of hydrophobic: hydrophilic regions proposed by Fitch (15), we were able to calculate, for successive segments of apoprotein, the relative thermodynamic stabilities of the most probable two-dimensional secondary structures, namely, α-helix, random coil, β-sheet, and β-turn. For the β-sheet, we assumed that successive amino acids were alternately in a hydrophilic or hydrophobic environment, whereas for the β-turn we used the two most likely side-chain geometries proposed by Ramachandran and Sasisekharan (21), having the amino acids at the end and in the middle of the turn in different environments.

To calculate the relative thermodynamic stability of the α-helical structure, we assumed that the axis of the helix is tangential to the lipoprotein surface and that, due to the large curvature of the HDL surface, the hydrophobic phase of the helix is only $^4/_{11}$th of the total surface. The actual search algorithm made use of the Edmondson helical wheel (15), and a computer program was designed for the calculation of the sum of the hydrophobic–hydrophilic interaction energies of such an amphiphilic helix. In the actual calculation, successive segments of 11 amino acids were chosen from the primary sequence. The relative location of the amino acids was derived from the Edmondson wheel, and the sum of the hydrophobic–hydrophilic free energy interactions was calculated for each of the 11 possible orientations of the helix with respect to the

[3] Shen, B., A. M. Scanu, and F. Kézdy. Physical properties of human apoA-I at the air–water interface. In preparation.

TABLE 2. Hydrophobicity parameters[a]

Amino Acid	Free Energy (kcal/mol)
Ala	0.5
Gly	0
Ile	1.8
Leu	1.8
Phe	2.5
Pro	1.4
Val	1.5
Asn	−0.2
Cys/2	1.0
Gln	−0.2
Met	1.3
Ser	−0.3
Thr	0.4
Trp	3.4
Tyr	2.3
Arg^+	−3.0
Asp^-	−2.5
Glu^-	−2.5
His^+	0.5
Lys^+	3.0

[a] Values taken from references 19 and 20. These data represent the free energy changes associated with the transfer of side chains from a polar to a nonpolar environment.

lipoprotein surface. Of the 11 total free energies of interaction, that with the free-energy minimum was chosen as the most probable orientation with respect to the surface of the lipoprotein. This free-energy value was divided by 11 to obtain the average free energy per amino acid and was then increased by 0.5 kcal, to take into account the energy for hydrogen bonds in the helical structure.

For the calculation of the relative free energy of the amphiphilic β-sheet, segments of six, eight, and ten amino acids, centered around each amino acid in the primary sequence, were chosen and their total free energy was calculated with respect to both possible orientations relative to the lipoprotein surface. Then, the optimal value was divided by the number of amino acids. To this value, 0.25 kcal was added to account for the hydrogen bonding of the β-sheet structure.

In the estimation of the relative free energy of the random-coil conformation, we assumed that all amino acid side chains are oriented according to their intrinsic lyophilicity, as defined by the free-energy values listed in Table 2. Finally, we calculated the free energy of solvation averaged over the entire protein by adding the absolute values of all free energies of solvation multiplied by the number of the corresponding amino acid in the apoprotein (apoA-I) and dividing by 245, to obtain the average value per amino acid. This free energy of solvation, which refers to a totally denatured apoprotein at $\pi = 0$ dyne/cm interfacial pressure, had to be corrected for the compressional energy of the apoprotein on the surface of the lipoprotein which probably has an interfacial pressure of 35 dyne/cm (9). For this correction, we used the measured compressional energy of the totally denatured apoproteins, which is 150 kcal/mol of apoA-I.[3] No such correction was necessary for the α and β structures, since indirect evidence shows that the compressional energies of the α-helix and the β-sheet from 0 to 35 dynes/cm are relatively small (22).

