CONSTRUCTION OF A THREE-DIMENSIONAL ISO-DENSITY MAP OF THE LOW-DENSITY LIPOPROTEIN PARTICLE FROM HUMAN SERUM

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SUMMARY: A three-dimensional iso-density map of negatively stained low density lipoprotein (LDL) from human serum has been constructed. Iso-density data were collected from electron micrographs of LDL by scanning at one-micron intervals with a Tech/Ops Isodensitracer. Isodensity contour data were plotted directly on X-Y coordinates. A direct 50-fold enlargement of the particle was achieved with a significant reduction in the signal to noise ratio when compared to conventional enlargement methods. The best three-dimensional distribution of the isodensity data was that of a pentagonal-dodecahedron with isodensity maxima at the vertices. Based on this and other studies (1) these maxima were thought to represent protein subunits.

The low density lipoprotein (LDL, $1.020 < d < 1.063$) from human serum has been shown to possess dodecahedral symmetry (1). The designation was based on isolation of characteristic two-fold, three-fold, and five-fold rotation axes from electron micrographs of negatively stained LDL particles. In this paper the construction of a three-dimensional iso-density map of LDL is described. This map independently verifies the dodecahedral symmetry property and places the analysis of LDL substructure on a quantitative basis. The map is based on isodensitometric analysis of electron micrograph images of negatively stained LDL. A quantitative estimation of the optical density distribution was obtained with a resolution of 20-30 Å on the X-Y plane. Optical density maxima, previously thought to represent protein subunits (1), were found to be distributed on the vertices of a pentagonal dodecahedron.

METHODS

Lipoproteins: Low density lipoproteins from human serum were prepared as previously described (1), and by flotation from human plasma using sucrose to modify density.

Electron Microscopy: LDL, PTA (phosphotungstic acid), and fenestrated grids were prepared as previously described (1). Electron micrographs of negatively stained LDL particles were obtained as previously described (1).

Isodensitometry: Single particles from both original electron micrographs and photographic enlargements were scanned on a Tech/Ops Isodensitracer (Tech/Ops, Burlington, Mass.) with optical density (O.D.) increments of .025-.06 O.D., and a magnification of 50X. Optical densities were collected at one-micron intervals, and displayed on an X-Y recorder. The display on the X-Y recorder consisted of isodensity contours corresponding to the distribution of stain on the particle being examined. A nearby region devoid of particles was scanned in order to provide a reference for background density. Data were also collected on magnetic tape.

RESULTS

Figure 1A shows a highly magnified view of an LDL particle, enlarged about 20-fold by conventional photographic procedures. An isodensity contour

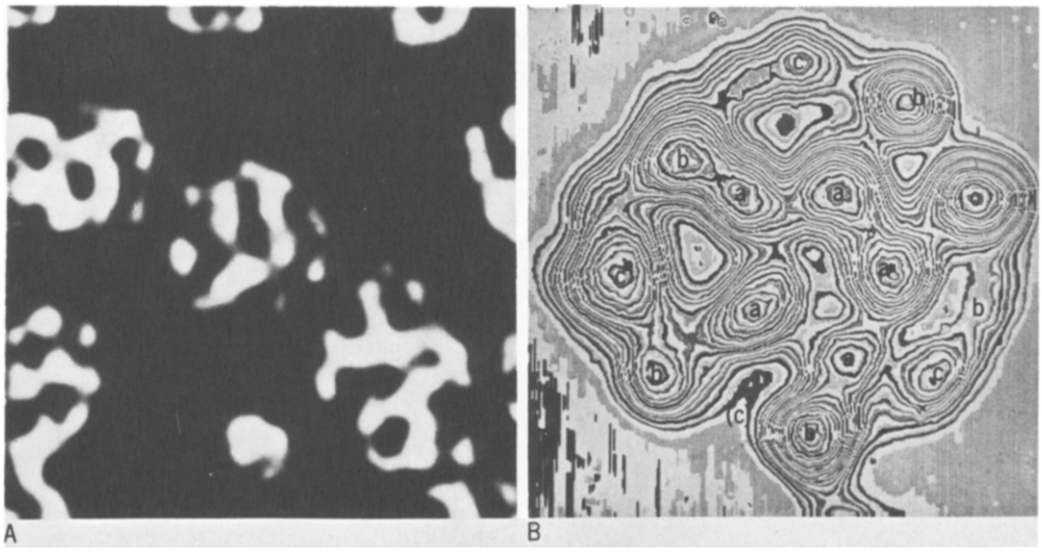

Figure 1 (A). A twenty-fold enlargement of an LDL particle produced by conventional photographic means, (B) A fifty-fold enlargement of the particle seen in Fig. 1A, made directly from the electron micrograph. Contour lines indicate isodensity maxima.

map of the same particle is shown in Figure 1B. The view is down a five-fold axis, and individual isodensity maxima are denoted by "a," "b," or "c." The map represents a 50-fold enlargement from the original image.

