X-RAY SMALL ANGLE SCATTERING OF HUMAN PLASMA HIGH DENSITY LIPOPROTEIN LpA FROM HDL_2: APPLICATION OF A NEW EVALUATION METHOD

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1. Introduction

In their recent study, Shipley et al. [1] presented data on the structure of HDL_2 as obtained from X-ray small angle scattering. The results were in good qualitative agreement with the results of our previous studies on LpA from HDL_3 [2, 3] indicating a common molecular architecture for these two species of human plasma high density lipoproteins. However, the proposed model for HDL_2 was based only on comparison of theoretical and experimental scattering curves, obtained from experiments in solvents of one single electron density. In this case where a non homogeneous electron density within the molecule must be assumed a priori, the fit of the data is only to be considered as a necessary but not sufficient condition. Therefore we extended our studies on lipoproteins also to the HDL_2 species. This article is a report on the results which were obtained from X-ray small angle scattering of LpA from HDL_2 in different solvents. For the translation of the scattering data into real space information we employed for the first time a novel procedure of Fourier transformation, which greatly reduces the potentially misleading influence of nonidealities in the experimental procedure, especially the termination effect. The results confirm the lipid core model and provide some additional information about the arrangement of the lipids within the molecule.

2. Materials and methods

2.1. *The sample*

LpA from human plasma HDL_2 containing only ApoAI and ApoAII as protein constitutents was prepared according to the procedure described by Kostner et al. [4, 5]. Concentrations were determined by dry weight and dilution series were prepared by mixing with equilibrium solvent (0.15 M NaCl, 0.1% NaN_3). Different solvent electron densities were obtained by exhaustive dialysis against sucrose solutions of various concentrations: 7%, 15%, 25%, 32%, 40% and 65% (w/w). All samples were found to be monodisperse by analytical ultracentrifugation.

Molecular weight, partial specific volume and chemical composition of the samples are given in table 1. Partial specific volume determinations were

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** Appendix.

Table 1
Molecular composition of LpA from HDL_2

	HDL_2
Total protein (% w/w)	42%
Total lipid (% w/w)	58%
Phospholipids	23% (55)
Cholesterol	4% (9.5)
Cholesterol ester	13% (31)
Trigly ceride	2% (4.5)
	58% (100)
Molecular weight (UZ)	3.6×10^5
Molecular weight (XSAS)	3.6×10^5
Partial specific volume 4°C (25°C)	0.903 (0.917) g/cm³)

performed by differential density measurements employing the Digital Density Meter DMA 02/C (Anton Paar, Graz) which allows an accuracy of 2×10^{-6} g/cm³ in aqueous solutions [6].

2.2. *X-ray measurements and data evaluation*

X-ray experiments were performed as described earlier [7, 8] using a Kratky camera and electronic step scanning [9] and counting devices. Monochromatisation was achieved by means of a pulse height discriminator and a subsequent mathematical procedure described by Zipper [10] which eliminates the influence of K_β radiation. The same number of pulses (5×10^5) was counted at each point of the scattering curve within the angular range of

$$8 \times 10^{-3} < h < 4 \times 10^{-1}\ \text{Å}^{-1}\ \left(h = \frac{2\pi}{\lambda} \cdot \sin 2\vartheta\right)$$

During the measurements the sample was kept at a constant temperature of 4°C by use of a Peltier cuvette [11]. The influence of interparticular interference was eliminated by measurements of a dilution series in the small angle region of the scattering curve and extrapolation to zero concentration. Desmearing of the data was obtained by an iterative numerical method recently described by Glatter [12]. Radii of gyration were determined from the Guinier plots log I vs. h^2 [13] (as well as from the electron density distribution and the correlation function).