The results of these calculations for apoA-I are shown in **Fig. 1.** In Fig. 1A, the baseline of the ordinate has been chosen as the free energy of the random coil at 35 dynes/cm, reduced to the average amino acid. Over the whole length of the apoprotein, only in isolated short segments are there β-structures significantly more stable than the random coil conformation. On the other hand, the stability of the α-helical conformation is much greater than that of the other conformations. Most notably, the small β-structure peaks, except for those centering about amino acids 46, 60, 100, 178, and 230, are overwhelmed by the stability of the α-structure. Therefore, on the basis of lyophilic considerations alone, one would predict that more than half of the molecule should be in the α-helix conformation.

The most important feature which justifies the

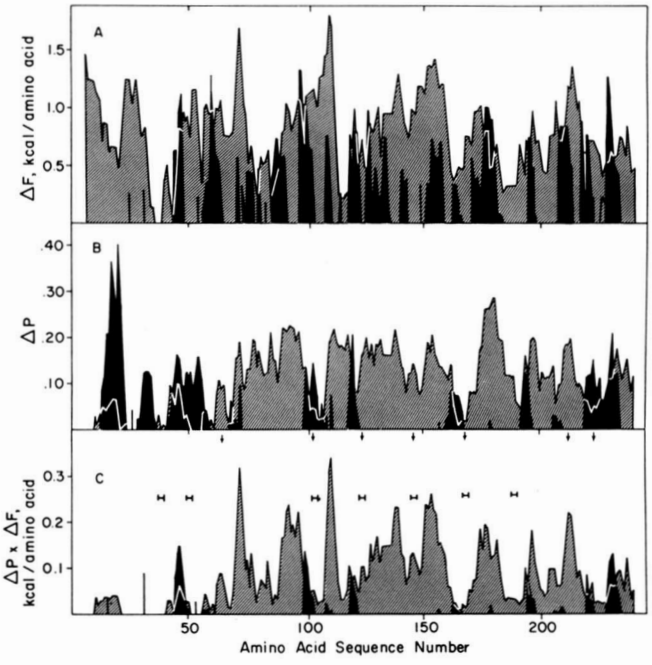

Fig. 1. Graphic representation of the predicted secondary structure of apoA-I at the interface. *A*. Average differential energy of stabilization of α-helix (shadowed area) and β-sheet (black area) with respect to random coil at the lyophilic interface. *B*. Average differential Fasman potentials of the same structures. *C*. Relative stability of α-helix and β-sheet based on the combination of analyses in *A* and *B*. The small horizontal bars indicate the positions of the β-turns. The arrows point to the positions of the proline residues.

method of our energy calculations is the fact that, in the region where an α-helix or β-sheet has been predicted, the energetically optimal orientation of the structure with respect to the surface does not change when one progresses from one amino acid to another. For example, in the interval between amino acids 85 and 102, the orientation of the plane dividing the two domains, as predicted by the optimal lyophilic free energy, does not vary by more than 33°.

Since the secondary structures had to satisfy in addition the conditions specified by the Chou and Fasman method, we also carried out the latter analysis for the same segmental lengths as the ones used in the energy calculations. The results are shown in Fig. 1B. Again, the dominating feature of this diagram is the high α-potential of a large portion of the molecule. Moreover, in the region from amino acid 60 to about 220, the predictions of the α-helical structure by the two methods largely coincide. With regard to the β-structure, only the segments centered around 230, 100, and 49 were predicted as potential β-structure by the two methods. In the remainder of the molecule, the two methods yielded contradictory predictions. Notably, in the region centered around tyrosine-18, the Chou and Fasman method indicates an unusually high β-potential, but this segment of the molecule is not amphiphilic in the β-sheet conformation.

