# SMALL-ANGLE X-RAY SCATTERING OF HUMAN SERUM HIGH-DENSITY LIPOPROTEINS

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*(Received February 23, 1972)*

Small-angle x-ray scattering (SAXRS) studies of the human serum high-density lipoprotein $HDL_2$ indicate a symmetrical particle with a radius of gyration Rg = 46 Å. The positions and intensities of subsidiary maxima in the scattering curves are not consistent with those of a uniformly electron dense sphere. Scattering curves calculated for spheres with a step-model radial electron density distribution, show good agreement with the experimental scattering curve for $HDL_2$ only for specific values of the step function used. The dimensions obtained for the electron-deficient core and electron-rich shell model are quantitatively consistent with a predominantly surface location for the $HDL_2$ protein and phospholipid head groups, the more hydrocarbon species being located in the interior of the particle.

## 1. INTRODUCTION

Although the chemical compositions and many of the physical properties of the various lipoprotein classes obtained from human serum have been defined, comparatively little is known about their structural organization and the modes of interaction between the major lipid and protein components (1,2). In the case of the high-density lipoproteins (HDL) significant advances have resulted from the preparation of a water-soluble, lipid-free apoprotein (3), its further fractionation into peptide sub-units (4,5) and the recombination of both apoprotein and major peptides with lipids to give reconstituted lipoproteins (6-8).

Recently, spectroscopic techniques including optical rotatory dispersion (9) and circular dichroism (10), nuclear magnetic resonance (11,12) and electron spin resonance (13) have been applied to native lipoproteins and in the case of the high-density fraction $HDL_2$ to a reconstituted lipoprotein. Although these techniques are providing valuable information on the conformation, environment and interactions of individual components, so far details of the overall structure have depended on the use of electron microscopy (14).

Small angle x-ray scattering (SAXRS), which has been used to probe the overall size and internal structure of micellar lipid solutions (15) and some model lipoproteins (16), would seem to be a technique suited to the investi-

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gation of these structural features of serum lipoproteins. In this paper we describe the SAXRS from native human serum high-density lipoprotein $HDL_2$. A similar approach to the study of reconstituted lipoproteins formed by the recombination of lipid-free water soluble protein, apo-$HDL_2$, with various lipids will be reported later.

## 2. METHODS

### (a) *Preparation of $HDL_2$*

$HDL_2$ was separated by ultracentrifugal flotation from sear of healthy, human Caucasian donors (Type A, $Rh^+$) as previously described (17) and dialysed for 48 hr at 4° against several changes of 0.15 M NaCl, pH 7, containing $10^{-3}$ M EDTA.

### (b) *Small angle x-ray scattering*

The x-ray scattering measurements were recorded using a Kratky camera (18), with divergent beam geometry, adapted to a highly stabilized Philips PW 1010 generator. The entrance slit-width, determining the resolution of the camera, was normally 100 μ with a corresponding counter slit-width of 217 μ. The distance between the sample cell and the recording plane was either 205 mm or 215 mm. The scattered radiation was detected by a xenon-filled proportional counter and measured using a transistorized counting chain. Monochromatisation of the copper radiation was achieved with a nickel β-filter in conjunction with pulse height discrimination.

$HDL_2$ samples were mounted in 1 mm diameter, thin walled glass capillary tubes and the particle scattering curves derived by subtraction of the solvent from the solution scattering curves. The scattering curves corrected for the effect of the line collimation system, "desmearing", were computed using the methods described by Taylor and Schmidt (19) and Lake (20).

X-ray scattering curves from a concentrated $HDL_2$ solution (19.8 mg protein per ml) were obtained using Elliot toroidal optics (21) and the high intensity focal spot (0.2 $mm^2$) of the Elliot GX 6 rotating anode generator.

## 3. RESULTS AND DISCUSSION

The scattering curve I (h) from $HDL_2$ (8.5 mg protein per ml) in 0.15 M NaCl, $10^{-3}$ M EDTA buffer is shown in Fig. 1(a). This scattering curve has been obtained reproducibly from many samples of $HDL_2$ and shows the usual decrease in scattered intensity with increasing h at low values of h. More importantly, it shows 2 clearly defined maxima centered at h equal to 0.075 $Å^{-1}$ and 0.152 $Å^{-1}$. The broad maxima in the region h equals 0.20 to 0.40 $Å^{-1}$ is probably due to further poorly resolved subsidiary maxima. This regions of the scattering curve was further examined using photographic methods, and the microdensitometer trace (insert Fig. 1(a)) showing a maximum at h equals 0.225 $Å^{-1}$ would appear to confirm the above interpretation.