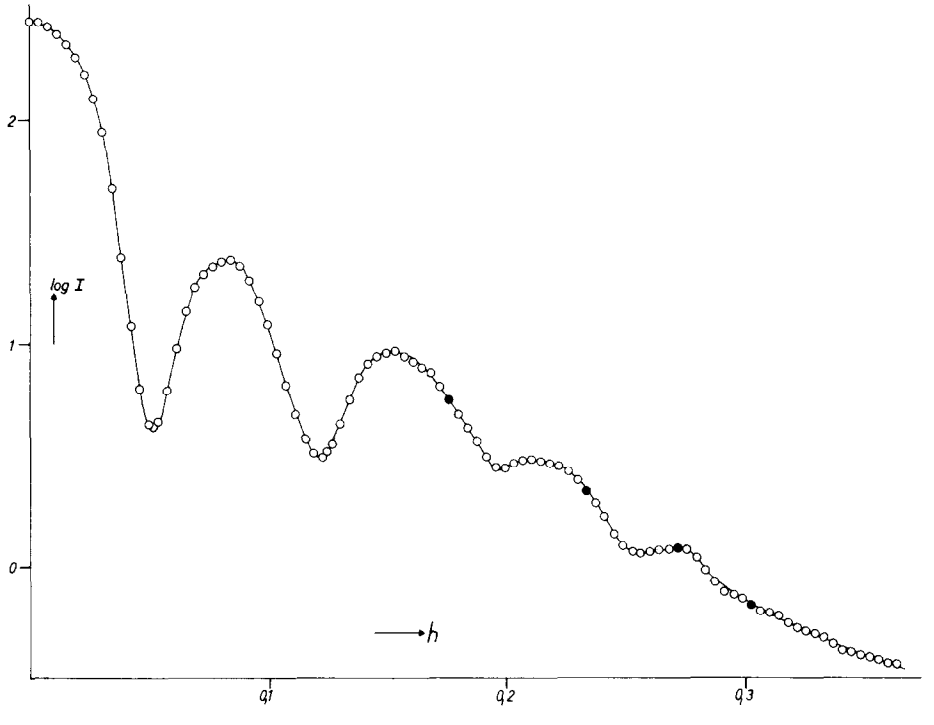

Fig. 1. Experimental scattering curve of LpA from HDL_2 in 0.15 M NaCl, 0.1% NaN_3, desmeared. The filled circles indicate the angular terminations for the Fourier transformation.

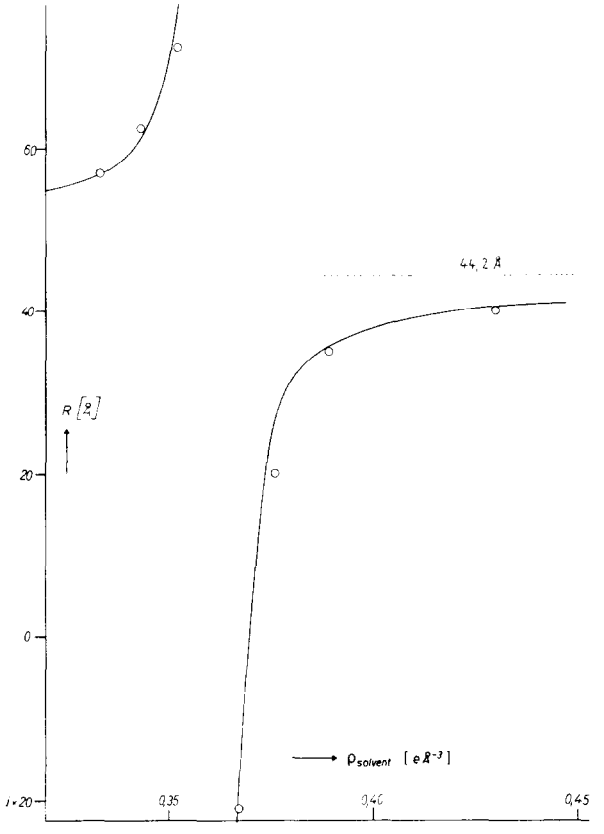

Fig. 2. Dependence of the radius of gyration on solvent electron density. (o) Experimental values; (–) theoretical values for the model resulting from alternating signs of the amplitudes.

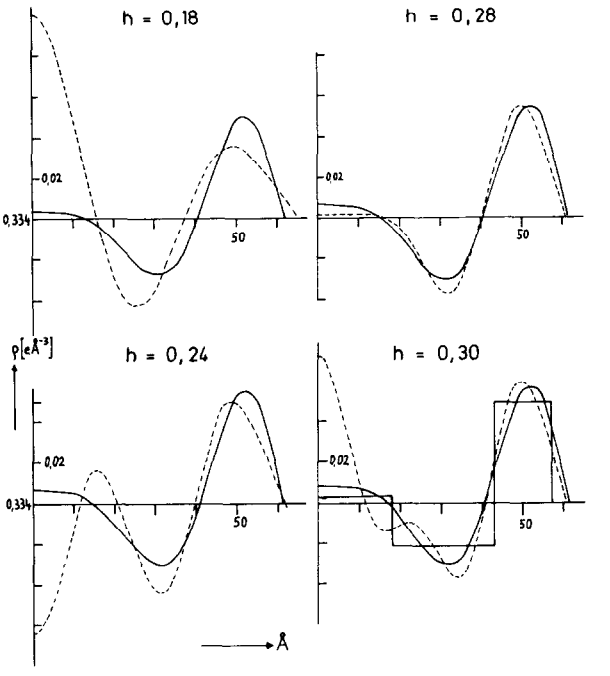

Fig. 3. Comparison of radial electron density distributions as obtained from direct (- - - -) and indirect (——) Fourier transformation with different termination angles. The lower right diagram shows also the correspondent step model.