The Chou and Fasman method is somehow related to the thermodynamic stability of the folding of the peptide backbone, whereas the lyophilic energy values represent the contributions of the individual amino acid side chains to the total free energy. Therefore, the relative stability of the various secondary structures should be given by the combination of the free-energy changes and of the average Chou and Fasman potentials corrected for the background (potential = 1). At the present, we do not know the appropriate scaling factor that would allow us to assess the relative importance of these two contributions; therefore, we have used the product of the two to estimate the relative total stability of the different secondary structures in apoA-I. The result of these calculations (shown in Fig. 1C) indicates that indeed the dominant secondary structure of apoA-I on the surface of the lipoprotein should consist of extended segments of α-helix separated by random coils or β-turns. The only segments that should be in the β-conformation are those centered around amino acids 47 and 230. In Fig. 1C, we also indicate the segments that have a high potential for β-turns. On the basis of the data shown in this figure, we would like to propose that a likely secondary structure for apoA-I at the hydrophobic–hydrophilic interface is that shown in **Fig. 2** (upper panel). The analysis does not specify the lengths of the various secondary structural segments. For the estimation of segmental length, we have taken into consideration the repeating characteristics of the helices revealed by the statistical analysis of the secondary structure (15). We also considered proline and glycine as potential helix breakers. The structure proposed in Fig. 2 is not

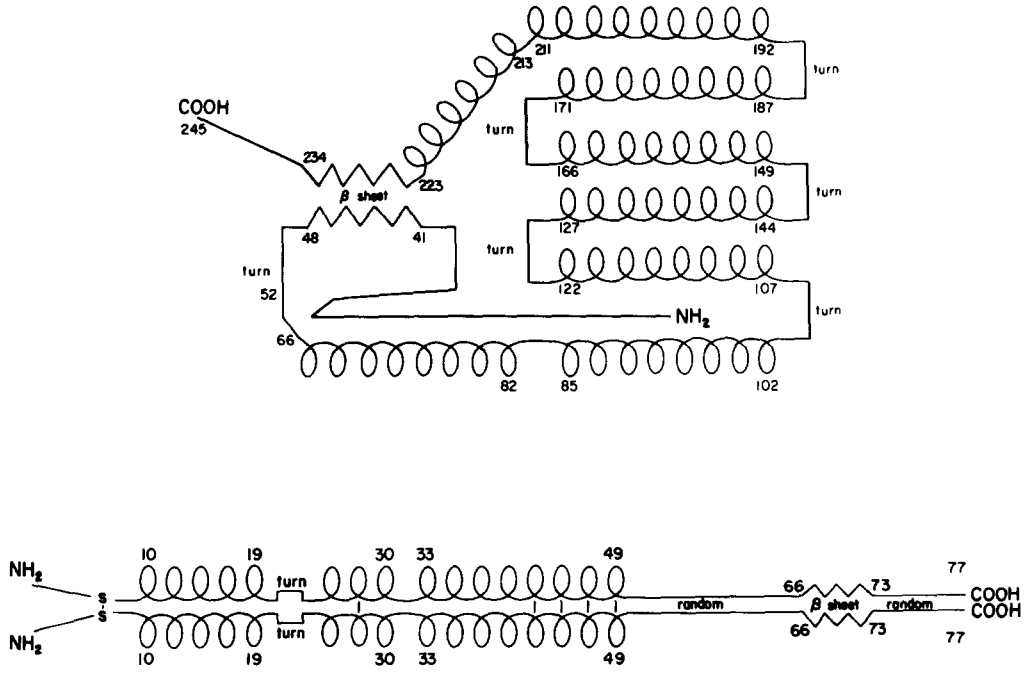

Fig. 2. Schematic drawing of the various predicted secondary structures in apoA-I (upper panel) and apoA-II (lower panel). α-helix ⌇⌇; β-sheet ⩘; β-turns]; and random coil —.

meant to be definitive. Nonetheless, it allowed us to construct space-filling models of apoA-I which were used in the final form of the HDL_3 model.

Similar analyses were carried out for apoA-II assuming the same average compressional energies as for denatured apoA-I. The conclusion from the analysis upon which we based our model is presented in Fig. 2 (lower panel).

