Characterization of the structure of polydisperse human low-density lipoprotein by neutron scattering

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Low-density lipoproteins (LDL) in plasma are constructed from a single molecule of apolipoprotein B-100 (M_r 512000) in association with lipid (approximate M_r $2\text{--}3 \times 10^6$). The gross structure was studied using an updated pulsed-neutron camera LOQ with an area detector to establish the basis for the interpretation of structural changes seen during dynamic studies of LDL oxidation. Neutron-scattering data for LDL in 100% $^2\text{H}_2\text{O}$ buffers emphasize their external appearance. Guinier analysis on a continuous-flux neutron camera D17 revealed pronounced concentration-dependences in the radius of gyration, R_G , and the intensity of forward scattering, $I(0)$ (equivalent to the M_r of LDL) between 0.5 and 11 mg of LDL protein/ml. LDL preparations from different donors gave different R_G values. When extrapolated to zero concentration, R_G values ranged between 8.3 and 10.6 nm and were linearly correlated with M_r , which is consistent with a spherical structure. The distance-distribution function $P(r)$ in real space showed a single maximum

at 9.1–10.9 nm, which is just under half the observed maximum dimension of 23.1 ± 1.2 nm expected for a spherical structure. The neutron radial-density function $\rho(r)$ exhibited a plateau of high and featureless density at the centre of LDL. LDL can be modelled by a polydisperse assembly of spheres with two internal densities and a mean radius close to 10.0 nm in a normal distribution of radii with a standard deviation of 2.0 nm. The data are consistent with recent electron-microscopy and ultracentrifugation data. Although D17 and LOQ gave similar results, the short acquisition times for full scattering curves obtained with LOQ at LDL concentrations close to physiological are advantageous for oxidation time-course studies of structural changes, and no radiation damage is observed. Structural changes during LDL oxidation are best studied at a concentration of 2 mg of LDL protein/ml or higher, and data on non-oxidized LDL are an important control.

INTRODUCTION

Low-density lipoproteins (LDL) constitute a major family of cholesterol transporters in plasma [1]. LDL are formed from single copies of apolipoprotein B mainly located on the surface, together with a phospholipid monolayer associated with free cholesterol, and a hydrophobic lipid core containing cholesteryl esters and triacylglycerols. They exist as a range of subfractions within and among individuals, and these may exhibit differences in biochemical properties [2,3]. Increased plasma levels of LDL correlate with the incidence of atherosclerosis, which is a leading cause of death in the Western world [4,5]. Oxidized LDL are thought to be involved in the formation of early atherosclerotic lesions [6]. Oxidation can be mimicked by transition metals alone, and the naturally occurring antioxidants in LDL and plasma protect it from oxidation [6].

An investigation of the time course of structural changes in LDL during oxidation requires a knowledge of the structure of native LDL and how this may best be studied during oxidation. Recent studies show that native LDL exhibit polydisperse structures. Electron microscopy with negative staining reveals that LDL are spherical with mean diameters ranging between 19.7 and 28.5 nm, where the range of diameters reflects polydispersity, the subfraction under study and sample preparation. Electron microscopy does not characterize LDL under physiological conditions. In solution, a range of LDL sizes have also been reported using photon-correlation spectroscopy, sedimentation coefficients and non-denaturing gradient gel electrophoresis. From these studies the polydispersity properties of LDL have been attributed to the variable lipid contents bound to apolipoprotein B.

Neutron- and X-ray-scattering experiments allows LDL to be analysed in solution to reveal details of its overall and internal structure. They have been well characterized by X-rays, with which their maximum diameter was determined to be between 23 and 25 nm, their lipid transition point was shown to be between 17 and 41 °C, and their internal structure at lower temperatures was found to be two concentric shells of cholesteryl esters and triacylglycerols in a core surrounded by a surface monolayer of phospholipids, free cholesterol and protein [7]. Relatively little neutron scattering has been reported with LDL [8,9]. Many of these scattering studies required unphysiologically high LDL concentrations for data collection (which may induce spontaneous aggregation), and none have described the general effects of polydispersity on the neutron-scattering curves of LDL.

Large changes occur in the neutron-scattering curves of LDL during oxidation [10]. Time-course studies of these changes require data acquisition within minutes, preferably at LDL concentrations close to physiological. New developments in scattering cameras, in particular LOQ at the pulsed neutron source at ISIS at the Rutherford–Appleton Laboratory [11], now permit the simultaneous measurement within 10–20 min of the full scattering curve $I(Q)$ for dilute LDL samples in 100% $^2\text{H}_2\text{O}$ buffers with excellent signal-to-noise ratios. To a good approximation, these neutron-scattering experiments provide information on the overall structure of LDL, as the lipid component (and less so the protein component) is emphasized in this high solute/solvent contrast. In the present study, by reference to data measured on both instrument D17 at the Institut Laue-Langevin, Grenoble, France and LOQ, we show how LDL polydispersity is reflected in its neutron-scattering curves. The native LDL structure obtained by this method can be used as the baseline for

Abbreviations used: LDL, low-density lipoproteins; DTPA, diethylenetriaminepenta-acetic acid; ITP, indirect transformation procedure.