For Fourier transformations the experimental scattering curve (fig. 1) was extrapolated to zero intensity in the region of the minima and the amplitudes were given alternating signs starting with a positive first maximum due to the net positive electron density of the molecule in 0.15 M NaCl solution. This sign combination was found to be valid by measurements of the radius of gyration in different solvent electron densities and comparison to the theoretical values calculated for the model which results from this assignment (fig. 2).

In order to reduce the obscuring effect of the termination at finite angles we employed a refined method of Fourier transformation [14] which is briefly described in the Appendix.

Apart from the ambiguities arising from different possible assignments of the amplitudes the optimal fit of the experimental and model scattering curve by itself cannot serve as a criterion for the validity of the model. We consider the relative invariance with respect to the termination angle as an additional and strong criterion for the proposal of a model. A comparative example of this invariance is given in fig. 3, where the results for different termination angles are shown for both, conventional Fourier transform and our indirect procedure.

3. Results and discussion

The comparison of the experimental and theoretical scattering curves (fig. 4) for the radial electron density functions (fig. 3) indicate that a spherical model is a good approximation to the structure of HDL_2. Angular positions as well as intensities of the maxima are in good agreement. The deviations in the region of the minima reflect that the assumption of

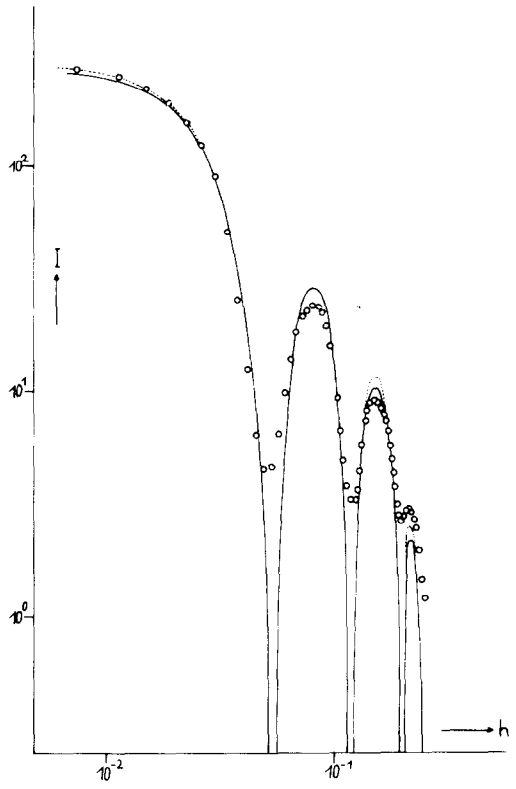

Fig. 4. Comparison of the experimental scattering curve (○, •) with the model scattering curves calculated from Spline functions (- - - - -) and step functions (——).

sphericity is an idealisation. However, since particularly in these regions the scattering curve is exceedingly sensitive to several factors (deviations from sphericity, small amounts of aggregates, imperfections in desmearing) a quantitation of the deviation from sphericity can hardly be achieved.

We found the radius of gyration in 0.15 M NaCl to be 57 Å. We are not in a position to discuss the discrepancy between this value and the value found by Shipley et al. [1] on a firm basis. It might be due to differences in the preparation as well as slight differences in solvent composition and temperature which have a strong influence on the radius of gyration of particles with nonuniform electron density.

The resulting electron density function and the radial dimensions show that LpA from HDL_2 has an average diameter of approximately 114 Å. The outer shell of 14 Å thickness indicates by its volume and electron density the surface location of the constituent peptides and the phospholipid polar head groups. The dimensions and electron density of the low electron density core corresponds in our opinion to a spherical micellar arrangement of the constituent lipid molecules with the cholesterol esters present in an extended conformation similar to the model obtained for LpA from HDL_3 [3]. This is in good agreement to the results from n.m.r. spectroscopic experiments [15, 16].

The slight increase of electron density towards the centre of the molecule cannot be discussed with any certainty, since valuable information about this region lies beyond the resolution of our experiments. From its invariance to the termination [fig. 3] it might be speculated that this increase might be due to a central position of the cholesterol moieties with the fatty acid chains of the cholesterol esters interdigitating with the phospholipid hydrocarbon chains.