The actual placement on the surface of the lipoproteins necessitated a knowledge of positioning of the phospholipid head groups. Most importantly, we had to decide whether the phospholipid head groups existed as small clusters or whether they were randomly dispersed in the protein matrix. We preferred the random distribution, since all data presently available, obtained both by physical (8) and by enzymatic probes (14), indicate that all phospholipids are equivalent. On the other hand, a clustering would require that some of the phospholipids be distorted because of the high curvature of the spherical surface. In the actual construction of the space-filling model, we found it virtually impossible to juxtapose the phospholipid molecules without introducing large strains on the alkyl chains. Also, a random distribution of the phospholipid head groups on the surface of a particle of the size of HDL would leave a spacing of 11–12 Å, which would be ideal for the intercalation of α-helical structures or a double-stranded β-sheet.

Once one comes to accept that phospholipids are randomly distributed at the surface, the only possible placement of the protein is between the head groups of phospholipids. Such a disposition excludes to a large extent the possibility of considerable intersegmental interactions. In fact, the actual construction of the space-filling model of HDL_3 proved to be unexpectedly easy, and the apoprotein molecules could be placed on the available surface in a quasi-infinite number of ways, without leaving large forbidden areas and without inducing unreasonable twists in the peptide chains. Selected areas of the space-filling model are shown in Plates 2A and 2B. In **Fig. 3** we give an overall schematic view of the HDL_3 model. Because of surface irregularities due to the outward projection of amino acid side chains and polar head groups of phospholipids, the definition of the actual diameter of HDL_3 may be difficult. Surface irregularities have also been noted by the techniques of small angle X-ray scattering (23) and electron microscopy (24). Thus the differences in particle size between those studies may only be apparent and attributable to the mode of measurement and the degree of resolution of the techniques employed. In contrast, there is a good correspondence between the size of the core in our model and that by Ohtsuki et al. (24). In **Fig. 4** we give a crude conceptual representation of one of the possible ways in which two apoA-I molecules occupy the HDL_3 surface. On this figure the entire HDL_3 surface is represented on a single plane with the help of an equal-area projection. Because of the limitations of the single-plane projection, the relative surface areas occupied by the apoproteins and lipids are only approximate.

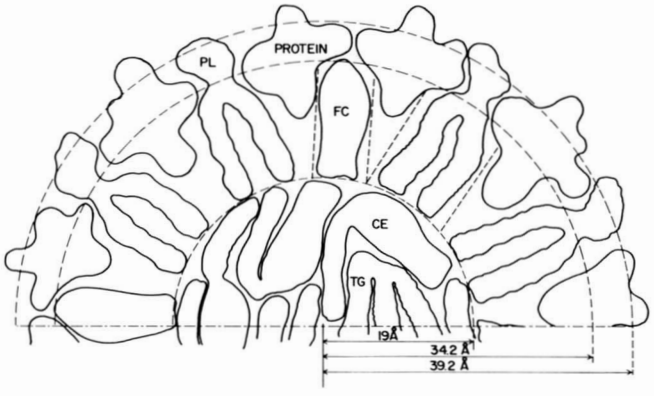

Fig. 3. Structural model of human HDL_3. The dimensions of all components were derived from the space-filling atomic models. The semicircles, from inside to outside, represent the boundary of the hydrophobic core, the midpoint of the protein monolayer, and the outer surface of the HDL_3. The broken lines radiating from the surface of the core represent the boundary of the volume occupied by either free cholesterol or phospholipid molecules in the outer monolayer. PL, phospholipids; FC, free cholesterol; CE, cholesteryl ester; TG, triglyceride. Note the surface irregularity due to the outward projection of amino acid side chains and polar head group of phospholipids from the dotted line defining the outer surface.

