MOLECULAR PACKING OF HIGH DENSITY LIPOPROTEINS: A POSTULATED FUNCTIONAL ROLE

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1. Introduction

A molecular model has been proposed for protein-lipid association in the plasma lipoproteins [1] that is based on the available amino acid sequence of plasma apolipoproteins [2–5] and the knowledge that lipid-free apolipoproteins recombined with phospholipid demonstrate an increase in α-helical structure as measured by circular dichroism [6]. According to the model, specific phospholipid-associating sites along the polypeptide chains of the apolipoproteins are α-helical. Each helical domain, termed an amphiphathic helix, is longitudinally divided into polar and nonpolar faces, and a specific distribution of charged residues is evident along the polar face. Amphipathic helixes, as defined, are complementary for the polar–nonpolar interface of hydrated bulk phospholipid; these lipid-associating domains have been postulated to interact with the phospholipid by partially immersing themselves at the interface between the fatty acyl chains and the polar head groups.

The predicted helical lipid-associating domains have proved consistent with the total helicity and location of known lipid-binding sites for each apoprotein [6], including apo A-I, whose sequence was determined after the publication of the theory [7]. Recently a computer analysis has supported the two basic features of the model: a hydrophobic face and a collection of complementary, topographically-close ion pairs on the polar face [8]. Further, the computer study predicted that amyloid A, a pathologically occurring protein of unknown origin and function, is associated with an amphipathic helical lipid-associating domain. Subsequent lipid binding studies with amyloid A have supported this prediction [9].

NMR studies by several investigators, especially those of Stoffel et al. [10] and Assman and Brewer [11], have challenged the amphipathic helix theory on the grounds of an inability to demonstrate electrostatic association. Stoffel et al. [10], utilizing ^{13}C spin lattice relaxation measurements (T_1) of ^{13}C-enriched lipids, suggested that phospholipid molecules associate with apolipoproteins in reassembled high density lipoproteins (HDL) via interaction of their fatty acyl chains but not their polar head groups. This interpretation was based on the observation that T_1 for the polar head groups did not change significantly after protein–lipid association, whereas T_1 for select portions of the fatty acyl chain clearly decreased [10].

The purpose of the present communication is to demonstrate that molecular mobility of the terminal 80% of the fatty chains of a given phospholipid molecule depends entirely upon the packing of the lipid molecules, particularly cholesteryl ester, in the high density lipoprotein (HDL) particle. On the other hand, the mobility of the polar head group and the first few methylene groups of the fatty acyl chains is substantially affected by protein–lipid associations. Based upon this demonstration, it is suggested that in HDL (a) the contribution of electrostatic forces to protein–lipid interactions varies with the nature of the lipid packing, and (b) the reversibility of amphipathic helix–phospholipid association (predominantly involving apo A-I) is the means of controlling the packing density of the polar phospholipid head groups. The packing density of the polar phospholipid head

Abbreviations: HDL, high density lipoprotein; VLDL, very low density lipoprotein; T_1, spin lattice relaxation time; AI and AII, major apoproteins of HDL.

groups in HDL, in turn, is related to the surface free energy of the particle; the surface free energy controls the rate of exchange of phospholipid and cholesterol between lipoproteins and cell membranes.

2. Experimental

Consideration of the packing of lipid and protein molecules in HDL particles requires that the geometric parameters of each molecular species be defined. A compilation of approximate numbers and molecular dimensions for phospholipid, cholesterol, and cholesteryl ester is given in table 1. The two forms of HDL (HDL_2 and HDL_3) are assumed to be spheres with diameters of 115 Å and 85 Å, respectively [12,13].

The analytical tool for evaluation of HDL molecular packing is the freedom parameter (f) defined as:

$$f = \left[\frac{A(r_i)}{A^*(r_i)}\right] - 1$$

where

$A(r_i)$ = total cross-sectional area of the i^{th} spherical shell with radius r_i centered on the HDL particle.

$A^*(r_i)$ = the sum of molecular van der Waals cross-sectional areas cut by the i^{th} spherical shell with radius r_i.