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oxidation studies. We show that a polydisperse spherical structure is a good model for LDL neutron-scattering curves, in agreement with other physical data on LDL. We then show in the following paper [12] that LOQ is a suitable instrument for the interpretation of neutron time-course studies of structural changes in LDL during oxidation.

MATERIALS AND METHODS

Preparation of human LDL for scattering experiments

LDL were prepared, using a procedure based on the method of Chung et al. [13], by discontinuous density-gradient ultracentrifugation in a fixed-angle rotor (70.Ti) using a Beckman XL-70 ultracentrifuge. Freshly drawn blood, anticoagulated with EDTA, was obtained with informed consent from healthy individuals and centrifuged for 20 min to obtain plasma. The plasma was brought to a density of 1.3 g/ml by the addition of NaBr containing the chelators 2 μ M diethylenetriaminepentaacetic acid (DTPA) and 300 μ M EDTA. Some 10–13 ml of this was underlaid beneath a 0.9% NaCl solution (deoxygenated with N_2 gas) and spun at 17000 g (48000 rev./min) for 2.5 h at 14 °C. The LDL band was removed with a syringe. Then 10 ml of this solution was mixed with 6.5 ml of NaBr solution (density 1.151 g/ml), overlaid with a NaBr solution of density 1.063 g/ml (both containing 2 μ M DTPA and 300 μ M EDTA), and centrifuged again at 17000 g at 14 °C for 14 h. The LDL were at the top of the tubes and were removed (density range 1.025–1.055 g/ml), concentrated using Amicon Centriprep-100 concentrators, and either desalted through a Sephadex G-25 column (20 cm $\times$ 1.1 cm diameter) equilibrated with 140 mM NaCl/12.5 mM Tris/HCl, pH 7.4, or dialysed into one of three buffers: modified Tyrode's (137 mM NaCl, 11.9 mM $NaHCO_3$, 4.2 mM $NaH_2PO_4 \cdot 2H_2O$, 2.7 mM KCl, pH 7.4), PBS (12.5 mM phosphate, 140 mM NaCl, pH 7.4) or Tris/saline (12.5 mM Tris, 140 mM NaCl, pH 7.4). LDL was stored under N_2 in the dark at 4 °C for no more than 4 days. LDL protein concentrations were determined by a modified Lowry assay [14]. Protein LDL concentrations are cited throughout the text, as the lipid content of LDL can be very variable. LDL was assayed for possible oxidation or degradation by SDS/PAGE [15] (4–16% polyacrylamide gradient).

Neutron-scattering-data acquisition and reduction

Neutron-scattering data were obtained in four sessions on instrument D17 at the high-flux reactor at the Institut Laue-Langevin. LDL was used from 17 blood samples from 14 healthy volunteers (six male and eight female; ages 20–45). The LDL samples were dialysed over 36 h at 6 °C with three changes into the corresponding 100% 2H_2O buffer. LDL samples (protein concentrations 0.5–6 mg/ml) were contained in rectangular 2 mm-thick quartz Hellma cells. Instrument D17 was set up at a sample-to-detector distance of 1.400 m and 3.457 m, with main beam-detector axis angles of 0° and 20° at the 1.400 m distance. The neutron wavelength λ was 1.106 nm (wavelength spread of 10% full-width-half-maximum). The full Q range (where $Q = 4\pi\sin\theta/\lambda$ and 2θ is the scattering angle) was 0.03–3.6 nm⁻¹. Data were recorded in a thermostatically controlled rack at 15, 20, 30, 40 or 50 °C. Counting times ranged from 1–2 min at 3.457 m to 7–14 min at 1.40 m/20°. Sample scattering curves $I(Q)$ were corrected for buffer backgrounds (using the LDL dialysate), and normalized using the incoherent scattering of a 1 mm-thick water sample, an empty cell and cadmium. All sample and buffer transmissions were measured. Reduction of the raw data utilized standard software (RNILS, SPOLLY, RGUIM and RPLLOT [16]).

