Crystallization of human low density lipoprotein (LDL), a large lipid–protein complex
Collection of X-ray data at very low resolution

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Abstract

Human LDL subfractions LDL-2 ($d = 1.031$–1.034 g/ml) and LDL-5 ($d = 1.040$–1.044 g/ml) were crystallized in different crystal forms using polyethylene glycol as a precipitant. Both fractions were from one donor. Crystals of LDL-5 were yellow, hexagonal, and showed no dichroism. Of LDL-2 two dichroitic crystal forms were obtained. One had a rod-like shape with deep notches at both ends (form A), the other was a more compact form with plain surfaces (form B). To be able to measure low order reflections down to 300 Å a special experimental setup was developed. One single crystal was used to obtain a complete native data set of LDL-2 (form A) with an overall internal R-factor of 4.5% for reflections from 100 to 28 Å. Data were collected under cryogenic conditions using synchrotron radiation. The space group is most probably C2 with unit cell dimensions of $a = 183$ Å, $b = 421$ Å, $c = 385$ Å, $\alpha = \gamma = 90°$, $\beta \approx 90°$. Further optimization of the crystallization conditions and the search for heavy metal derivatives are in progress. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Low density lipoprotein (LDL) particles play a major role in the development of coronary heart disease [1,2]. LDL is a large complex of lipid and protein, with a particle diameter of about 200 Å and a total molecular mass of about 2000–3000 kDa depending on the number of associated lipid molecules. According to the different lipid/protein ratios total LDL can be divided into different subfractions using equilibrium density gradient centrifugation. These subfractions are assumed to have a different atherogenic potential [1]. The lipid moiety of the LDL particles is made up of a core of

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cholesterol esters and triacylglycerol. This core is surrounded by a monolayer of phospholipids. The lipids constitute about 75% of the total molecular mass of the particle. The protein moiety consists of a single polypeptide chain (apoB) with a molecular weight of 510 kDa (550 kDa with carbohydrate). The primary structure of apoB is known [3,4], and its secondary structure has been investigated using spectroscopic methods. A high content of beta sheet is commonly accepted for apoB, while other apolipoproteins are predominantly α-helical [5]. The radially averaged structure of LDL particles has been studied by small-angle X-ray and neutron scattering [6–11]. Only a limited amount of information is available on the tertiary structure of apoB. In order to explain the molecular mechanisms involved in the development of atherosclerosis, the knowledge of the three-dimensional structure of apoB in LDL particles would be an important approach. LDL has been crystallized by two different groups [12,13]. Here we report further progress in the crystallization of this large lipid–protein complex. In addition we describe a special setup for the collection of data at very low resolution (>300 Å), and discuss synchrotron diffraction data of these unusual crystals.

2. Materials and methods

2.1. Sample preparation

EDTA plasma from one single donor was obtained using standard procedures. Total LDL ($d = 1.019$–1.063 g/ml) was prepared by sequential flotation and then fractionated into six density classes via equilibrium density gradient centrifugation [8]. The density ranges of the subfractions as determined by precision refractometry of blank gradients were: LDL-1: < 1.031, LDL-2: 1.031–1.034, LDL-3: 1.034–1.037, LDL-4: 1.037–1.040, LDL-5: 1.040–1.044, LDL-6: > 1.044 g/ml. Phospholipid (PL), free cholesterol (FC), total cholesterol (TC), and triglycerides (TG) were measured using automated (EPOS, Eppendorf, Hamburg, Germany) enzymatic methods. Cholesterol ester (CE) was calculated as the molar difference between TC and FC. Apolipoprotein B was measured with endpoint nephelometry (Behring, Marburg, Germany).

2.2. Crystallization

All subfractions were dialyzed for 24 h against a buffer which consists of 20 mM PIPES, 150 mM $(NH_4)_2SO_4$, 2 mM ascorbate, 2 mM sodium azide, 2 mM EDTA at pH 7.5. Crystallization conditions for LDL subfractions were first established using the sparse matrix screening method [14] and optimized to obtain crystals suitable for X-ray diffraction experiments. Screening was performed with the hanging drop vapor diffusion technique at room temperature and 4°C. The protein concentration was about 5–6 mg apoB/ml. The droplet volume was 8 µl, and the reservoir volume was 200 µl.