The 2 methods used for desmearing the experimental curves show good agreement although sometimes 30 iterations of the Lake procedure (20) were required to produce convergence. As shown in Fig. 1(b) desmearing shifts the positions of the maxima, accentuates the minima and due to termination effects, slightly distorts the scattering curve at values of h near to the maximum value of h. This last effect probably accounts for the slight assymmetry of the 2 subsidiary maxima in the desmeared curve. In order to eliminate the effect of interparticle interference on the scattering curves, the inner parts of the curves were recorded at several $HDL_2$ concentrations in the range 2.00 to 8.50 mg protein per ml.

According to the treatment of Guinier (22) the radius of gyration Rg is

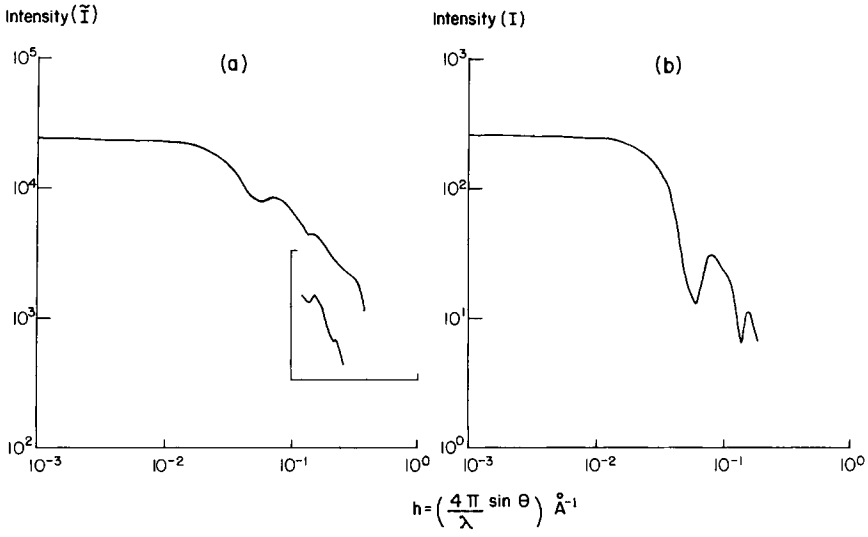


Fig. 1. X-ray scattering curve of human serum $HDL_2$ (8.50 mg protein per ml) in 0.15 M NaCl, $10^{-3}$ M EDTA at pH 7. (a) Scattering curve $\tilde{I}$ (h) containing smearing effect. Insert: microdensitometer trace from x-ray photograph (for details see text) of $HDL_2$ (19.8 mg protein per ml). (b) Scattering curve as in (a) with the smearing effect removed.

obtained from the initial slope of a plot of log I versus $h^2$. Linear extrapolation to h equals 0 gives the scattered intensity at zero angle ($I_0$) and Rg is determined from the following relation:

$$\log_e I = \log_e I_0 - 1/3\ Rg^2h^2 \text{ where } h = \frac{4\pi}{\lambda}\sin\theta$$

"Guinier plots" of the smeared and desmeared scattering curves, producing $\tilde{R}g$ and Rg respectively, are extremely linear at low values of $h^2$. The Guinier plots of the desmeared scattering curves at 3 $HDL_2$ concentrations are shown in Fig. 2. In Fig. 3 the radii of gyration obtained from both the smeared and desmeared scattering curves are plotted as a function of concentration. We observe that desmearing has only a small effect on the radius of gyration as anticipated from the extensive linear region of the Guinier plots. Furthermore, the linear dependence of the radius of gyration on concentration is typical of that resulting from interparticle interference. The radii of gyration extrapolated to zero concentration give $\tilde{R}g_{C=0}$ equals 44 Å and $Rg_{C=0}$ equals 46 Å for the smeared and desmeared data respectively.

Consideration of the resolved subsidiary maxima in the x-ray scattering curve, together with the extensive linear region shown in its Guinier representation, would suggest that $HDL_2$ is a highly symmetrical particle. By assuming both a spherical shape for the particle and a uniform electron density distribution, using the zero-concentration radius of gyration ($Rg_{C=0}$) of 46 Å we calculate a particle diameter of 119 Å. Making the same assumption regarding shape we can calculate a molecular diameter using a molecular weight of 386,000 obtained by sedimentation equilibrium ultracentrifugation and the partial specific volume 0.914 ml $g^{-1}$. This gives a molecular diameter equal to 104 Å. Both of these values for the diameter of $HDL_2$ exceed those obtained by electron microscopy of negative stained preparations of $HDL_2$ which gave particle diameters in the region 90 to 100 Å assuming again that the particles viewed are spheres and not rods (14). Thus, if we make the assumption of sphericity there is

