HIGH-DENSITY LIPOPROTEIN RECOMBINANTS: EVIDENCE FOR A BICYCLE TIRE MICELLE STRUCTURE OBTAINED BY NEUTRON SCATTERING AND ELECTRON MICROSCOPY

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1. Introduction

Apo A-I, the major protein of HDL, interacts spontaneously with hydrated dimyristoyl phosphatidylcholine (DMPC) to form complexes smaller than 200 Å in greatest dimension [1,2]. These A-I : DMPC recombinants have been suggested on the basis of negative stain electron microscopy [3] and low angle X-ray scattering [4] to be discoid in shape. Nascent HDL synthesized in the liver [5] and gut [6], and also the mature HDL of patients with the heritable disorder lecithin : cholesterol acyl transferase deficiency [7], all are discs of similar size and shape. These observations suggest that such a particle may be an intermediate in the formation of mature, spherical HDL. Based on thermodynamic considerations and differential scanning colorimetry, Segrest [8] and Tall et al. [9], respectively, have suggested that the A-I : DMPC recombinants represent unilamellar bilayer discs whose otherwise thermodynamically-unstable edges are lined by amphipathic helical domains [18] of apo A-I. In this study we analyze the size, shape and structure of the recombinants by a combination of Guinier region neutron scattering, electron microscopy and column chromatography.

Abbreviations: HDL, high density lipoprotein; apo A-I, major apoprotein of HDL; d-DMPC, dimyristoyl phosphatidylcholine deuterated in fatty acyl chains; h-DMPC, hydrogenous dimyristoyl phosphatidylcholine

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In the last five years, low-angle neutron scattering has been used to study a number of macromolecules in solution. In almost all cases, including the studies of low density lipoprotein [10], chromatin [11], ferritin [12] and ribosomes [13], contrast variation was achieved by measurement of scattering in solutions containing variable ratios of H_2O to D_2O. These measurements have two major drawbacks. First, the degree of hydrogen exchange differs with the ratio; second, incoherent scattering by hydrogen atoms contributes high background, thus decreasing the effective sensitivity of measurement. To circumvent these problems, we changed contrast by using deuterated (d-DMPC) and hydrogenated (h-DMPC) lipids to form recombinants, and did all of the scattering experiments in D_2O.

2. Experimental

h-DMPC was purchased from Avanti Biochemicals and was shown to be pure on the basis of thin layer chromatography (100 μg/spot) and high resolution differential scanning colorimetry (Microcal MC-1). d-DMPC was purchased from Serdary Research Laboratories but required extensive further purification by silica gel chromatography, with a final yield of 30 mg from a starting 200 mg.

HDL was isolated by sequential density floatation [14]. Apo A-I was isolated from apo HDL by Sephadex column chromatography in 0.01 M Tris/HCl, 8 M urea, pH 8.5, as described previously [15].

This preparation gave a single band by urea polyacrylamide gel electrophoresis.

The apo A-I was dissolved in 0.01 M Tris, 0.15 M NaCl, pH 9.0 in D_2O at a concentration of 15.5 mg/ml, as measured by Lowry [16]. A-I solution was added to lyophilized h-DMPC or d-DMPC to give a DMPC : A-I molar ratio of 40 : 1, and the A-I : lipid mixtures were then incubated at 23°C for 12 h. The resulting optically clear solutions were used for column chromatography, neutron scattering studies, and electron microscopy.

A-I : DMPC recombinants, sonicated DMPC, and free A-I were chromatographed on Sepharose 6B equilibrated with the 0.01 M Tris, 0.15 M NaCl, pH 9.0 buffer. The recombinants were then negatively stained with 2% phosphotungstate [17] and examined at a DMPC concentration of 0.1–0.5 mg/ml with a Philips 400 transmission electron microscope.

Neutron scattering data were collected using a low-angle diffractometer at the National Bureau of Standards reactor, operated at 10 MW. Neutrons were moderated to room temperature, passed through a liquid nitrogen-cooled 25 cm beryllium–10 cm lead filter and monochromatized by a helical velocity selector. A wavelength of 5.85 Å was chosen, with $\Delta\lambda = 1$ Å. Samples of 0.2 ml were placed in sealed quartz cuvettes and the temperature was kept at 30°C by a circulating water bath. Measurement time for each spectrum ranged from 12 to 48 h. Spectra were corrected for background and slit geometry. All calculations were done on a VAX 11/780 computer.

3. Results and discussion

The A-I : DMPC recombinants eluted on Sepharose 6B in an intermediate position between sonicated DMPC vesicles (void volume) and free A-I (fig.1). No large recombinant particles or unreacted vesicles were seen, but there was a small amount of free A-I, reflecting the reversibility of the A-I : DMPC interaction [13].