Neutron-scattering data were also obtained in two sessions on the LOQ instrument at the pulsed-neutron source at ISIS at the Rutherford-Appleton Laboratory, Didcot, U.K. [11]. LDL (1–6 mg of protein/ml) was used from 11 blood samples from 10 donors (five male and five female, aged 20–40 years). For one session, aliquots of seven LDL samples from seven different donors measured 9 days previously on D17 were stored at 6 °C in the dark under N_2 in the presence of 300 μ M DTPA. DTPA was dialysed out immediately before LOQ measurements. Neutron pulses were moderated and monochromatized using time-of-flight techniques, and detected using either a BF_3 LETI scintillation or a 3He ORDELA wire detector at a fixed sample-to-detector distance of 4.3 m. Samples and buffers were measured in Hellma cells (as on D17 above) thermostatically controlled at either 20 and 50 °C or 15 and 40 °C, for a fixed total of 0.5×10^6 to 1.5×10^6 monitor counts for 30–35 min (50 μ A beam; U target) or 9–18 min (110 μ A beam; Ta target). Intensities were normalized relative to the standard scattering of a calibrated partially deuterated polystyrene sample [17]. Transmissions were measured for samples, buffers, empty beam and the standard. Data reduction of each run containing 100 time frames of 64×64 cells utilized COLETTE software [18]. Intensities were binned using wavelengths of 0.22–1.00 nm in linear steps of 0.02 nm or logarithmic steps of 0.08%, corrected for transmissions, and merged to give scattering curves in a Q range between 0.05 and 2.2 nm⁻¹. Intensities were calculated using 0.04 or 0.08% logarithmic increments of Q , which were optimal both for Guinier R_g and $I(0)$ analysis at low Q and better signal-to-noise ratios at large Q .

Scattering curve analyses

Guinier analyses of the scattering curves $I(Q)$ at low Q give the radius of gyration R_g and the forward scattering $I(0)$ [19,20]:

$$\ln I(Q) = \ln I(0) - R_g^2 Q^2/3$$

For a spherical macromolecule, this expression is valid in a Q range extending to a QR_g of 1.3. For a given solute/solvent density contrast difference, the R_g is both a measure of particle elongation and the internal arrangement of different scattering densities, i.e. the arrangement of protein and lipid within LDL. $I(0)/c$ (c = sample concentration) is proportional to the M_r . The M_r is calculated as an absolute value for neutron-scattering data, as it is referenced to the incoherent scattering from water as a known standard on D17 [21], or to the forward scattering $I(0)$ of the standard polymer on LOQ [17]. In this study, c is the LDL protein concentration.

The indirect transformation procedure (ITP) of Glatter [19] (versions ITP-81 and ITP-91) was used to transform the scattering curves $I(Q)$ in reciprocal space into the nuclear-pair distance-distribution function $P(r)$ in real space [9]:

$$P(r) = \frac{1}{2\pi^2} \int_0^\infty I(Q) Q r \sin(Qr) dQ$$

$P(r)$ corresponds to the distribution of distances r between any two volume elements within one particle weighted by the product of their respective nuclear densities relative to the solvent density. For D17 data, $P(r)$ calculations were based on the merger of three neutron subcurves for a given sample in the Q range 0.08–3.06 nm⁻¹ (a total of 87 $I(Q)$ data points), and fitted using 20 splines, and optimized using stability plots [22]. The assumed maximum dimension D_{max} of native LDL was 30 or 35 nm, which is 30 or 50% larger than the expected D_{max} of 23 nm, although Müller and Glatter [22] state that this need only be

Figure 1 Neutron and X-ray R_g and $I(0)$ Guinier plots for native LDL

The $I(Q)$ data points used for Guinier fits are denoted by straight lines, and the QR_g ranges are indicated. The Guinier data were fitted in the Q range 0.08–0.19 nm⁻¹. (a) A neutron dilution series at LDL protein concentrations of 0.7 (Δ), 1.2 ($\blacktriangle$), 1.9 ($\square$), 3.1 ($\blacksquare$), 3.8 ($\circ$) and 5.1 ($\bullet$) mg/ml for one LDL sample is shown, measured at 20 °C in modified Tyrode's buffer in 100% ²H₂O, using instrument D17. (b) A neutron dilution series at LDL protein concentrations of 1 (Δ), 2 ($\blacktriangle$), 3 ($\square$), 4 ($\blacksquare$), 5 ($\circ$) and 6 ($\bullet$) mg/ml for an LDL sample is shown, measured at 15 °C in Tris buffer in 100% ²H₂O, as in (a), using instrument LOQ.

20% larger. For LOQ data, $P(r)$ was calculated from $I(Q)$ data in the Q range 0.06–2.00 nm⁻¹ either with 99 $I(Q)$ data points in linear steps of Q , or 88 $I(Q)$ points in logarithmic steps of Q . Dilution series of six $I(Q)$ curves were normalized and linearly extrapolated to zero concentration, fitted using 20 splines, and transformed assuming $D_{\max}$ of 30 nm. The minimum Q value required for ITP was determined to be 0.13 nm⁻¹ from the stricter of the two relationships $Q_{\min} D_{\max} \leq \pi$, where $D_{\max}$ is assumed to be 23 nm [22]; this condition is satisfied by both instruments in use. The calculation of $P(r)$ can be affected by termination errors in the transformation. Criteria for the correct choice of the parameters for the $P(r)$ calculations were that: (i) $P(r)$ should be zero when r is zero; (ii) the R_g and $I(0)$ values calculated from $P(r)$ should agree with those from the Guinier analyses; (iii) $P(r)$ should be stable and reproducible for different $I(Q)$ curves obtained under similar experimental conditions.