For cryoexperiments the crystals were harvested, and subsequently incubated in reservoir solution containing 30% (w/v) glycerol for some seconds. The crystals were frozen and stored in liquid nitrogen for further use. LDL crystals are stable under these conditions for at least six months.

2.3. X-ray data collection

Data were collected at the EMBL beamlines X11 and BW7B (DESY, Hamburg, Germany), and on beamline ID14-3 at the European Synchrotron Radiation Facility (ESRF, Grenoble, France). Here we report on the data collected at ID14-3 under cryogenic conditions using the MarCCD detector. The crystal to detector distance was 524 mm which corresponds to a maximum resolution of 6 Å. The exposure time was 10 s per frame, with $\Delta\phi = 1°$. In order to collect data down to very low resolution a small lead beam stop (diameter 2 mm) was placed directly in front of the detector. The edge of the beamstop was at approximately 300 Å. The beamstop was mounted on a second goniometer head using the x–y translation for fine adjustment. To avoid air scattering the use of a helium path between sample and beamstop was necessary. In addition background scattering was minimized by direct contact of the beamstop with the second Mylar film of the helium path. For autoindexing and data reduction the program XDS [15] was used.

3. Results

3.1. Crystallization

LDL-5 crystals were obtained using polyethylene-glycol-monomethyl-ether 2000 (PEG 2000MME) as a precipitant. The concentration was 6% (w/w) PEG 2000MME and 75 mM ammonium sulfate in 75 mM acetate buffer (pH 4.6). This solution was equilibrated against reservoir solution containing 7% (w/w) PEG 2000MME, 100 mM ammonium sulfate in 100 mM acetate buffer (pH 4.6). We obtained compact, hexagonal crystals (Fig. 1) which showed no dichroism and grew to a size of $80 \times 80 \times 50 \ \mu m^3$ after three weeks.

LDL-2 crystals of were grown under slightly modified conditions. These crystals grew as long thin plates with large notches at one or both ends (Fig. 2). They were dichroitic, and grew to full size ($100 \times 200 \times 1500 \ \mu m^3$) after four weeks (form A).

Both types of crystals were of the same yellow color as LDL and floated in the reservoir solution, indicating a low density, as expected for LDL crystals.

In order to optimize the crystallization conditions different additives were included in the crystallization buffer. We obtained a different dichroitic crystal form of LDL-2 using Ampholyte 5-7 (Serva, Heidelberg, Germany) at a concentration of 3% as an additive. In contrast to the original crystals the new crystal form showed a compact shape without notches (Fig. 3). Crystals grew to a size of $250 \times 80 \times 30 \ \mu m^3$ after three weeks (form B).

3.2. X-ray diffraction

Despite the fact that crystals of the LDL-5 and LDL-2 (form B) subfractions showed a compact crystal form with plain surfaces and sharp edges no diffraction was detectable, even down to the very low resolution limit achieved in this study. We, however, reproducibly observed diffraction with LDL-2 (form A) crystals. The same diffraction pattern was obtained for crystals with one or two notches.

