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A LOW TEMPERATURE STRUCTURAL TRANSITION IN HUMAN SERUM LOW DENSITY LIPOPROTEINS

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Summary

Freezing human serum low density lipoprotein solutions to temperatures below 0°C induces reversible changes in the small angle X-ray scattering patterns. These changes are interpreted as being due to a low temperature structural transition not previously detected.

During the last few years the structure of human serum low density lipoprotein (LDL) has been investigated with a variety of physical and chemical techniques [1—4]. LDL is a quasi-spherical particle with a maximum diameter of 280 Å. The external surface is highly convoluted allowing the solvent to penetrate to within 55 Å of the center. Most of the proteins, polar groups of phospholipids and free cholesterol are exposed to the aqueous environment covering the cholesterol esters and triacylglycerols which are located towards the center of the particle. Moreover, some authors [5, 6, 7] have suggested the presence of globular subunits arranged at the surface with icosahedral symmetry.

Deckelbaum et al. [8] have recently published a remarkable contribution to the field of serum lipoproteins. Based on differential scanning calorimetry and X-ray techniques these authors have shown that on heating native LDL, two thermal transitions occur. The first one takes place near body temperature; it is completely reversible and it has been associated with the melting of the cholesterol esters from an ordered smectic liquid crystal to a more disordered isotropic state. The second transition occurs at 70—90°C, it is irreversible and it has been associated with 'lipoprotein denaturation' and release of cholesterol esters from the particles.

In this communication we want to report a new thermal transition in LDL. This transition takes place at low temperature and it has been detected by

small-angle X-ray scattering techniques. The experiments were performed using an Elliott rotating anode X-ray generator provided with a nickel-coated bent glass mirror operating with linear collimation. The scattered intensity was recorded with a proportional position-sensitive detector. The experimental curves were extrapolated to zero concentration and corrected for collimation distortions [9]. In Fig. 1, we show four small-angle X-ray scattering curves, obtained on the same lipoprotein sample* at different temperatures. Curves a and b are shown for comparison purposes with the low temperature experiment (see below). They correspond to LDL at 37 and 1°C and are similar to those previously reported by Deckelbaum et al. [8]. The major differences between the two curves are found in the resolution range above $2 \cdot 10^{-2}$ Å^{-1}. A strong band centered at $s = 2.7 \cdot 10^{-2}$ Å^{-1} is present at 1°C (curve b) and it is absent at 37°C (curve a)**. It should be stressed that the origin of this band has been

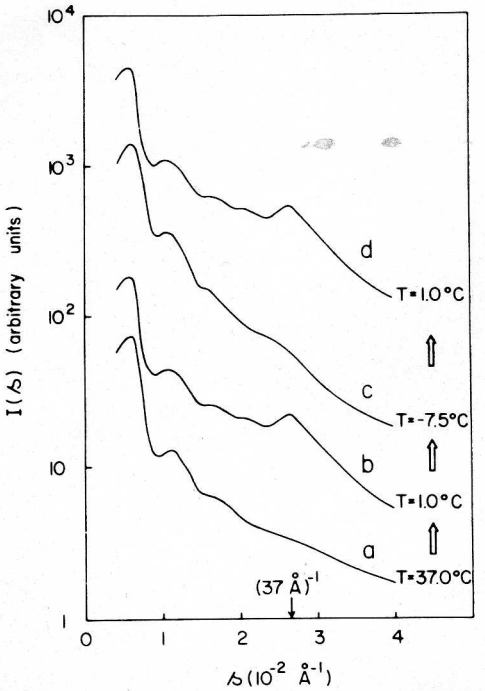

Fig. 1. Logarithmic plot of the small angle X-ray intensity curves of LDL, extrapolated to zero concentration and corrected for collimation distortions. The relative scaling is arbitrary. The scattered intensity was recorded with a position-sensitive proportional detector (30-min counting). The temperature of the experiment is shown for each curve. Open arrows indicate the experimental sequence of the temperature steps. The closed arrow indicates the position of the maximum at $s = 2.7 \cdot 10^{-2}$ Å^{-1} present at 1°C (curves b and d), but absent at 37 and −7.5°C (curves a and c). $s = 2(\sin\theta)/\lambda$ (2θ, scattering angle; λ, wavelength).

*Low density lipoprotein (LDL) of d = 1.019—1.063 was prepared in pure form, from fresh human serum from male donors, by the method previously described [10]. The preparation was dialysed exhaustively against the solvent: 150 mM NaCl/5 mM Tris·HCl/0.5 mM Na_2EDTA, pH 7.4. The concentrations were determined by protein analysis (biuret) and were adjusted by dilution. When needed, the LDL solutions were concentrated by extensive centrifugation, recovering the pellet on the bottom of the tube. The samples were inserted in a temperature-controlled sample holder (1-mm thick) and exposed to X-ray after 15 min of thermal equilibration.

**The intensity of the $2.7 \cdot 10^{-2}$ Å^{-1} band as recorded at different temperatures with the position-sensitive detector progressively decreases until it disappears at the end of the transition (Kirchhausen, T., Padrón, R. and Mateu, L., to be published).

interpreted as being due to the long-range organization of the cholesterol esters [8]. At 37°C these molecules are in an isotropic-liquid organization, whereas at 1°C they are in a smectic-like state, producing the strong diffraction band at $2.7 \cdot 10^{-2}$ Å^{-1} [8].

With this concept in mind, we have decreased the temperature of the LDL below 0°C, searching for new X-ray reflections indicative of a smectic → crystalline transition of the cholesterol esters. Unexpectedly, we have found that when the LDL sample freezes the $2.7 \cdot 10^{-2}$ Å^{-1} band disappears. This may be seen in curve c, where the intensity curve obtained with LDL at −7.5°C is shown. The strong maximum at $2.7 \cdot 10^{-2}$ Å^{-1} has disappeared and it may be observed that this curve is very similar to curve a obtained at 37°C with the same lipoprotein sample (note the absence of the band in both a and c curves). This low temperature transition was found to be completely reversible on reheating the sample to 1°C (curve d), even after repeatedly going backwards and forwards across the transition. We have also found that this phenomenon occurs at the same temperature for LDL obtained from different donors and it seems not to be correlated with the lipid composition of the samples (Kirchhausen, T., Mateu, L. and Camejo, G., to be published).

The aim of this communication has been to report for the first time low temperature structural transition in LDL. The question now is to explain how the freezing of the LDL solution can affect the long-range organization of the neutral constituents (cholesterol esters) which are supposed to be sequestered from the aqueous environment in the neutral lipid core.

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