the layer at about $r = 2.0$ nm would be too small to accommodate the acyl or iso-octyl chains of the ordered cholesteryl ester molecules. The arguments for a single-layered organization are essentially the same as those discussed for a slightly smaller cholesterol-rich particle, HDL_c (Atkinson *et al.*, 1978).

For the proposed single-layered spherically symmetric organization of the ordered core in LDL₂, the separation of the two maxima of the radial electron density function is about 3.9 nm. This value is essentially the same as that found for other cholesterol-rich lipoproteins below the transition temperature. The constant separation of about 3.8 nm between the surface layer and the adjacent cholesteryl ester layer, independent of size and perhaps even of class of lipoproteins, may mean that the surface layer serves as a boundary or a template at which an ordered layer of cholesteryl esters is formed. Whether or not the ordering of lipids will take place depends on the conditions in the core (concentration of triacylglycerols, temperature, volume, etc.).

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The cholesteryl-ester core of porcine low-density lipoproteins

In the evaluation of X-ray data, model-dependent assumptions are normally avoided as far as possible until the state is reached where a given shape, size and electron-density distribution has to be filled with chemical constituents. Therefore, apart from the justifiable assumption of quasi-spherical symmetry (see discussion on pp. 3235 and 3236 of Jürgens *et al.*, 1981) our results on the structure of porcine LDL₂ are independent of *a priori* hypotheses. The electron-density profiles showing two maxima around 3.0 and 6.3 nm (Fig. 4 of Jürgens *et al.*, 1981), both for LDL₁ and LDL₂, have been obtained from direct deconvolution of the distance distribution function $p(r)$ (Glatter, 1981) involving no phase ambiguity and providing the optimal weighted-least-squares approximation to the experimental data. Thus, despite the intrinsic low-resolution nature of X-ray small-angle-scattering results, they are, at present, the most reliable data on porcine LDL structure and hence the yardstick against which any model of molecular architecture has to be put.

The primary criterion in the search for a suitable molecular model is the agreement between the volumes required for any chemical constituent and the volume available within the structure. In LDL₂, with 31.6% (by weight) cholesteryl esters and 3.6% triacylglycerols, and a molecular mass of $(2.0 \pm 0.2) \times 10^6$, the volume required for those apolar lipids is $(1.23 \pm 0.12) \times 10^3$ nm³, using a value of 1.06 cm³/g for their specific volume. Packed into a sphere, this would correspond to a radius of 6.6 nm. Note that this estimation involves no assumption on the molecular conformation. Errors in the specific volume are negligible as they are strongly diminished by the third-root correlation to the radius. The agreement of this radius with the dimensions of the temperature-sensitive domain in the radial electron density distributions is very good (see Fig. 3 in Jürgens *et al.*, 1981). Accepting that mainly the cholesteryl esters are responsible for the thermotropic behaviour of LDL, it must be concluded, as in all other cases of LDL subfractions, that the apolar constituents fill

Abbreviation used: LDL, low-density lipoprotein.

the particle core. The only electron-dense material is the cholesteryl-ring systems and therefore their locations correspond to the maxima at 3.0 and 6.3 nm.

Assuming, further, that the shells at these radii are only occupied by the cholesteryl esters (neglecting the small amount of triacylglycerols), an average area of 0.56 nm^2 per molecule is obtained, which is about 50% higher than the value in close crystal packing (Craven & De Titta, 1976). Since a radial arrangement involves different packing constraints from that in the antiparallel multibilayer crystal structure of cholesteryl myristate, this difference is not surprising; only the opposite would be a catastrophe which, incidentally, appears to be the case with the assumed shell at 4.6 nm invoked by Herak (1984). Furthermore, our results have shown that the outer electron-density peak at 6.3 nm has only one-third of the amplitude of the peak at 3.0 nm. This, indeed, confirms that, at least in the second shell, the cholesteryl moieties are not closely packed, contrary to Herak's (1984) basic assumption. Finally, it has to be emphasized that any surface-area calculations in a spherical particle are strongly (by the square) dependent on the radius, and hence error-prone.

With the notion of partial deviations from close packing, the inconsistency seemingly borne out by the surface-area calculations of Herak (1984) loses its substance.

Regarding the 2.7 nm separation between the second core-layer and the surface layer, no packing

difficulties arise, since this value is in good agreement with the thickness of a phospholipid monolayer into which apoprotein is interspersed. The polar constituents, protein, phospholipids and cholesterol, require about 80% of the volume of the outer shell ($\sim 3.1 \times 10^3 \text{ nm}^3$), as estimated from their specific volumes, thus allowing a reasonable amount of solvation.

Because of this consistency between required and available volumes on the one hand, and of the model-dependent nature of Herak's (1984) arguments on the other hand, there is no reason to consider a revision of our interpretation.

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