The described results confirm the view that HDL_2 and HDL_3 are formed according to a common structural principle. Together with the fact that they represent different molecular species with different protein to lipid ratio this indicates that the apoproteins ApoAI and ApoAII are capable of binding and solubilising discrete portions of phospholipids, cholesterol and cholesterol esters.

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Appendix

Indirect Fourier transformation

This new method for Fourier transformation was presented in Grenoble 1973 [1]. A detailed paper is in preparation and will be published elsewhere.

The indirect Fourier transformation minimizes the termination effect and gives a weighted least squares approximation to the measured data. No artificial 'temperature' or 'convergence'-factor is used (B.E. Warren [2]). No extrapolations beyond the limits of the measuring range are introduced.

The indirect Fourier transformation can be used

for all nonperiodic scattering media, i.e. for scattering media which have a restricted extension in R-space, as for example scattering for globular particles and scattering of amorphous substances where the variations of the electron density go to zero beyond a certain distance.

Theory

The Fourier transformation in small angle X-ray scattering is given by the following formulae:

$$A(h) = 4\pi \int_0^\infty r \cdot \rho(r) \cdot \frac{\sin(hr)}{h}\, dr \qquad (1)$$

respectively:

$$\rho(r) = \frac{1}{2\pi^2} \int_0^\infty h \cdot A(h) \cdot \frac{\sin(hr)}{r}\, dh \qquad (2)$$

The scattering amplitude is known only in a finite range

$h_1 \leqslant h \leqslant h_2$.

If one calculates the electron density distribution ρ_τ

$$\rho_\tau(r) = \frac{1}{2\pi^2} \int_{h_1}^{h_2} A(h) \cdot h \cdot \frac{\sin(hr)}{r}\, dh \qquad (3)$$

the amplitude $A(h)$ should be zero for $h_1 > h > h_2$. This leads to the well-known termination effect (B.E. Warren [2] Becherer and Weber [3]).

The indirect Fourier transformation uses the information, that the electron density distribution $\rho(r)$ is identically zero for $r > R_{max}$ and that the measured part of the amplitude curve $A(h)$ includes only partial information about the particle.

Starting with

$$\rho_I(r) = \sum_{\nu=1}^{N} c_\nu \varphi_\nu(r) \qquad (4)$$

where $\rho_I(r)$ is the approximated electron density distribution resulting from the indirect Fourier transformation. The function system $\varphi_\nu(r)$ is defined in the subspace $\phi \leqslant r \leqslant R_{max}$ of the whole R-space. The N functions have to be linearly independent in this subspace.

This set of functions φ_ν can be transformed into the reciprocal space using equation [1]

$$\psi_\nu(h) = 4\pi \int_0^\infty r \cdot \varphi_\nu(r) \cdot \frac{\sin(hr)}{h}\, dr$$

$$\equiv 4\pi \int_0^{R_{max}} r \cdot \varphi_\nu(\nu) \frac{\sin(hr)}{h}\, dr \qquad (5)$$

The coefficients c_ν of eq. (4) can be determined now in the reciprocal space fullfilling the following constraint:

$$\int_{h_1}^{h_2} \frac{[A(h) - \Sigma_{\nu=1}^{N} c_\nu \psi_\nu(h)]^2}{\delta(h)^2}\, dh = \text{Min} \qquad (6)$$

The variance function $\sigma(h)$ represents the accuracy of the amplitude curve $A(h)$.

For a set of sampling points h_i ; $i = 1, M$ eq. (6) goes over to

$$\sum_{i=1}^{M} \frac{[A(h_i) - \Sigma_{\nu=1}^{N} c_\nu \psi_\nu(h_i)]^2}{\delta(h_i)^2} = \text{Min} \qquad (7)$$

The coefficients c_ν, resulting from this minimization problem can be used to construct the solution $\rho_I(r)$ in the R-space according to eq. (4). It should be mentioned that the amplitudes are not forced to zero outside the measuring range. The restricted measuring range leads to a finite number of expansion coefficients in the serie [4]. It would be possible to define an infinite number of linearly independent functions φ_ν in the subspace $O \leqslant r \leqslant R_{max}$. But these transformed functions ψ_ν would be linearly dependent in the subspace $h_1 \leqslant h \leqslant h_2$ of the reciprocal space. A real or numerical dependency in this subspace leads to singular or unstable matrices in the least squares problem [7]. The problem of error propagation cannot be treated here. It should be mentioned, that a preliminary smoothing of the amplitude curve from the statistical oscillations is not necessary.

Until now the best results were obtained with cubic B-splines (Greville) [4] as $\varphi_\nu(r)$ in eq. (4). These B-splines were introduced into the field of small angle scattering by Schelten and Hossfeld [5].

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