The distribution of optical density in an electron micrograph of a negatively stained particle represents a planar projection (2) of all regions of the particle that are inaccessible to the negative stain (3). Therefore, the approach to the analysis of the isodensity contour map was to compare it to planar projections of various geometric figures. The choice of a "one-sided" particle that was approximately oriented with regard to a specific rotational axis facilitated analysis. It was found that the isodensity maxima were quantitatively distributed according to co-ordinates for the twenty vertices of a pentagonal dodecahedron.

The first step in the analysis was to compare the planar projection of a regular pentagonal dodecahedron, as viewed down a five-fold axis, with the isodensity contour map of LDL. Figure 2A shows a view of a pentagonal dodecahedron as seen along a two-fold axis. By assuming that the particle is analogous to a unit cell in a crystal, we can designate particles in planes perpendicular to the five-fold axis as constituting pseudo-lattice planes. These are indicated by "a," "b," "c" or "d" in Figure 2A. Figure 2B is a planar projection of the dodecahedron down a five-fold axis with small circles indicating vertices. Vertices belonging to different pseudo-lattice planes shown in Figure 2A are labeled accordingly. A comparison of Figures 1B and 2B reveals a quantitative relation between the planar isodensity map of the LDL particle and the planar projection of the dodecahedron as viewed down a five-fold axis.

As a second step in the analysis, isodensity maxima in the two-dimensional isodensity map were assigned to specific spatial co-ordinates. This was accomplished by tracing "a," "b," and "c" contours on different sheets of lucite and positioning them according to the "a," "b," and "c" pseudo-lattice planes shown in Figure 2A. Continuing the analogy of the particle to a unit cell of

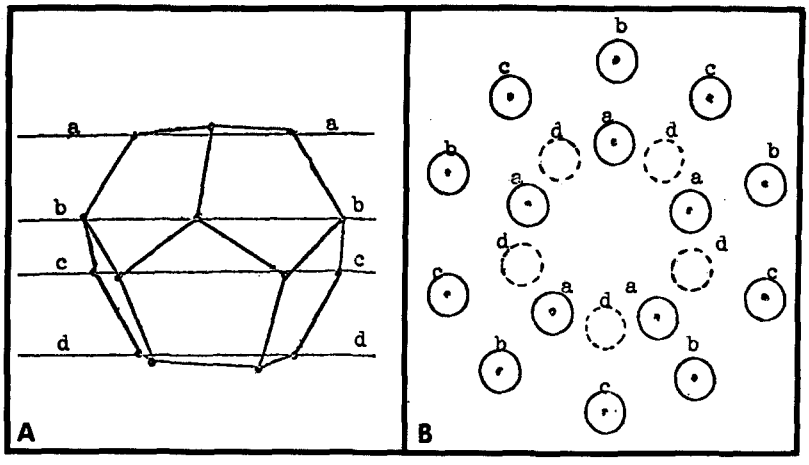

Figure 2 (A). View of the two-fold axis of a pentagonal dodecahedron showing pseudo-lattice planes (——) perpendicular to a five-fold axis. (B) Planar projection of a pentagonal dodecahedron form a five-fold axis with vertices indicated by small circles. Vertices intercepted by each of the pseudo-lattice planes shown in Figure 2A are indicated by either "a," "b," or "c."

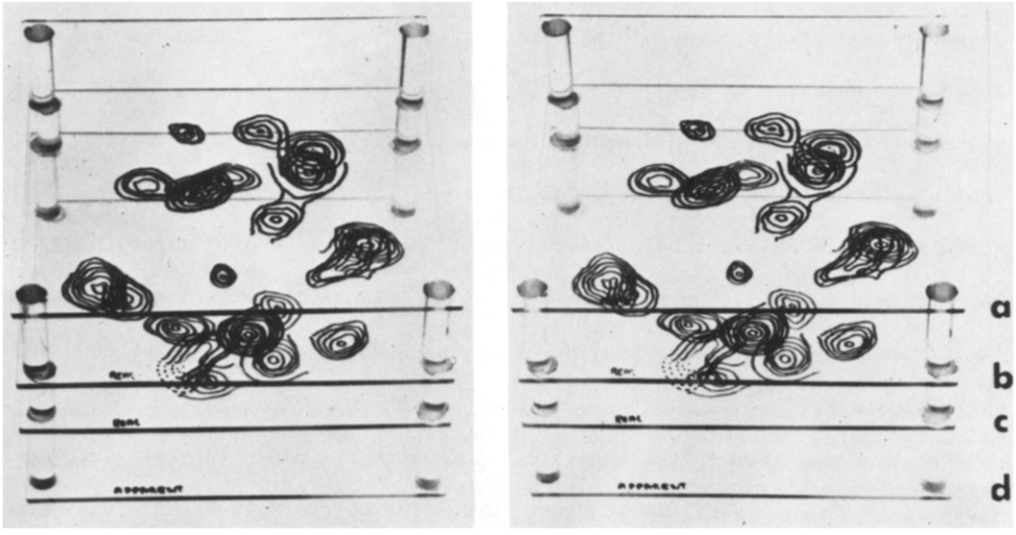

Figure 3. Stereo photographs of the three-dimensional reconstruction of LDL based on the two-dimensional isodensity map shown in Figure 1B. Convenient viewing is achieved with a stereo viewer (Taylor-merchant Corp., N.Y., N.Y.).

a crystal, we were able to define the distance between opposite faces as 2π, and fractional distances as fraction of 2π. This allowed us to make an explicit designation of the phase for each pseudo-lattice plane. The resulting three-dimensional structure is shown in stereo in Figure 3.