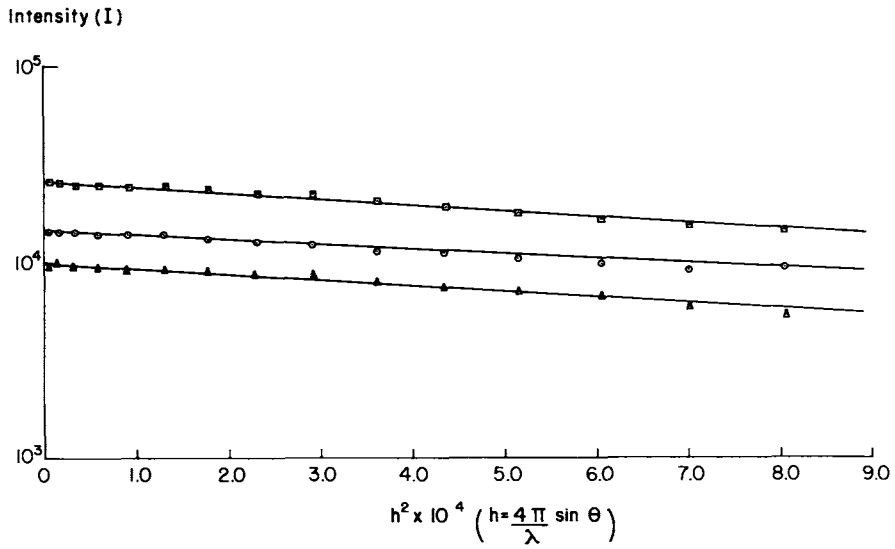


Fig. 2. Guinier plots (log I versus $h^2$) for human serum $HDL_2$. △, 2.0 mg protein per ml; O, 4.25 mg protein per ml; □, 8.5 mg protein per ml.

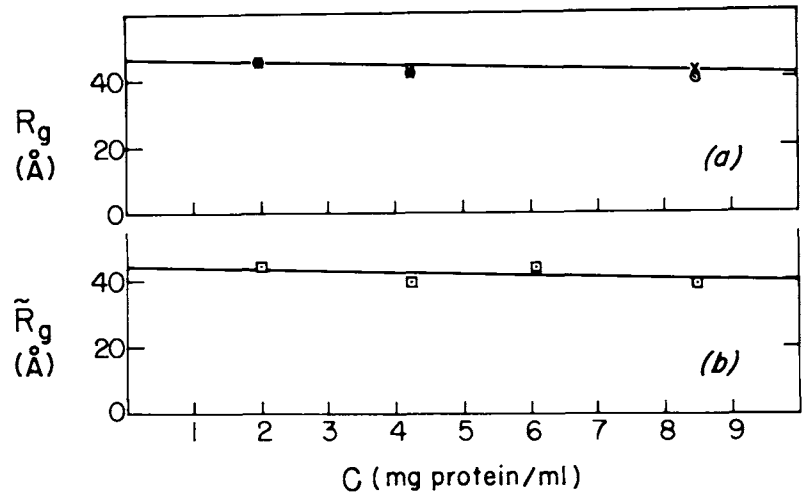


Fig. 3. Plot of radius of gyration as a function of concentration c (mg protein per ml). (a) $\tilde{R}g$ versus c; (b) Rg versus c; ⊙ indicates scattering curve desmeared using Lake procedure, x indicates scattering curve desmeared using Schmidt procedure.

some disagreement in the values obtained for diameters of $HDL_2$ using these independent techniques.

Returning to the x-ray data, comparison of the experimentally observed scattering curves with the theoretical scattering curves for model globular particles (e.g. spheres, ellipsoids) of uniform electron density reveals that the positions and, more strikingly, the relative intensities of the subsidiary maxima do not coincide with those expected for this type of particle. For example, the relative intensities of the subsidiary maxima of the experimental

curve may exceed by a factor of 10 those of the calculated curves (23). This appears to confirm the view that the uniformly electron dense, spherical model for $HDL_2$ is of doubtful validity.

An extension of this approach has involved the calculation of scattering curves for symmetrical particles with a non-uniform electron density distribution. In particular, a detailed study has been made of spherical particles with radial electron density distribution within the particle, the distribution being defined by a 4-parameter step model (see Fig. 4). Theoretical scattering curves were calculated for spherical particles in which the external radius R, the internal radius $\gamma R$, and a parameter $\beta$ relating the electron densities of the core and shell were varied systematically and changes in the positions and relative intensities of the subsidiary maxima were monitored. Compared with the uniform sphere model, enhancements of the relative intensities of the subsidiary maxima were obtained. Detailed comparisons with the desmeared $HDL_2$ experimental curve has led to the optimal agreement shown in Fig. 5, the theoretical curve being calculated using the parameters R equals 54 Å, $\gamma$ equals 0.80, and $\beta$ equals -0.263. The agreement in terms of peak positions and peak intensities is excellent, although the peak widths are rather different and the minima in the theoretical curve are deeper. Reasonable agreement is observed in the small-angle region of the scattering curves.