By definition, if $A^*(r_i) = A(r_i)$, $f = 0$; i.e., space available for molecular motion in the spherical shell r_i is equal to zero. Since molecular cross sections as a first approximation can be considered circular, maximal packing density will result in an f greater than zero. As a working approximation, molecular motion will be defined as zero (i.e. $f = f_0$) in the state of close square packing. Assuming the presence of circular van der Waals cross sections with identical diameters equal to r and packed in a close square array. then $A(r_i) = (2r)^2 = 4r^2$, $A^*(r_i) = \pi r^2$, and $f_0 = (4/\pi - 1) = 0.27$.

3. Results and discussion

On the basis of experiments with real and artificial membranes [14], cholesterol and phospholipid have been postulated to form a complex in which the hydroxyl of cholesterol is hydrogen bonded to an ester carbonyl of the fatty acyl chains of phospholipid; the steroid ring would be oriented parallel to the fatty acyl chains (fig.1A and 1B). This manner of cholesterol

Table 1
Approximate molecular parameters for components of high density lipoprotein[a]

Parameter[b]		Components				
		Phospholipid		Cholesterol	Cholesteryl ester	Amphipathic helixes
Number	HDL_2	147		44	90	40–50
	HDL_3	59		19	37	25–35
Length		30 Å		19 Å	40 Å	15–20 Å
Cross sectional diameter		–		–	–	14 Å
Cross sectional area						
	Head	55 Å^2	Hydroxyl end	20 Å^2	–	–
	Acyl chain	20 Å^2	Steroid ring middle	50 Å^2	–	–
			Tail	25 Å^2	–	–

[a] HDL_2 = 115 Å diameter; HDL_3 = 85 Å diameter.
[b] From Verdery and Nichols [17] and measurements of CPK space filling models. There are assumed to be from 8–12 amphipathic helixes per molecule of apo A-I and 2 amphipathic helixes per apo A-II [5,6].