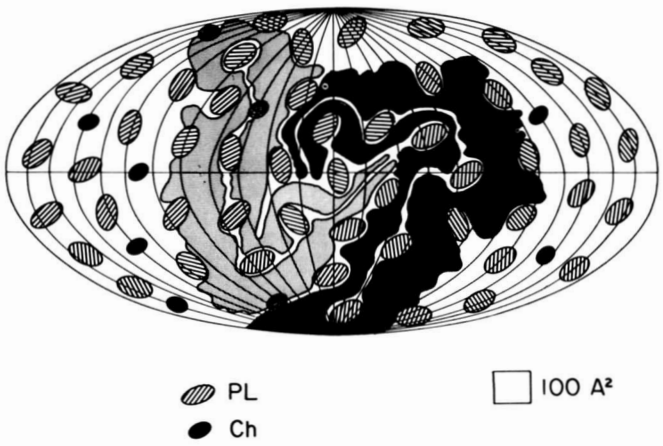

Fig. 4. Schematic cartographic view of HDL_3 (equal-area projection). The entire surface area of HDL_3 is unfolded into a single plane. One of the possible types of surface organization of two apoA-I molecules is sketched out. In the area occupied by apoA-I, the hydroxyl group of cholesterol is beneath the polypeptide chains. ApoA-II is not represented. PL, phospholipid; Ch, cholesterol.

DISCUSSION

The present studies have shown that, if semiempirical guidelines are followed, it is possible to construct a space-filling model of HDL_3 that conforms with both theoretical and experimental findings. The salient features of the model are as follows. *1*) The surface of the HDL particle is tightly packed with apoproteins and phospholipid head groups, thereby segregating hydrophobic structural units from the aqueous environment. *2*) The phospholipids are distributed randomly at the lipoprotein surface and interact neither with themselves nor with the apoproteins in any specific way. *3*) The best packing arrangement for the cholesterol head groups is a conformation in which the alcohol function is shielded from the solvent by the helical polypeptide segments. *4*) The helical structure of the apoproteins is ideally suited to occupy the wedge-shaped gaps created by the large curvature of the HDL particle and by the disposition of phospholipids. *5*) The random distribution of the phospholipid head groups does not favor specific tertiary or quaternary structure of the apoproteins.

From these structural features one can draw conclusions concerning the forces responsible for the overall structural organization of the lipoprotein particle. The information regarding the apolipoproteins is the most revealing. The predictive secondary structural analyses presented in Fig. 1 indicate that, in apoA-I, the amphiphilic helix is the predominant secondary structure at the interface. In apoA-II, the helical segments appear to have a comparatively lower stability, followed in decreasing order by apoC-II and apoC-III (shown in **Table 3** for comparison). ApoC-I is rather similar to apoA-I; it is, however, a minor constituent of HDL_3, and its contribution, if any, to lipoprotein stabilization must be minimal. By estimating the stability of the α-helical structure in an amphiphilic environment, we stressed the lyophilic interactions of the amino acid side chains and gave less emphasis to other types of interactions, e.g., electrostatic, whether occurring within each helix or with phospholipids. In other words, we propose that the stability of the lipoprotein surface is predominantly due to the intrinsic amphiphilicity of the surface molecules and not to group-specific interactions among them. This may not be the case when apoproteins and phospholipids interact in aqueous solutions. In the formation of "discs" (7), for example, the predominant stabilizing forces may be different from those in the intact lipoprotein. Thus, experiments on protein–phospholipid interactions in solution may not be directly relevant to an understanding of the organization of the lipoprotein surface.

TABLE 3. Average differential energy of stabilization of α-helix with respect to random coil at the hydrophobic–hydrophilic interface

Protein	$\Sigma(\Delta F \times \Delta P)/n$, kcal/mol of amino acid
A-I[a]	0.15 ± 0.01 ($n = 7$)
A-II	0.11 ± 0.02 ($n = 2$)
C-I	0.16 ± 0.03 ($n = 2$)
C-II	0.09 ± 0.02 ($n = 3$)
C-III	0.08 ± 0.005 ($n = 2$)

[a] α-Helix #7 (192–211) is not included in the calculations because of the ambiguity of its assignment. n, number of helices; ΔF, differential free energy of transfer; ΔP, differential α-helical potential.