The neutron $P(r)$ curves were converted using DECON-91 into the nuclear radial-density functions $\rho(r)$ assuming that LDL are spherically symmetrical and monodisperse (however, see below). This corresponds to the Fourier transformation of the scattering amplitude $A(Q) = \pm \sqrt{I(Q)}$, where the radial-density function is given by:

$$\rho(r) = \frac{1}{2\pi^2} \int_0^\infty A(Q) Q^2 \frac{\sin Qr}{Qr} dQ$$

Neutron $P(r)$ curves were transformed into $\rho(r)$ curves using 20 radial nuclear-density steps, each of width 0.65 nm, assuming that LDL have a radius of 13 nm. Stabilization plots were again used to optimize the transformation. Criteria for correctly calculated $\rho(r)$ curves were: (i) $\rho(r)$ should be zero or close to zero when r is at its maximum; (ii) back-calculation of $\rho(r)$ to $P(r)$ and $I(Q)$ should give curves similar to the starting $I(Q)$ and $P(r)$ curves; (iii) $\rho(r)$ should be stable and reproducible for different $I(Q)$ curves obtained under similar experimental conditions.

ITP-91 was also applied to study LDL polydispersity via the size-distribution functions $D_v(r)$ [23]. The $D_v(r)$ method was applied to the LDL neutron-scattering data measured in ²H₂O buffers, as the high contrast means that LDL are well approximated by a sphere of uniform density with a range of radii about a mean. Polydispersity was simulated by assuming that

LDL were spheres of uniform density from which $I(Q)$ was calculated on the basis of a standard normal distribution of LDL radii r , which takes the form of a Gaussian curve:

$$y = \sqrt{(2\pi\sigma^2)} \exp[-(r-M)^2/2\sigma^2]$$

where M is the most frequently occurring radius, σ is the standard deviation of the distribution, and the full-width-half-maximum of the normal distribution is given by 2.3548 σ .

RESULTS AND DISCUSSION

Continuous-flux neutron scattering of native LDL measured on instrument D17

The structure of LDL was characterized first on instrument D17. A total of 17 different native LDL preparations in 100% ²H₂O buffers from 14 donors was studied. The Guinier R_g and $I(0)$ analyses were linear in a satisfactory QR_g range of 0.7–1.7 (Figure 1a). No samples exhibited non-specific aggregation as would be demonstrated by curved Guinier plots at low Q . Individual R_g or $I(0)/c$ parameters were unchanged between 15 and 50 °C. Pronounced concentration-dependences of the slopes of the Guinier fits of Figure 1(a) are visible, and these are summarized in Figures 2(a) and 2(b) for six samples. Different linear dependences were observed for different LDL samples between 0.5 and 6 mg of LDL protein/ml. These effects are attributable to the high negative charges found on phospholipid headgroups on the LDL surface. These cause repulsive effects between different molecules, the extent of which is different for different preparations. At zero concentration, the R_g values range between 8.3 and 10.6 nm. The corresponding $I(0)/c$ values vary likewise. This reflects variable amounts of lipid associated with apolipoprotein B, as individuals are known to vary in their profile of LDL subfractions for dietary and genetic reasons [3]. The observed R_g and $I(0)/c$ values for LDL are thus mean values.

Figure 3(a) shows that the 17 pairs of R_g and $I(0)/c$ values are linearly related with a regression coefficient of 0.85, irrespective of buffer. This can be attributed to changes in the lipid/protein ratio in the LDL samples. If LDL are spherical, the slope of Figure 3(a) corresponds well to the expected increase in the

Figure 2 Concentration-dependence of the R_g and $I(0)$ parameters for native LDL

Neutron R_g (a) and $I(0)/c$ (b) data measured on D17 in two different sessions for LDL in 100% $^2\text{H}_2\text{O}$. Six independent preparations were used, denoted by the symbols, ∇ , $\blacktriangledown$, $\square$, $\blacksquare$, $\circ$ and $\bullet$. Each point corresponds to the average of four R_g and $I(0)$ values measured between 20 and 50 °C ($\square$, $\blacksquare$, $\circ$ and $\bullet$) or 15 and 40 °C (∇ and $\blacktriangledown$). Statistical error bars (S.D.s) are shown only when large enough to be visible.