Negative stain electron microscopy of the 40 :1 (M : M) recombinants showed discoid structures (see fig.4). The h-DMPC : A-I recombinants measured 99 ± 9 Å (standard deviation, $n = 150$) in diameter and 32 ± 3 Å ($n = 50$) in thickness; the d-DMPC : A-I recombinants measured 98 ± 10 Å ($n = 150$) by 33 ± 5 Å ($n = 50$).

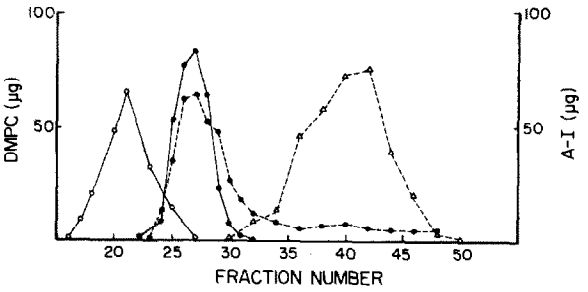

Fig.1. Gel filtration of sonicated DMPC vesicles, A-I : DMPC recombinants, and free apo A-I on Sepharose 6B: (○——○), sonicated DMPC vesicles; (●——●), DMPC in A-I : DMPC complex (1 : 40 M : M) measured by counting trace ^{14}C-DMPC; (●——●), apo A-I in A-I : DMPC complex measured by Lowry [16]; (△ - - - -△), free apo A-I measured by A_{280}. Concentrations in each experiment were 5 mg of protein and/or 5 mg lipid.

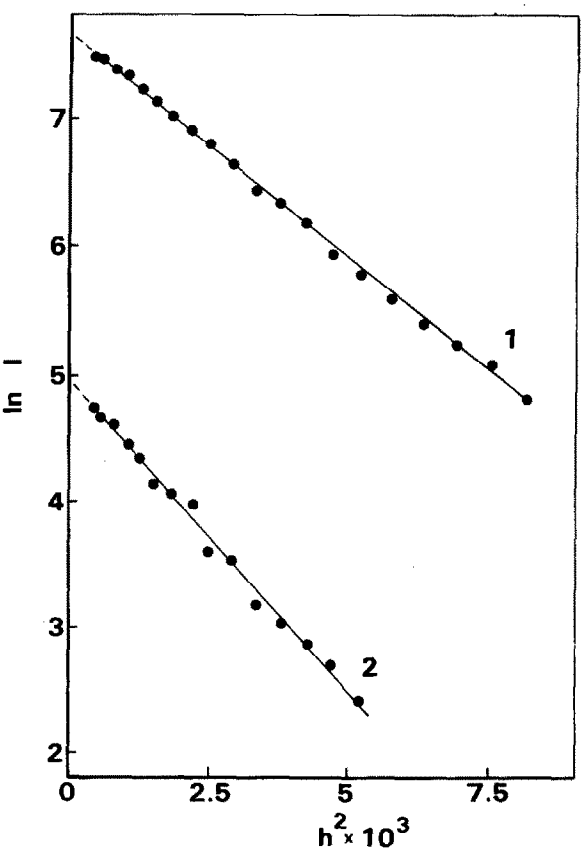

Fig.2. Guinier plots for apo A-I recombinants containing h-DMPC (curve 1) and d-DMPC (curve 2). Intensity is measured in arbitrary units, but is scaled for the two samples. $h = 4\pi \sin\theta/\lambda$. Concentrations of recombinant were 24 (curve 1) and 30 mg/ml (curve 2).

In the first neutron scattering experiments, h-DMPC : A-I recombinants were studied at concentrations of 5.75, 11.5, and 23 mg dry weight lipid per ml. Calculation of the radii of gyration showed no concentration dependence, within experimental error.

Figure 2 shows Guinier plots of low-angle scattering from h-DMPC and d-DMPC recombinants. Radii of gyration calculated from the slopes were 33.5 ± 0.2 Å for h-DMPC and 38.0 ± 0.5 Å for d-DMPC. The normalized square roots of zero-angle intensity were 3.8 and 1.0, respectively. The electron microscopic studies indicate that this difference in radii of gyration cannot be explained simply by different diameters of the h- and d-particles ($P < 0.01$, using the delta method [24] for estimation of variances to obtain a *t*-test on log-transformed ratios of the measurements).