Though the LDL-2 crystals did also diffract at room temperature, establishing cryoconditions was advantageous for handling the crystals, for their stability in the beam, and for the obtainable resolution. For the collection of data sets cryoconditions proved to be essential.
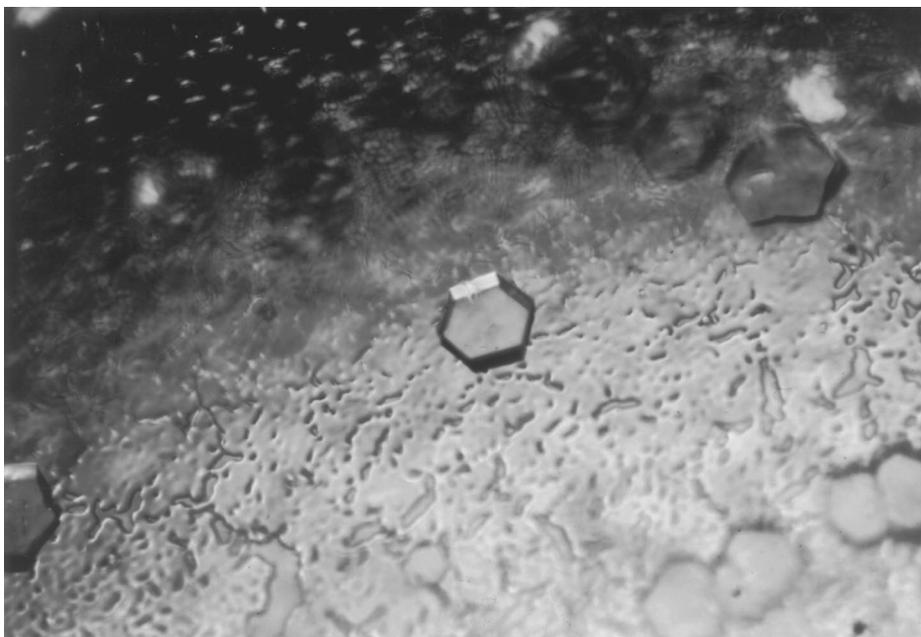

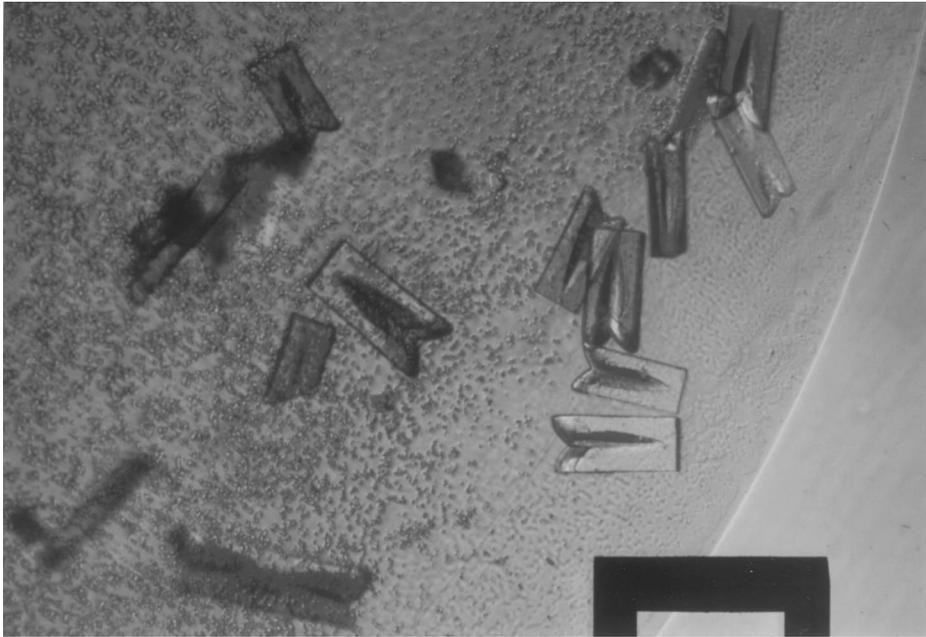Fig. 1. Hexagonal crystals of LDL-5 with PEG 2000MME as a precipitant.