From our analysis, it appears that the apoproteins examined have a two-dimensional amphiphilicity and that this property is an important stabilizing force in lipoproteins. However, this conclusion does not readily account for the structural and functional specificity of these apoproteins. For instance, in the case of the major HDL apolipoproteins, the types of particles generated by apoA-I or apoA-II, when they are incubated with HDL lipid, differ significantly from one another (25, 26). Hybridization studies of canine HDL, which contained only apoA-I, with human lipid-free apoA-II have also shown that apoA-II has a greater affinity than apoA-I for the HDL_3 surface (27). Can our analyses provide criteria for such specificity? For instance, in the case of apoA-I these analyses indicate that the helical segments cannot be much longer than 20 amino acids, since the peptide chain, in order to be accommodated on the surface of HDL, must follow the curvature of the particle. Moreover, our predictive analyses require that the helical segments must be linked by flexible, short, interconnecting segments which are themselves amphiphilic. None of the other apolipoproteins examined thus far meet these requirements. Hence, it would appear that apoA-I has an essential role in HDL structure, at least under normal, steady-state conditions. This conclusion is indeed supported by the observation (6) that, in all animal species examined thus far, the HDL's within the 100 Å diameter range contain apoA-I as their most important constituent. HDL species outside the 100 Å range have been described, such as the so-called HDL_C. This lipoprotein contains arginine-rich peptide as its predominant apoprotein (8) and thus may exhibit a structural organization different from that discussed in the present work in regard to HDL containing apoA-I. Then the question arises whether this specificity of apoA-I for HDL is due to the structure of the apoprotein, the structure of the lipoprotein, or to both. A useful piece of information which is obtained from our model is that the average distance

between the phospholipid head groups (D) is 11 Å. In general, D is determined by the curvature of the particle, as approximated by the relation

$$D = \frac{9.3r}{r - 20.2} - 8.9. \qquad \text{Eq. 4}$$

where r = 39 Å for HDL_3. The value of D obtained for HDL_3 corresponds roughly to the average Van der Waals diameter of the apolipoprotein helices. We mentioned earlier that this close fitting does not imply specific interactions between apolipoprotein side chains and phospholipids, but that it is a consequence of the intrinsic properties of each of the constituents at the lipoprotein surface. The preceding considerations appear to provide a reasonable mechanism by which apoA-I could fulfill its role of unique size-determining factor in HDL.

The close interplay between HDL_3 curvature and the surface organization of both apoproteins and polar head groups of phospholipids suggests that the basic structural features discussed for HDL_3 can be extended to the other HDL subclasses as well. The variation in the lipid content of these various lipoproteins should result only in a slight increase of the diameter of the hydrophobic core, and in a corresponding increase of the surface molecules, leading to a surface monolayer with the same structure and packing as in HDL_3. It is tempting to extend these considerations to LDL and to lipoproteins of lower density, such as chylomicrons and VLDL. Unfortunately, predictions for these lipoproteins must await clarification of the primary structure of apoB. We must stress, however, that the lipoproteins of lower densities have an extremely broad size distribution which probably reflects the absence of stringent size-determining factor(s). It is likely that lipids, rather than apoproteins, are the molecules that play a dominant stabilizing role in these particles.

In conclusion, by a combination of theoretical considerations and the actual construction of a space-filling model, information can be derived concerning the structural organization of serum lipoproteins. Whereas previously model-building has been utilized to yield structural information on rigid, well-defined, three-dimensional structures, such as globular proteins and nucleic acids, it is evident that it has a justified place in the study of fluid noncovalently bound molecular assemblies with a complexity comparable to that of plasma lipoproteins.

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