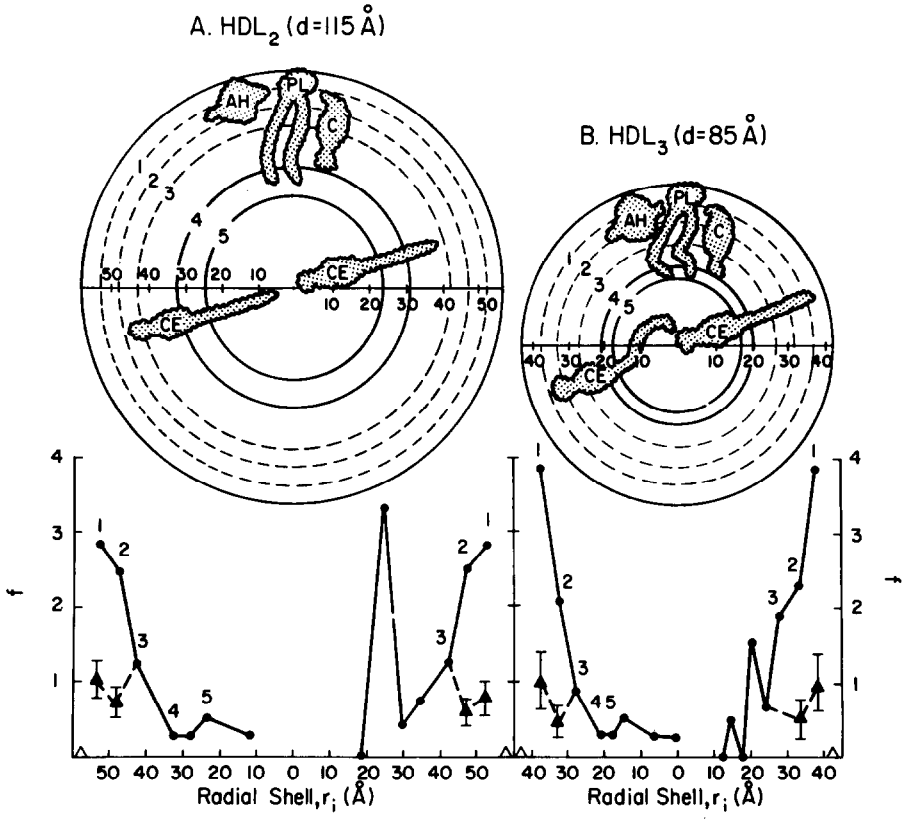

Fig.1. Plots of the freedom parameter ($f = A(r_i)/A^*(r_i) - 1$, see text) versus r_i for HDL_2 and HDL_3 using the molecular parameters for the constitutents of HDL listed in table 1. The plots on the left side of each graph are for cholesteryl ester oriented steroid-ring outward, the plots on the right of each graph for cholesteryl ester oriented steroid ring inward. (●——●) freedom parameter for lipids alone; (▲ – –▲) freedom parameter for lipids plus protein in the form of amphipathic helixes; AH, amphipathic helix; PL, phospholipid; C, cholesterol; CE, cholesterol ester; radial position 4, minimal overlap shell; radial position 5, minimal volume shell.

phospholipid association will be assumed also to hold for HDL. It is further assumed that the basic structure of HDL is that of a micelle, with the phospholipid polar head groups oriented outward and the center of the micelle occupied by cholesteryl ester molecules (fig.1A and 1B). This basic model for HDL has been suggested by a number of different laboratories on the basis of X-ray diffraction studies [15], molecular packing models [6,10,11,16–18], and thermodynamic considerations [18]. The basic uncertainty in HDL structure concerns the orientation of the cholesteryl ester and the apoprotein molecules.

In HDL, cholesteryl ester has three possible orientations. As an extended molecule, cholesteryl ester could be oriented with its steroid ring pointed outward and the fatty acyl chains at the HDL center or, in a reverse position, with the steroid ring at the HDL center. As a third possibility cholesteryl ester could exist in a compact folded conformation; because of a bulkiness of the compact conformation, the cholesteryl ester would likely exist in this form for only a small percentage of the time, e.g. mostly during synthesis and catabolism of the molecule [17,18].

The mean freedom parameter for an individual phospholipid molecule in HDL_2 and HDL_3 (containing only the lipid components) has been plotted in fig.1A and 1B as a function of r_i for the two extended orientations of cholesteryl ester. The range of radial positions available to a particular segment of each lipid molecule is narrow because of volume restrictions

Thus the assigned position for any given segment of a lipid molecule is considered to be a mean radial position.

As seen from fig.1, the steroid ring does not pack well with cholesteryl ester in the ring-inward orientation. Therefore, such an orientation for this molecule is considered an unlikely model for HDL packing.

For both forms of HDL, the radius of the minimal volume sphere that will encompass the fatty acyl chains of the cholesteryl ester molecules in the ring outward orientation and the radius of the minimal van der Waals shell allowing overlap of phospholipid fatty acyl chains with the cholesteryl ester molecules have been indicated in fig.1. The concept of the overlap shell is derived from the assumption that the junction between the acyl and steroid ring portions of every cholesteryl ester molecule usually overlaps the methyl end of every phospholipid fatty acyl chain. The $A(r_i)$ for this overlap shell (and subsequently r_i) is calculated assuming a minimal freedom parameter equal to $[4/\pi] - 1$. The radius of the minimal volume and overlap shells determines the innermost, average, radial extent allowable for the cholesteryl ester rings. Because of the smooth f plot for ring-outward packing, this orientation for cholesteryl ester is favored for HDL_2 and HDL_3.

The polar head group of an individual phospholipid molecule in the outer leaflet of a 250 Å single bilayer liposome can be calculated to have an f of approximately 0.9. (Each liposome contains approximately 2500 phospholipid molecules, and a phospholipid ratio of 7/3 between the outer and inner liposomal leaflets [19,20].) In fig.1, the f plots for a given phospholipid molecule in HDL_2 and HDL_3, ignoring packing contributions by apoprotein, indicate that HDL phospholipid head groups have an enormously high potential f. The NMR data of Stoffel et al. [10] and Assman and Brewer [11] indicate that little difference exists between the T_1 of polar head groups of phospholipid in sonicated liposomes versus reconstituted HDL. Therefore, one is compelled to conclude that freedom at the HDL surface is markedly restricted relative to the liposomal surface and that this restriction must be produced by apolipoprotein. Further, the only potential volume available for protein–lipid association in HDL is in the outer 10–15 Å portion of the HDL particle (fig.1).