Fig. 2. Crystals of LDL-2 with PEG 2000MME as a precipitant. The bar represents a length of 0.6 mm. Deep notches at one or both ends are clearly visible.
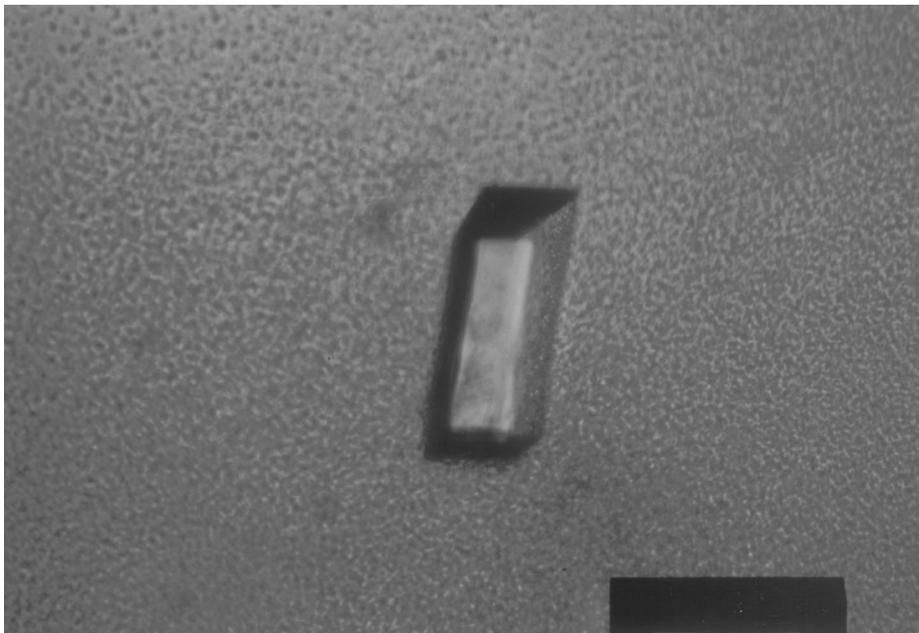

Fig. 3. Crystals of LDL-2 with PEG 2000MME as a precipitant in the presence of 3% (w/v) Ampholyte 5–7. The bar represents a length of 0.3 mm.
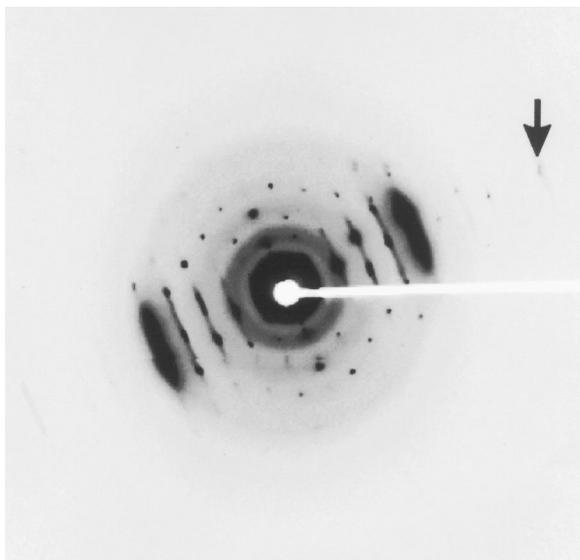
Fig. 4. Diffraction pattern of LDL-2 crystals. The arrow indicates a reflection at 15 Å resolution.

The diffraction pattern (Fig. 4) shows the strongly anisotropic diffraction of LDL-2 crystals. In one direction the diffraction pattern shows well-resolved series of discrete spots and single spots up to 15 Å. The intense arcs observed are superimposed by discrete spots, which becomes apparent when the exposure time for one frame is reduced considerably [12].

The diffraction data could be autoindexed by XDS. The Bravais lattice of the observed diffraction pattern indicates space group C2 with unit cell dimensions of $a = 183$ Å, $b = 421$ Å, $c = 385$ Å, $\alpha = \gamma = 90°$, $\beta \approx 90°$. Measured diffraction intensities at low resolution (Table 1) are also consistent with C222 or C222$_1$. Reducing the data in C222 produces similar merging statistics as data reduction in C2. However, the size of an individual LDL particle, which is believed to resemble a sphere of about 200 Å in diameter, does not allow for a model with 8 LDL particles (one per asymmetric unit) in a $183 \times 421 \times 385$ A**3 cell. Therefore we assume that an LDL particle has an approximate internal 2-fold symmetry axis which is oriented along a crystallographic axis, thus producing a diffraction pattern with pseudo-orthorhombic symmetry. Though the true space group must be monoclinic based on biochemical evidence, data reduction in an orthorhombic space group is also adequate at the current state and resolution of the analysis.