Figure 3 Correlations between the neutron Guinier analyses and distance-distribution functions for native LDL measured on Instrument D17

(a) A total of 17 pairs of $I(0)/c$ and R_g values extrapolated to zero concentration for samples measured in four independent D17 sessions are shown ($\blacksquare$, $\square$ modified Tyrode's buffer; $\bullet$, $\circ$, PBS). The data were fitted to a regression line: $I(0)/c = 22(\pm 4)R_g - 149(\pm 32)$; with a correlation coefficient of 0.85. For comparison, four further pairs of $I(0)/c$ and R_g values measured from LOQ data ($\triangle$, Tris/saline buffer) are shown also; these data leave the regression line almost unchanged. (b) A total of 34 values of r at the maximum M in the $P(r)$ curves at LDL protein concentrations of 3–4 mg/ml are compared with the corresponding $I(0)/c$ values extrapolated to zero concentration. The correlation coefficient of the line is 0.69. (c) The 34 values of M as in (b) are correlated with the corresponding observed R_g values extrapolated to zero concentration. The correlation coefficient of the line is 0.62.

radius of LDL. When the R_g value increases from 8.32 nm to 10.62 nm (a factor of 1.28), the $I(0)/c$ (or M_r) value is increased by a factor of 2.49. The radius of LDL (and in turn the R_g of LDL) would have increased by a factor of 1.36 if LDL are spherical and had increased by this factor in M_r . This is close to what is observed. The protein content of LDL can vary by as much as 14.6 ± 2.3 to $30.3 \pm 1.8\%$ in different LDL subfractions [24–26]. This means that the M_r of LDL will range by a factor of two between 1.77×10^6 to 3.67×10^6 . Although lipid/protein ratios for the LDL samples of Figure 3(a) are not available, the neutron-scattering data agree with known variations in the relative amounts of buoyant, intermediate and dense LDL subfractions from different donors [3,27]. The nuclear densities of phospholipid, cholesterol, cholesteryl ester and triacylglycerols are very similar and equivalent to $10.2\text{--}12.1\%$ $^2\text{H}_2\text{O}$ [28]. This shows that variation in the total M_r of the LDL lipids, not in the

relative proportions of LDL lipids, accounts for the observed changes in $I(0)$.

Conversion of the full scattering curve $I(Q)$ (Figure 4a) into the density distribution function $P(r)$ (Figure 4b) provides information on the overall dimensions of LDL and their internal structure. Stable transformations from $I(Q)$ to $P(r)$ were obtained at 15–20 °C and 40–50 °C for 46 curves from 22 different LDL preparations. No temperature-dependence was seen. Comparisons show that the R_g and $I(0)$ values calculated from $P(r)$ agree satisfactorily with those from the Guinier analyses to within $17 \pm 14\%$ and $8 \pm 6\%$ respectively (32 values). $P(r)$ is symmetrical about its maximum M . It resembles the X-ray $P(r)$ curve of LDL measured in high contrast in 55% sucrose solutions [29]. At higher LDL protein concentrations (4 mg/ml), the $P(r)$ curve showed a negative feature at large r of 23–25 nm. This is characteristic of concentration effects [21], as already discussed

Figure 4 Full scattering curves $I(Q)$ for native LDL by neutron scattering, with the corresponding distance-distribution functions $P(r)$ and radial nuclear-density functions $\rho(r)$ for native LDL

(a–c) The neutron $I(Q)$ curve at 15 °C and 1.0 mg of LDL protein/ml was measured on D17. The curve fits were based on 20 splines and $D_{\max}$ of 30 nm to obtain $P(r)$, all of which were optimized using stability plots. (d–f) The neutron $I(Q)$ curve at 15 °C was measured on LOQ and extrapolated to zero concentration. The final curve fit was based on the parameters of (a–c). (a, d) The starting $I(Q)$ curve (●) with error bars is compared with the back-calculated $I(Q)$ curve obtained from the final $\rho(r)$ curve (—). (b, e) The starting $P(r)$ curve (—) with error bars is likewise compared with the back-calculated $P(r)$ curve (○). The position of the maximum in $P(r)$ is denoted by M and the maximum dimension by L . (c, f) The final $\rho(r)$ curve is shown in 20 steps of 0.65 nm each (radius 13 nm), together with associated error bars (S.D.s).

(Figures 2a and 2b). The maximum dimension L of LDL is obtained when $P(r)$ becomes zero at large r . The mean of 11 good determinations of L when concentration effects were minimal was 23.1 ± 1.2 nm. The maximum M in $P(r)$ is at $r = 9.1$ – 10.9 nm, which is just under half the value of L . The $P(r)$ curve is thus symmetrical about its maximum, as expected for a spherical structure. Analysis of Figures 3(b) and 3(c) suggests that increased Guinier R_g and $I(0)/c$ values were also linearly correlated with increased M values, although the regression coefficients were weaker at 0.69 and 0.62. This again shows that LDL are spherical.