This model with a relatively electron-deficient central core (diameter 86 Å) surrounded by an electron-rich outer shell (thickness 11 Å) is consistent with the evidence favoring a surface location for the protein sub-units (24), particularly the glutamic and lysine amino acid residues, and the polar moieties of the constituent phospholipids (12,25) of $HDL_2$. Also it might be expected that the apolar parts of the molecular constituents, e.g. the hydrocarbon chains of the phospholipids and the cholesterol esters, might be located in the interior of the particle, this region being identified with the electron deficient core in the step-model. However, although this is undoubtedly the best description of a spherical $HDL_2$ particle, the fact that the minima in the scattering curves do not reach zero on the abscissa probably indicates that small deviations from ideal spherical symmetry do occur. Similar effects in the scattering curves of other highly symmetrical particles, for example, latex spheres (26), plant viruses (27) and macrophages (28) have been reported. Thus further work embodying calculations of the scattering curves for non-uniform, non-spherical particles and eventually a Fourier inversion of the intensity distribution giving an electron density profile of the $HDL_2$ particle is necessary before an unequivocal commitment to the present structure is made.

The detailed description of the structure of serum lipoproteins has so far depended upon the use of electron microscopy. The x-ray scattering study

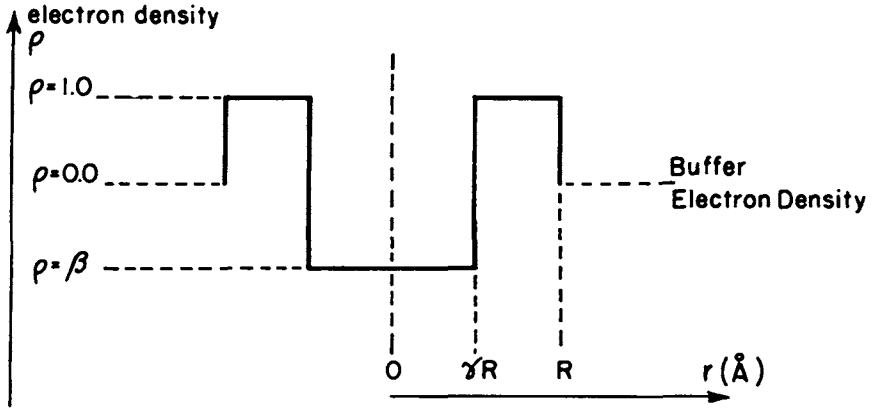


Fig. 4. Step-model of spherical particle with radial electron density distribution.

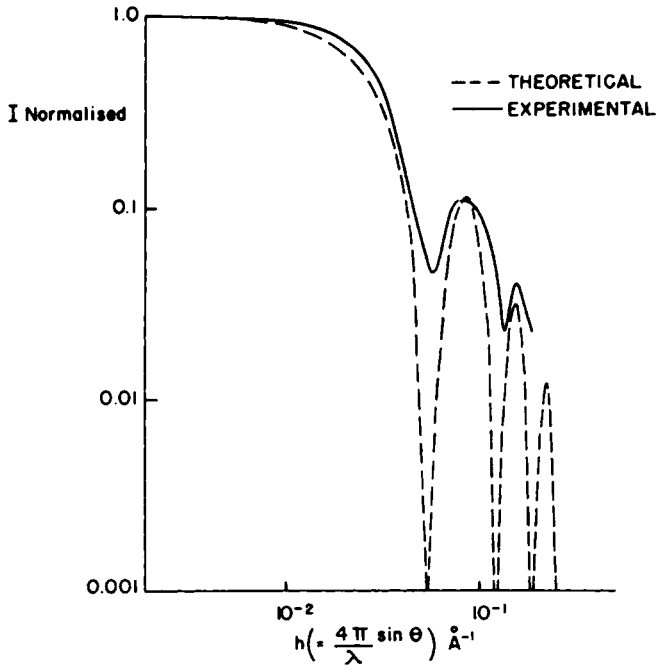


Fig. 5. Comparison of experimental and theoretical x-ray scattering curves. ——, desmeared experimental curve for $HDL_2$ (extrapolation to zero concentration); ----, theoretical scattering curve for spherical particle with radial electron density distribution (R = 54 Å, $\gamma$ = 0.80, $\beta$ = -0.263).

reported here shows that this technique is capable of providing a quantitative structural characterization (size, shape, internal structure) in the case of $HDL_2$ and indicates its applicability to other soluble, native and reconstituted lipoprotein systems.

## ACKNOWLEDGMENTS

The interest and assistance of Mr. M.A.F. Davis, Dr. R.B. Leslie and Prof. R.E. Burge is gratefully acknowledged.

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