Table 1 shows the statistics for the diffraction data indexed in C2 in the range from 100–15 Å resolution. The whole data set (rotation range $\phi = 190°$, $\Delta\phi = 1°$) could be collected using only one crystal. The data is of good completeness and quality up to a resolution of 28 Å. Nevertheless a reasonable amount of reflections $> 3\sigma$ are found up to 15 Å. This is consistent with the observed strongly anisotropic scattering of the crystal.

Table 1
Merging statistics for a native data set of a LDL-2 measured at ESRF beamline ID14-3. Values were calculated using the program *xscale* of the XDS package [15]. Two summary statistics are given: 100–28 Å and 100–15 Å to account for the anisotropic scattering of the LDL crystals

Resolution (Å)	Observed	Unique	Unique ($I > 3\sigma$)	Completeness	Completeness ($I > 3\sigma$)	R_{int}	R_{int} ($I > 3\sigma$)
100–80	24	12	12	66.7%	66.7%	2.5%	2.5%
80–50	244	89	89	89.0%	89.0%	3.8%	3.8%
50–28	1910	543	441	93.8%	76.2%	5.7%	5.4%
100–28	2178	644	519	90.0%	72.5%	4.5%	4.3%
28–25	1007	268	92	94.0%	32.3%	56.3%	33.7%
25–20	3169	846	99	92.2%	10.8%	80.4%	39.8%
20–15	8836	2407	196	92.9%	7.6%	83.8%	36.9%
100–15	15190	4165	929	92.4%	20.6%	10.2%	4.7%

4. Discussion

We report the crystallization of two human LDL subfractions and a setup for the collection of data at very low resolution. Very narrow density cut subfractions of LDL are a major prerequisite for the crystallization of this lipoprotein. Although this particle contains a large amount of lipid, we were able to crystallize two subfractions (LDL-2 and LDL-5) which show different crystal forms, size and diffraction. The addition of additives resulted in the observation of a new dichroitic, more compact crystal form of LDL-2 (form B). Despite the better optical quality these crystals did not diffract, even to very low resolution. Whether this is due to the size of the crystals, an increased number of LDL particles in the asymmetric unit of the compact crystal or some sort of disorder, remains to be determined. Up to now LDL-2 crystals diffract to a resolution of 15 Å. Several reasons for this observation have to be discussed. These are a variability in the diameter of the particles arising from compositional heterogeneity with respect to lipids, glycosylation, or minor differences in the lipid/protein ratio. In addition the inherent flexibility of this particle may lead to positional disorder of apoB within the crystal lattice. Based on the observation of good merging statistics in space group C222 together with space filling considerations one might also assume a random orientation of the two similar halves of the LDL particle with respect to the pseudo noncrystallographic symmetry axis.

Standard experimental setups do not allow the collection of X-ray scattering data at resolutions below about 50 Å. This is a disadvantage especially for crystals with large unit cells and limited diffraction power, as one might miss the intense low order reflections, which give valuable information for the refinement of crystals otherwise classified as "non-diffracting". Therefore measures were taken to also allow for data collection in the 300–50 Å resolution range. In this way it was possible to adequately characterize the scattering behavior of LDL crystals grown under different conditions and obtain information on a possible internal pseudo-symmetry of LDL particles. Also, this type of experimental setup could be of interest for the collection of very low resolution data. The importance of these data for structure determination has probably been underestimated for a long time [16]. The major challenge will now be to improve the crystal quality in order to solve the molecular structure of apoB in LDL.

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