Data obtained from the Guinier region cannot determine uniquely the structure of particles. However, their consistency with other data may be examined using models based on geometric parameters and the scattering densities of the components. A-I : DMPC discs may be described in terms of three parts, with the scattering density of each assumed homogeneous. The lipids have no readily exchangeable hydrogens, and the average scattering densities calculated for their non-polar parts are -0.08×10^{-14} cm/Å^3 (h-DMPC) and 7.3×10^{-14} cm/Å^3 (d-DMPC). The scattering density of the polar head group is 0.76×10^{-14} cm/Å^3 in each case.

The scattering density for a protein is more difficult to calculate because of uncertainties in hydration and hydrogen exchange. We arrived at the value to be used here by considering the scattering intensity at zero angle. The square root of intensity is proportional only to the sum of scattering lengths of all atoms in the complex, less the sum for displaced solvent molecules; it is not influenced by the distribution of atoms. If the level of hydration of protein is 0.4 g/g [4], it follows that the volume fraction of protein in the complex is 0.5, that of non-polar part of the lipid is 0.375, and that of the polar lipid is 0.125. With these values, the scattering density of A-I can be calculated from the zero angle intensity as 5×10^{-14} cm/Å^3. This corresponds to exchange of 30% of hydrogen atoms for deuterium, in good agreement with a figure of 25% obtained from the amino acid composition.

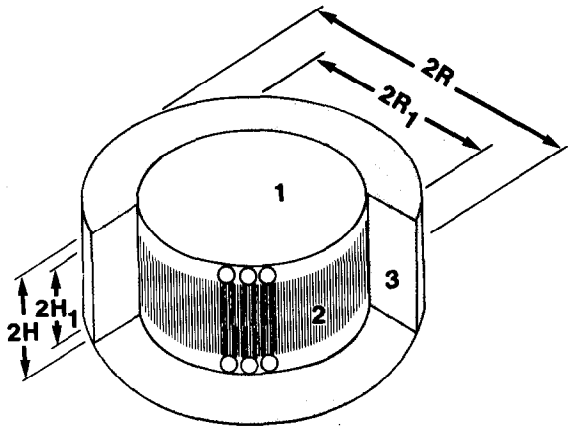

Fig.3. A model for apo A-I : DMPC discoidal recombinants. Region 1 is occupied by DMPC polar head groups, region 2 by DMPC fatty acyl chains, and region 3 by apo A-I. Detailed discussion in text.

The larger radius of gyration for d-DMPC (in which the scattering density of the non-polar lipid closely resembles that of D_2O) clearly indicates that the protein resides in the outer part of the complex. Using the above values for the volume fractions and scattering densities of the components, we searched for model structures in best agreement with the experimental radii of gyration. We considered models based on spherical, spheroidal, and cylindrical geometries with either three separate scattering regions or protein uniformly dispersed [4] throughout the lipid polar head regions. Predicted dimensions for the deuterated and hydrogenous recombinants were in best agreement for a three-component cylindrical model (fig.3) with R = 49 Å and H = 16.3 Å (R_1 = 34.6 Å, H_1 = 10.9 Å). Good agreement was not obtained with spherical or mixed protein–lipid models. The best spheroidal model had dimensions similar to those of the optimal cylindrical one, but the fit was not as good. Thus the AI : DMPC recombinant can be approximated by a disc with diameter of 98 Å and height of 33 Å, in excellent agreement with the dimensions determined by negative stain electron microscopy. In this model the A-I protein forms an outer rim 15 Å thick, the polar DMPC head groups are on the top and bottom and the non-polar fatty acid chains are buried inside. This is the bicycle tire micelle, first suggested by Segrest [8] and Tall et al. [9].

High resolution negative stain electron microscopy shows two additional features compatible with the bicycle tire model. First, when the discoid particles orient with their flat faces parallel to the plane of the grid, contrast rims can be seen around the perimeters of the discs (fig.4). Second, examination of discs oriented edgewise (the usual manner of orientation) reveals two parallel electron-lucencies running the length of the disc rim. These parallel lucencies are each approximately 16 Å thick. As evidence against artifact, both features are independent of the grain size and level of focus; however, artifact cannot be ruled out.

