SMALL ANGLE X-RAY SCATTERING OF LpA, THE MAJOR LIPOPROTEIN FAMILY OF HUMAN PLASMA HIGH DENSITY LIPOPROTEIN HDL_3

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1. Introduction

Most of the present knowledge of size and shape of human plasma high density lipoproteins (HDL) is based on their visualization by electron microscopy using negative staining techniques [1, 2]. These experiments indicate a subunit structure for both the classes of high density lipoproteins, HDL_2 and HDL_3. Unfortunately the resolution has been limited to about 30 Å, which did not allow to give precise dimensions of the subunit structure. Moreover, these pictures revealed a great variety of possible arrangements for the subunits within the particles of each class.

The assumption of a subunit structure of the constituent protein moiety was strongly supported by delipidation experiments. Several groups of investigators have shown [3–5], that the apoprotein can be fractionated into nonidentical polypeptides.

It is a well established fact, that lipoprotein density classes are heterogeneous with respect to their chemical, immunochemical and physicochemical properties [6–8]. In fact there does not seem to exist any density fraction, from which a single, homogeneous lipoprotein fraction can be isolated solely by ultracentrifugation. We therefore applied additional purification procedures to isolate a lipoprotein A (LpA) preparation from HDL_3 fraction of human serum, homogeneous with respect to its immunochemical and physicochemical behaviour.

This paper is a first report of our X-ray small angle scattering experiments on LpA. The results given are confined to the most unambiguous molecular parameters as the radius of gyration, the molecular weight and the particle volume. The results indicate a subunit structure for LpA.

2. Experimental

2.1. *Isolation and characterization of LpA from HDL_3*

Blood samples were collected from apparently healthy female subjects after fasting overnight. The isolation of LpA from HDL_3 was performed according to Kostner and Alaupovic as described in detail elsewhere [9]: VLDL plus LDL were removed from serum at a solution density of 1.073 g/cm^3. The HDL from the infranatant were isolated at d=1.22 g/cm^3 and the separation of HDL_2 from HDL_3 was performed at d=1.107 in the preparative ultracentrifuge. This HDL_3 preparation represents a mixture of LpA, LpC and a third lipoprotein family not characterized so far [10]. The isolation of pure LpA could be achieved by column chromatography on hydroxylapatite [10]. This LpA was further purified by passing over a column packed with Sepharose 4B in 0.15 M NaCl. A very narrow region of the peak maximum from the eluate was collected in order to cut off lipoproteins with smaller or

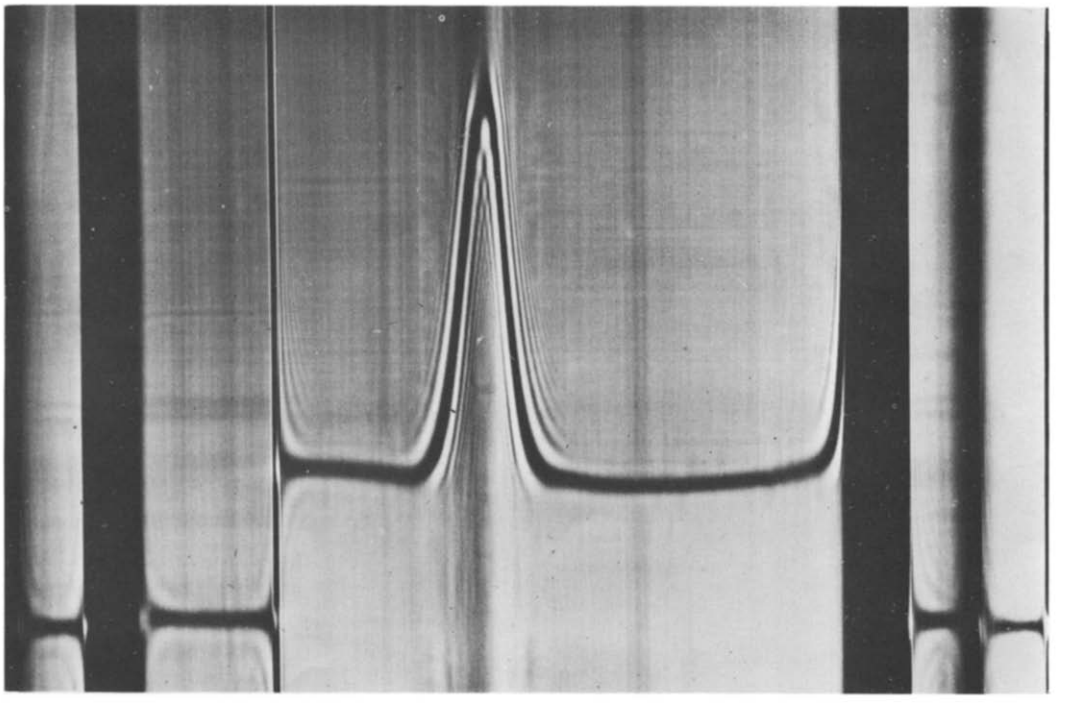

Fig. 1. Sedimentation pattern of LpA from HDL_3 in the analytical ultracentrifuge. Concentration: 14 mg/ml. The picture was taken 50 min after reaching maximum speed (52 000 rpm).