The three-dimensional isodensity map in Figure 3 contains a "d" layer of isodensity contours labelled "apparent." By reference to Figure 2B, it is seen that the pentagons on the "a" pseudo-lattice and the "d" pseudo lattice differ by $2\pi/10$ radians. However, the planar isodensity contour map of LDL shown in Figure 1B shows only one of the two possible sets of pentagons. Due to the fortunate circumstance that many PTA-stained particle images are one-sided (3), the bottom parts of particles are not often clearly superimposed on the top parts of particles. This was the case for all LDL images selected for analysis. However, two-fold axis-projections contained isodensity maxima

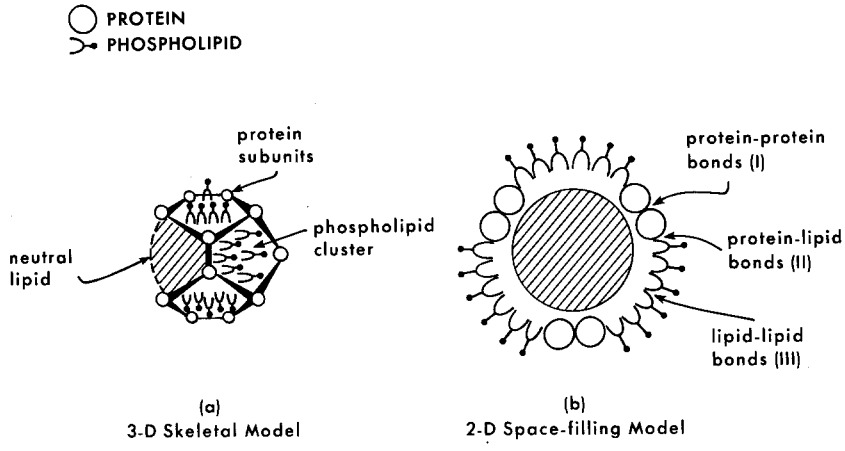

Figure 4. (a) A three-dimensional skeletal model for the LDL molecule, with protein subunits in dodecahedral array. Subunits are drawn smaller than their true proportion to emphasize the surface network of protein. The holes in the protein network are filled with clusters of phospholipids. Only a few of the approximately 60 lipid molecules per face are shown. Delipidation or digestion with lipases distorts the symmetry but leaves subunits clearly delineated. The cross-hatched central core is neutral lipid. Free cholesterol is assumed to be associated with phospholipid. (b) A two-dimensional space-filling model of the LDL molecule with protein subunits and phospholipids drawn in perspective. Interactions between protein subunits (I), among phospholipids (II), and between protein and lipid (III) are specified. The relation of neutral lipid to the other components is not well understood. Subunits interact at two-fold axes, while lipid-filled holes occur at five-fold axes of the dodecahedron.

of the "a" and "d" pseudo lattice planes.

Of the particles studied, most have proved to be slightly distorted from the form expected of a perfect dodecahedron. The distortion, as seen in Figure 3, consists mainly of twisted pentagonal faces. The diameter of the particle was found to be 200 Å ± 5 Å when the perimeter chosen was one that just included all isodensity maxima. Many isodensity maxima, probably representing protein subunits as previously shown (1), were found to have three-fold axes.

DISCUSSION

The structure of LDL has been of great interest, not only to students of lipid transport and metabolism, but also to those interested in lipid-protein interactions in general. Previously, it was shown that LDL possessed dodecahedral symmetry, and it was proposed that protein subunits were located on the twenty vertices of the dodecahedron. The present study verifies the dodecahedral symmetry property in an independent fashion. It also places the analysis of substructure on a quantitative basis, since there are precisely twenty isodensity maxima in the isodensity map. The isodensity maxima are thought to represent protein subunits. The subunits probably interact, as indicated by the local three-fold symmetry axes, thereby forming a network. Phospholipids would be located in the holes of this dodecahedral network as previously proposed (1). The central core would contain the neutral lipid component. These concepts are represented in Figure 5, where LDL models are shown in two and three dimensions.

The particles that have been mapped have, in general, had distorted forms. This may represent drying artifacts, or may represent the inherent instability of the LDL structure. In any event, the LDL structure is not as regular as some viral structures (2). In addition, some subunits appear to be assymetric. However, definitive information on this point will await the construction of maps of higher resolution.

The technique used to generate the isodensity map of LDL from electron

micrographs is quite general. The analysis neither depends upon specimen translation and re-photography, as do the Markham procedures (4), nor presupposes any special symmetry. The data is appropriate for eventual use in Fourier reconstructions once the symmetry principle is known, though the possession of symmetry is not essential (2). Future experiments on LDL will be directed towards analysis by Fourier reconstruction.

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