The $P(r)$ curve was transformed into the radial-density function $\rho(r)$. This reflects the distribution of lipid and protein within LDL (Figure 4c). $\rho(r)$ exhibited a high plateau at $r = 0$ – 4 nm, which decreased to $r = 11$ nm, and levelled out to 13 nm. The lack of detail in $\rho(r)$ corresponds to the high solvent contrast in use, which masks the differences between protein and lipid. The higher $\rho(r)$ values at low r correspond to lipid hydrocarbon at the centre of LDL. The decrease in $\rho(r)$ at higher r is attributed to increasing amounts of cholesterol oxygen and the interpenetration of protein with phospholipid headgroups at the surface of LDL. The $\rho(r)$ curves show that, to a good approximation by neutrons, LDL have a spherical structure with an almost uniform internal scattering density. This agrees with

calculations of the nuclear densities of LDL lipid and protein [9,28].

Pulsed neutron scattering of native LDL measured on instrument LOQ at ISIS

Less-extensive neutron Guinier plots from LOQ in Figure 1(b) show that the data were equivalent to those from D17. Full dilution series gave Guinier analyses similar to those in Figures 2(a) and 2(b) for D17 (Table 1). The use of 22 pairs of R_g values measured for the same samples on D17 and LOQ leads to a ratio of LOQ/D17 R_g values of 0.941 ± 0.021 . If the slight difference is real, this may be due to an instrumental effect [11,30] or to possible LDL degradation on aging [31,32].

The main advantage of LOQ is that the full $I(Q)$ curve is obtained at once, unlike with the D17 instrument when three subcurves are separately measured then merged. This facilitates extrapolation of the $I(Q)$ and $P(r)$ curves to zero concentration. Improved agreements between the Guinier and $P(r)$ calculations were obtained with LOQ compared with D17. In two sessions, the R_g and $I(0)/c$ values agreed to $5 \pm 1\%$ and $3 \pm 3\%$ respectively (30 values) and $9 \pm 8\%$ and $3 \pm 4\%$ (32 values). Concentration effects were clearly detected in $P(r)$ by the

Table 1 Structure of human LDL assessed by neutron scattering in 100% $^2\text{H}_2\text{O}$

R_g values were extrapolated to zero concentration, using 17 dilution series on D17 and four dilution series on LOQ (Figure 3a). L can be compared with the sizes reported in Table 2. L was determined from 11 D17 $P(r)$ curves at 1–4 mg of LDL protein/ml, and from four LOQ $P(r)$ curves after extrapolation to zero concentration.

Instrument	Property	Value (nm)
D17	Radius of gyration range (R_g)	8.32 – 10.62
	Maximum dimension (L)	23.1 ± 1.2
	Most frequent distance range (M)	9.1 – 10.9
	S.D. of distribution (σ)	1.68 ± 0.11
LOQ	Radius of gyration range (R_g)	8.03 – 8.46
	Maximum dimension (L)	22.8 ± 25.2
	Most frequent distance range (M)	9.9 – 10.2
	S.D. of distribution (σ)	1.47 ± 0.19

observation of an increased negative feature at r of 20–25 nm with increased concentration [21,33]. These were minimal at LDL protein concentrations of 1 and 2 mg/ml, showing that these concentrations are suitable for LDL oxidation studies. A total of 32 $P(r)$ curves extrapolated to zero concentration for four LDL samples gave M at r of 9.9–10.2 nm (Figure 4e). L ranged between 22.8 and 25.2 nm. Calculation of the $\rho(r)$ curves (Figure 4f) gave results similar to those obtained with D17, where the different shapes of the curves are attributable to larger

statistical errors at low r for $\rho(r)$ measured on instrument D17 (Figure 4c) [33]. Although D17 and LOQ produce similar results (Table 1), LOQ offers more stable $P(r)$ transformations and is more suitable for dynamic studies of LDL modification by oxidation.