higher molecular weights, and concentrated by vacuum dialysis against 0.15 M NaCl containing 0.05% Na_2 EDTA and NaN_3, respectively. Immunochemical and chemical analyses of LpA were performed as described in earlier publications [11]. The LpA from HDL_3 reacted only with antibodies specific to ApoAI and ApoAII and no reaction with antisera to LpB, LpC or the third lipoprotein family was obtained [12].

The chemical analysis yielded a protein:lipid ratio of 1.38. Analytical ultracentrifugations were carried out in a Beckman Model E (fig. 1). Molecular weights were calculated from S_{obs} and D_{obs} values using Svedbergs formula, and from equilibrium runs. A single symmetrical peak with an S value of $S^0_{25,w} = 4.53$ and a D value of $D^0_{25,w} = 4.36$ was observed. Molecular weights of $M_{S,D} = 18.45 \times 10^4$ and $M_{equil.} = 18.78 \times 10^4$ were calculated.

2.2. *Partial specific volume*

The partial specific volume ($\bar{\nu}$) was determined using a precision density measuring device DMA 02/C [13], allowing an accuracy in density determination of aqueous solutions of $\pm 3 \times 10^{-6}$ g/cm^3. The determination was carried out at the two different temperatures, where ultracentrifugation and X-ray measurements were performed, namely 4° and 25°. The $\bar{\nu}$ values found for 25.05° and 4.00° were 0.871 cm^3/g and 0.859 cm^3/g, respectively.

2.3. *Small angle X-ray measurements*

The X-ray technique employed in the experiments was essentially the same as described in detail earlier [14, 15]. Automatical operation was allowed by an electronically programmable step scanning device [16]. During exposure to the X-ray beam the samples were kept at a constant temperature of 4° using a thermoelectrically cooled capillary cuvette [17]. The influence of the K_β-line was eliminated by a computer program based upon a mathematical procedure reported by Zipper [18]. To avoid errors arising from interparticle interference, the inner part of the scattering curve was

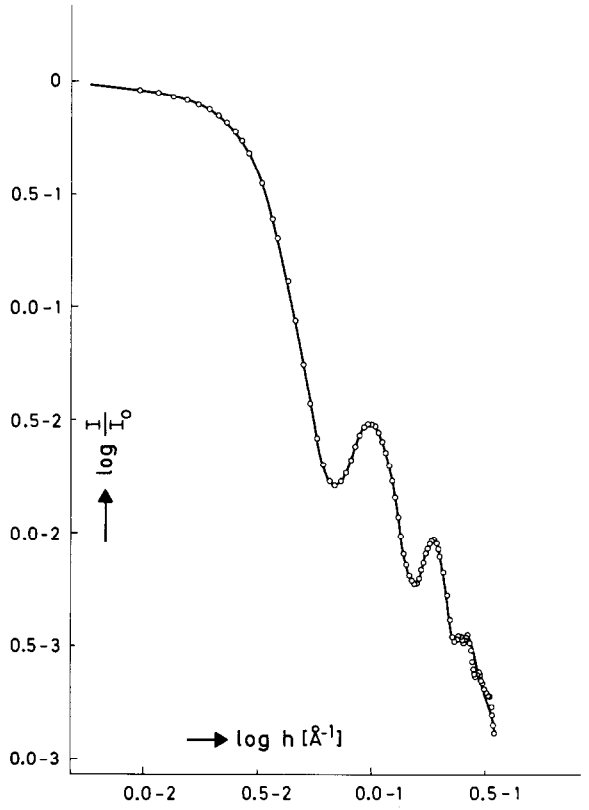

Fig. 2. Experimental scattering curve of LpA corrected for the collimation error.

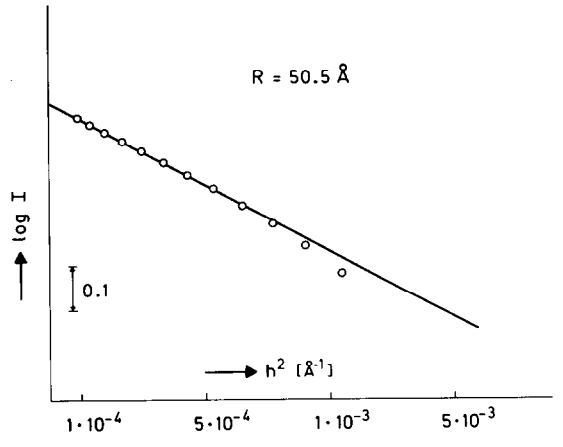

Fig. 3. Guinier plot of the inner part of the scattering curve of LpA for the evaluation of the radius of gyration R.

measured with 4 dilutions (53.6 mg/ml; 26.8 mg/ml; 13.4 mg/ml and 7.7 mg/ml) and extrapolated to zero concentration.