The electron microscopic features and neutron scattering data are compatible with a molecular model consisting of a unilamellar DMPC disc 68 Å in diameter ($2R_1$), whose rim is lined with A-I. The dimensions of the protein rim ($R-R_1$ = 15 Å; $2H$ = 33 Å) indicate two A-I molecules per recombinant, consistent with cross-linking studies reported

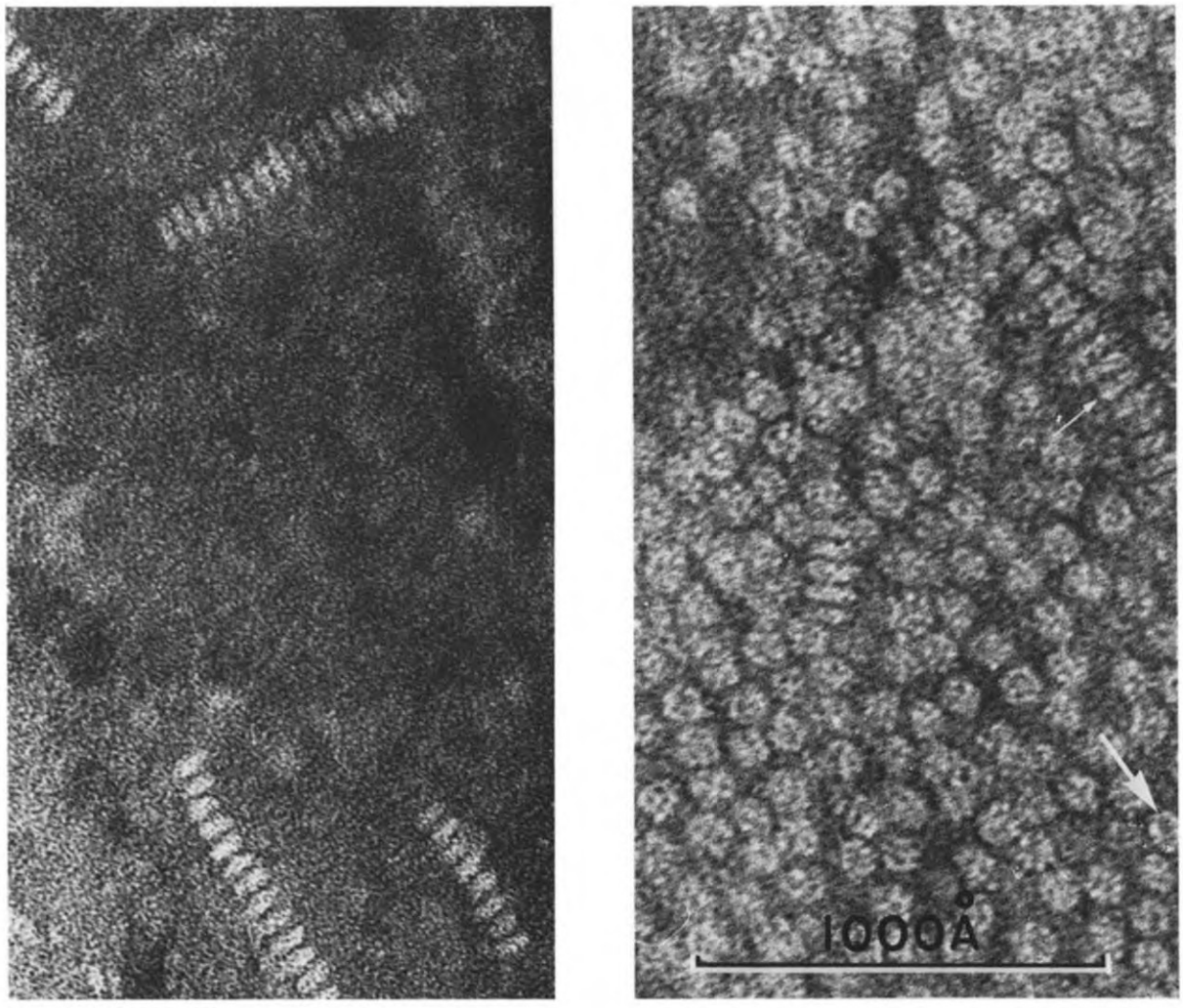

Fig.4. Negative stain electron microscopy at approximately 400 000 × of apo A-I : DMPC recombinants (1 : 40 M : M); (left) d-DMPC; (right) h-DMPC. Discs preferentially orient edge on (small arrow on right). Occasionally discs are seen oriented face on (large arrow on right). The latter orientation has been observed in d-DMPC, as well as h-DMPC, recombinants.

by Swaney [22]. The A-I could form a pair of parallel amphipathic helixes wrapped around the circumference of the disc; alternatively, 22-residue antiparallel amphipathic helix segments [23] might be oriented perpendicular to the plane of the disc, as suggested by Tall et al. [13]. Given its characteristic distribution of positive and negative ion pairs [18], the amphiphatic helix has the potential to associate with adjacent helixes, either in a parallel or an antiparallel configuration, via lateral electrostatic interactions.

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