Polydispersity analysis of LDL by modelling and size-distribution functions

The neutron $\rho(r)$ analyses show that the LDL structure can be approximated as an object of uniform density with low internal-density variations. If it is represented by spheres of different radii, the population of radii $D_v(r)$ can be assessed by simulations (Figures 5a–5c) and ITP analyses (Figure 6). Sphere radii in 13 steps of 0.5 nm were weighted according to a symmetrical normal distribution about a mean radius of 10 nm, with standard deviations σ ranging from 0 to 4.5 nm. Distributions with σ of 1 nm or less are too narrow to account for the LDL scattering curve (Figure 5a), whereas those with σ of 2 nm or more are too wide (Figure 5c). That with σ of 1.5 nm in Figure 5(b) gave reasonable agreement with the experimental curve. ITP analyses of $D_v(r)$ from D17 data gave a mean maximum M of 9.90 ± 0.18 nm from 38 $I(Q)$ curves at LDL protein concentrations between 1.0 and 4.4 mg/ml (Figure 6b). The full-width-half-maximum of the $D_v(r)$ curves led to a mean σ of 1.68 ± 0.11 nm. LOQ data extrapolated to zero concentration gave a mean M of 9.64 ± 0.24 nm and a mean σ of 1.47 ± 0.19 nm. The σ values ranged between 1.30 and 1.87 nm

Figure 5 Comparison of a LOQ neutron-scattering curve with theoretical curves for a polydisperse range of spheres of mean radius 10 nm

In (a–c), the theoretical curves (—) correspond to a Gaussian-weighted population of 13 spheres of uniform (one-step) density with radii 7–13 nm (in increments of 0.5 nm) with standard deviations, σ , of 1.0, 1.5 and 2.0 nm as shown. The σ values correspond respectively to full-width-half-maxima of 2.35, 3.53 and 4.71 nm in the Gaussian population. Each curve is compared with the experimental LDL curve extrapolated to zero concentration (●). In (d–f), the theoretical curves are now calculated from spheres with a two-step internal density (see the text).

Figure 6 Particle-size distribution functions $D_v(r)$ for LDL in 100% $^2\text{H}_2\text{O}$ buffers from neutron-scattering data

Representative calculations using D17 and LOQ data are shown in (a, b) and (c, d) respectively. These were based on 30 splines (from which the innermost seven were removed) and a maximum radius r of 17 nm. The D17 curve was measured at a protein concentration of 1 mg/ml at 15 °C. The LOQ curve was measured at 15 °C and extrapolated to zero concentration. In (a) and (c), experimental $I(Q)$ data with error bars (○) are compared with the fitted $I(Q)$ curve (□) back-calculated from the $D_v(r)$ curves in (b) and (d) respectively. The r values at the maximum in the $D_v(r)$ curves are denoted by M in (b) and (d). The σ values are 1.68 and 1.30 nm respectively [corresponding to full-width-half-maxima (FWHM) of 4.0 and 3.1 nm in that order], and represent the extremes of σ values observed in the $D_v(r)$ calculations for D17 and LOQ.

(Figures 6b and 6d), which is in agreement with the simulations of Figure 5(b).

The limit of the approximation of uniform densities is the interpenetration of surface protein and phospholipid headgroups with lower densities into the lipid core at large r (Figures 4c and 4f). If LDL are represented by a simple two-step density to correspond to a core of lipid within a 0.5 nm-thick outer shell of protein and phospholipid, with relative densities of -1.84 and -1.00 respectively [10], a much improved fit to Q of 0.9 nm^{-1} and the appearance of two further submaxima at larger Q were obtained when the mean radius was 10 nm with σ of 2 nm (Figure 5e). Values of σ of 1.5 nm or less, or 2.5 nm or more, gave poorer fits. The σ values are in better agreement with the distribution of LDL diameters observed by electron microscopy from which σ is calculated to be 2.5 nm [34], and also with that seen by ultracentrifugation [34]. Calculations based on the skewed distributions of Krauss and Blanche [3] gave poorer fits. The neutron analyses are thus consistent with other physical data on LDL structures.

These distribution curves can be used to assess the M_r range of lipid bound within LDL. For σ of 1.5 nm, 96% of the LDL

sample will be found between $r = 7$ or 13 nm at the extremes of the curve. The mean apparent volume of an LDL molecule at $r = 10 \text{ nm}$ is 4189 nm^3 , and will range from 1437 to 9202 nm^3 from $r = 7$ to 13 nm. This shows how variations in the relative amounts of LDL subfractions [3] can lead to the large range of R_G , M_r and M values shown in Figure 3.

Conclusions

Polydisperse structure of LDL by neutron scattering

The size of the LDL particle may be of clinical relevance in that it defines different biochemical affinities [2]. Other physical methods have been used to define the size of LDL (Table 2). Electron microscopy has generally viewed LDL as spherical objects with no protrusions but with variable diameters. The apparent diameter of LDL depends on the stain used, and there is the possibility that LDL may be flattened in the electron microscope [34,35]. The extensive study of LDL subfractions revealed LDL subfractions that ranged from 19.7 to 27.0 nm [36]. The mean ($\pm$ S.D.) LDL diameter is $23.6(\pm 3.2) \text{ nm}$ from Table

Table 2 Measurement of LDL size by electron microscopy, hydrodynamics and other methods

The numbering of fractions is not consistent between different studies.