To plot f versus r_i for intact HDL, it has been assumed that all protein–lipid associations involve amphipathic helical domains of HDL proteins AI and AII [1,6,8,17]. Table 1 gives assumptions concerning the number and dimensions of the amphipathic helixes. Because of some uncertainty concerning the length and number of these presumed lipid associating domains of AI and AII, ranges are estimated for each [1,6,8,17]. Figure 1 shows the effect on f of association of amphipathic helixes with the HDL lipids. The changes in f are limited to the outer 10–15 Å segment of each HDL particle. The polar head group of an individual phospholipid molecule in both forms of HDL has a freedom parameter comparable to phospholipid head groups in liposomes ($f \simeq 0.9$). On the other hand, the f for the first several carbons of the phospholipid fatty acyl chains of the phospholipid is low in both HDL_2 and HDL_3, suggesting restrictions in mobility. These predictions are supported by [^{13}C]-NMR studies of Hauser [21].

Single bilayer liposomes have the maximal spacing of phospholipid head groups ($f \simeq 0.9$), and thus maximal surface free energy, that is compatible with stability [22]. A further increase in spacing is energetically unfavorable because of the increased exposure of water to underlying fatty acyl chains [22]. The similarities of the freedom parameters of phospholipid head groups in HDL and liposomes indicate small likelihood of a tight electrostatic interaction between the head groups and amphipathic helixes in the intact lipoprotein since the surrounding water would be competing for charged groups. However, there is no reason to assume that electrostatic interactions would not be important under conditions in which low concentrations of amphipathic helixes are interacting with phospholipid bilayers. Microcalorimetry experiments showing a large negative enthalpy of association at low protein–lipid ratios support this possibility [23,24]. Electrostatic interaction thus might be important in initiation of protein–lipid interactions (e.g., in the synthesis of nascent HDL [18]) but relatively less important in the mature HDL particle.

Finally, it is clear from fig.1 that the freedom parameter of phospholipid head groups in HDL will be sensitive to the number of amphipathic helixes buried in the outer, 10–15 Å thick, surface volume. This suggests a mechanism for controlling the rate of exchange of phospholipid and cholesterol between individual lipoprotein particles and between HDL and

cell membranes. Interaction of HDL with receptors (located on other lipoproteins or on membranes) might produce a conformational change in AI and/or AII that would cause disassociation of a number of weakly bound amphipathic helixes from the surface of HDL. A self-aggregation tendency noted for AI and AII in solution [25–28] might be important in controlling helix association–disassociation [18] by modulating the free energy differences between apolipoprotein in the lipid-free and lipid-bound states. A 50% reduction in amphipathic helixes bound to HDL_2 would increase the f of the phospholipid head groups to 1.8. HDL_3 would be even more sensitive to helix disassociation ($f = 2.4$). The net result of helix disassociation would be an increase in surface free energy resulting in an instability in the HDL surface structure. This instability would potentiate phospholipid and cholesterol exchange between lipoproteins (including VLDL and chylomicrons) and membranes. Based on the greater sensitivity of HDL_3 to helix dissociation (fig.1), it seems likely that HDL_3 would play a more important role in lipid exchange (and/or fusion) than HDL_2.

A role for amphipathic helix-containing apolipoproteins in the disruption of membranes has been discussed in detail elsewhere [18]. The postulated role for apolipoproteins in controlling lipid exchange (and/or fusion), as well as membrane disruption, is supported by preliminary work underway in another laboratory (A. Tall, personal communication). Incubation of liposomes with intact HDL particles results in bilayer disruption apparently due to movement of A-I protein from the HDL particles to the liposomes. The resultant loss of A-I from HDL produces enlargement of the HDL particles, presumably due to either phospholipid uptake from the liposomes (asymmetric exchange) or self-fusion of the HDL particles. The role of A-I in modulation of lipid exchange and fusion is also supported by work by Nichols et al. [29] in which quanidine-HDL results in self-fusion of the A-I depleted HDL particles.

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