The experimental scattering curves were corrected for the collimation error [19] using a mathematical method given by Glatter [20].

3. Results

Fig. 2 shows the scattering curve in the logarithmic plot normalized to an intensity at zero angle of $I_0 = 1$.

According to Guinier and Fournet [21] the electronic radius of gyration R can be determined from the slope of the scattering curve at lowest angles in the log I versus $(2\theta)^2$ plot (fig. 3). The value of R obtained by this method is 50.5 Å. The error due to the graphical evaluation is less than ± 3%.

As previously described in detail [22, 15] the molecular weight can be obtained from the absolute value of the scattered intensity at zero angle. This value is found by extrapolation of the scattering curve in the $\log I$ *vs.* $(2\theta)^2$ plot and determination of the absolute intensity of the primary beam using a calibrated Lupolen sample [23]. The molecular weight determined by this method is $2.1 \times 10^5 \pm 0.15 \times 10^5$ daltons. The difference between this value and the values found by ultracentrifugation might partially be ascribed to the uncertainty in the calculation of the scattering power of the molecules which is based upon the relative electron densities of all components present in the solution.

From the invariant of the scattering curve $\tilde{Q} = \int_0^\infty \tilde{I}\ (2\theta)\ \mathrm{d}(2\theta)$ and the intensity at zero angle the volume of the hydrated particle V_h can be determined [24]. This calculation yielded a volume $V_h = 3.65 \times 10^5 \pm 0.15 \times 10^5$ Å^3. In order to make possible the integration to infinity the scattering curve has been extrapolated to larger angles using the equation

$$\tilde{I} = \frac{K_1}{(2\theta)^3} + K_2$$

($\tilde{I}$ . . . scattered intensity not corrected for the collimation error). From the plot $\tilde{I}\ (2\theta)^3$ *vs.* $(2\theta)^3$ the values of K_1 and K_2 are easily obtained. In this relation $K_1/(2\theta)^3$ represents the theoretical decrease of the scattering curve to larger angles according to Porod [24] and K_2 is an additional term caused by the

"liquid structure" within the particle [25]. However, the actually measured part of the scattering curve contributes about 75% to the total value of the invariant. Therefore an error of 10% in the extrapolated part would cause an error of only 2.5% with respect to the volume.

From the comparison of the dry volume calculated on the basis of M and $\bar{v}$ to the hydrated volume V_h a value for inner solvation of the particles can be obtained. Thus we found a value of 0.21 g solvent/g solute.

4. Discussion

All previous experiments for the structural elucidation of this class of high density lipoproteins have been performed on structurally heterogeneous preparations as represented by HDL_3. The structural homogeneity of the system under investigation is strongly reflected by the shape of the scattering curve with its distinct subsidiary maxima: a mixture of molecules having identical mass and volume but varying in their shape would cause a suppression of the subsidiary maxima.

Generally, a scattering curve showing considerable side maxima indicates a high degree of regularity of the shape of the particle. In order to judge whether this regularity arises from a simple spherical or cylindrical overall shape or from subunits which are symmetrically arranged, we have performed the following considerations.

A sphere having the observed volume of $3.65 \times 10^5 \pm 0.15 \times 10^5$ Å^3 would have a radius of 44.3 ± 0.6 Å, which is clearly inconsistent with the observed radius of gyration of 50.5 Å. From this it becomes evident, that the assumption of a closely packed spherical shape is not valid. A similar consideration leads to the exclusion of a cylindrical model. Assuming a rectangular cylinder with a diameter to height ratio of 1:1 consisting of a lipid core with a relative electron density of 0.0083 e.Å^{-3} surrounded by a protein shell of 0.0767 e.Å^{-3} (these electron densities are average values based on the chemical analysis) a radius of gyration of 41 Å is obtained, which is also inconsistent with the experimental value. All these calculations show that the lipid moiety, which contributes only a small amount to the total scattering power must be situated in the center of the molecule, whereas the comparedly strong scattering protein part is located farther from the common center of gravity than it would be the case for simple spherical or cylindrical core-shell models.

The angular position of the first side maximum does not coincide with the one to be expected of spherical or cylindrical particles having the observed radius of gyration [26], which independently from the above mentioned considerations confirms the findings.

This situation leads to the conclusion that the particle consists of a lipid core surrounded in a more or less symmetrical way by protein subunits. This is in good agreement with the experimentally found dissociation of the apoprotein into different subunits [3–5].

The calculation of theoretical scattering curves for such models is presently in progress. A refined measurement of the outer part of the scattering curve as well as experiments with varying electron density of the solvent should bring about more detailed data on the structure of this class of high density lipoproteins.

Acknowledgement

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