LDL sample	Diameter of LDL (nm)	Reference
Electron microscopy		
Fraction 1	27.1 ± 2.3	[37]*
Fraction 5	20.3 ± 2.5	
	21.9 ± 0.6, 22.0 ± 0.7	[38]
	28.1 ± 0.23 (uranium acetate)	[35]
	21.5 ± 0.62 (potassium phosphotungstate)	[40]
Large LDL	26–30	
Small LDL	18–22	[41]
	20.4 ± 2.0	
	28.5 (range of 24–34)	[34]
Fraction 1	27.0 ± 2.6	[36]*
Fraction 7	19.7 ± 1.8	
	22.7 ± 1.9	[42]
	24.5 ± 2.0 (fish oil LDL)	[43]
	25.0 ± 1.5 (corn oil LDL)	[32]
	20.1 ± 2.1	
Sedimentation/photon correlation spectroscopy		
	20.1–21.5	[34]
	24.9 ± 0.9 (fish oil LDL)	[43]
	24.9 ± 0.4 (corn oil LDL)	
Non-denaturing gradient gel electrophoresis		
Fraction 4	22.9 ± 0.3	[24]*
Fraction 10	21.4 ± 0.4	
Fraction 5	27.5 ± 0.2	[44]*
Fraction 9	26.3 ± 0.3	
Fraction 1	28.1 ± 1.0	[26]*
Fraction 6	24.5 ± 0.3	

* For reasons of brevity, only the upper and lower diameters found in these studies are listed.

2. Solution studies by sedimentation coefficient analysis or photon correlation spectroscopy have yielded similar average LDL diameters of 22.9 ± 2.4 nm. Non-denaturing gradient gel electrophoresis also yielded a similar average LDL diameter of 24.8 ± 2.4 nm in a range encompassing 21.4–28.1 nm (Table 2). The present neutron $P(r)$ data in Table 1 show that LDL have a maximum dimension L (or diameter) that is in good agreement with these determinations.

Neutron scattering on both D17 and LOQ indicates that LDL are well represented as a spherical structure. It is possible that deviations from sphericity will result in ellipsoidal structures, but the time-averaged structure would remain spherical. However, the marked variability in the D17 neutron-scattering data from standard LDL preparations from 14 different donors shows that LDL are polydisperse. Earlier scattering studies had not considered this aspect. The correlations between the mean size of LDL and its M_r (Figure 3) is as expected from a spherical polydisperse structure. The existence of polydisperse LDL structures shows that the 4536-residue apolipoprotein B has the remarkable property of being able to accommodate lipids that range in total M_r between 2×10^6 and 3×10^6 , in structures that vary from ≈ 20 nm to ≈ 30 nm in diameter. The polypeptide chain must act as a nucleation site for phospholipid and cholesterol to maintain the structural integrity of LDL. Indeed the use of monoclonal antibodies suggests that the conformation of apolipoprotein B changes with LDL size [45]. Larger LDL particles are less dense, and contain typically 21% protein, 41% cholesteryl ester, 21% phospholipid, 11% cholesterol and 6% triacylglycerol [24]. Smaller LDL particles are more dense, and

contain typically 30% protein, 40% cholesteryl ester, 19% phospholipid, 9% cholesterol and 3% triacylglycerol [24]. X-ray-scattering studies of LDL subfractions [25] showed that the outer radius of the cholesteryl ester core is reduced by 0.91 nm in the smallest LDL particle compared with the largest one studied, and that this is consistent with packing requirements for lipid within LDL.

Study of LDL oxidation by neutron scattering

The present study has defined the neutron structure of LDL in order to use it to monitor the time course of changes seen in LDL during oxidation [12]. In summary, LOQ is able to measure full LDL scattering curves with 10–20 min run times for LDL protein concentrations of 2 mg/ml in 100% $^2\text{H}_2\text{O}$ buffer, and this is advantageous over work with D17. It should be noted that other $\text{H}_2\text{O}/^2\text{H}_2\text{O}$ buffers could be used for data collection, but this would increase data-acquisition times to the extent that kinetic studies would be precluded. It is useful that the concentration range of 0.5–2.0 mg of LDL protein/ml corresponds to its physiological and pathophysiological values [46,47]. In 100% $^2\text{H}_2\text{O}$ buffer, the external form of LDL is viewed, not its internal structure, as shown by the lack of detail in the $\rho(r)$ curves (Figures 4c and 4f). The neutron R_g is low in 100% $^2\text{H}_2\text{O}$ buffer because of the relative contributions of protein and lipid to the scattering curve, compared with that measured by X-rays. This makes the R_g an accessible and useful monitor of LDL oxidation. The neutron-scattering data also permit LDL particle-size-distribution analyses through determinations of σ (Figures 5 and 6), and this measure of polydispersity can also be monitored during LDL oxidation.

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