THE ELECTRON MICROSCOPY OF FERRITIN CRYSTALS

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Ferritin is a protein that readily forms crystals which can successfully be examined by the electron microscopic methods for visualizing their molecules. The work described here demonstrates the molecular arrangement thus determined.

METHODS

Ferritin was obtained from fresh horse spleen and subsequently crystallized[1] from solutions containing $CdSO_4$. The crystals that rapidly form when a concentrated aqueous solution is made 4–5 % with respect to $CdSO_4$ are exceedingly soft, optically isotropic octahedra. Under the optical

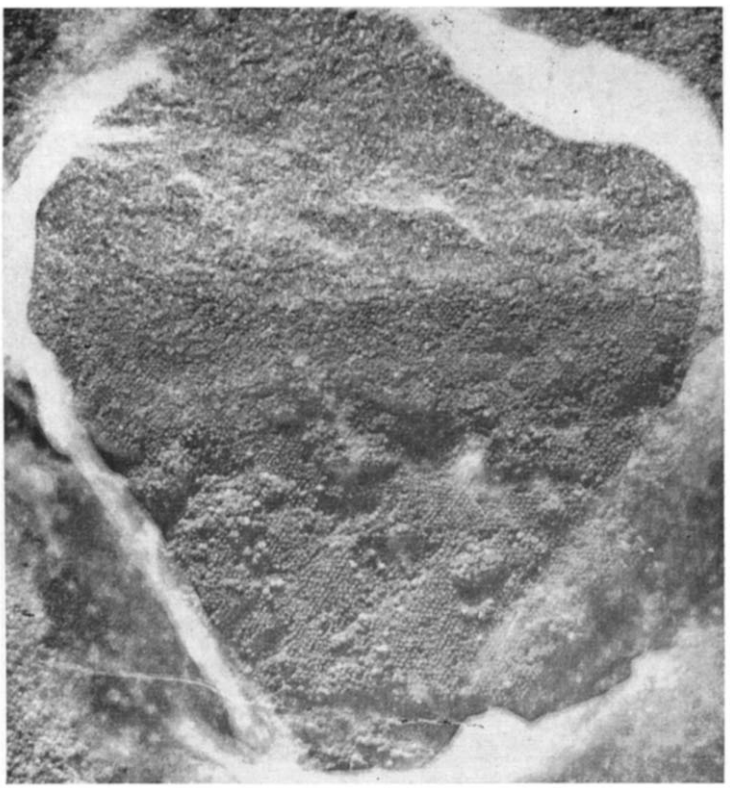

Fig. 1. An octahedral crystal of ferritin showing the rough character of its faces, on one of which the molecular order is clearly visible. Magnification 65,000 ×.

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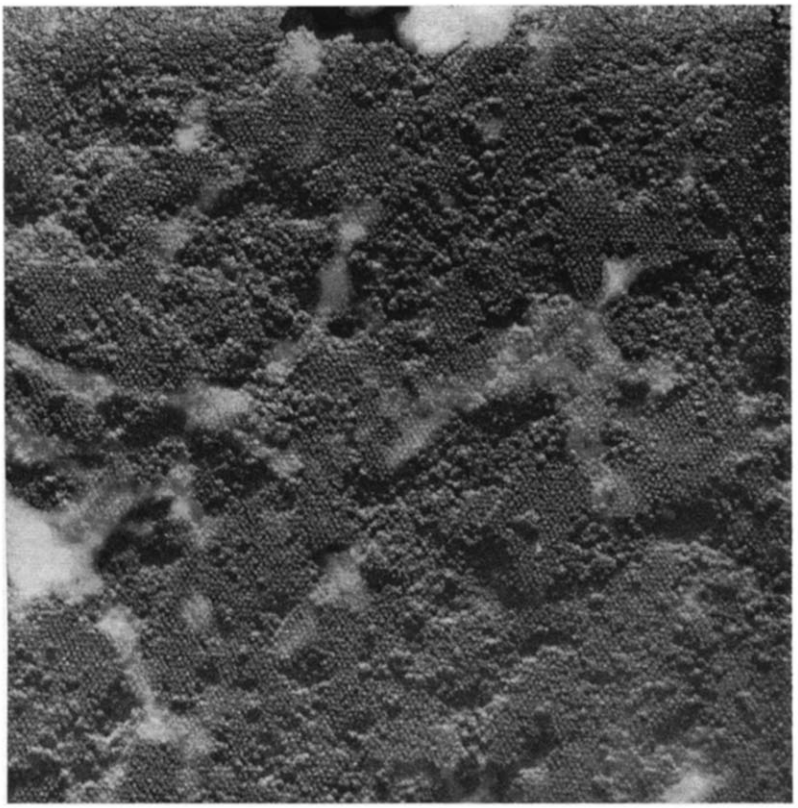

Fig. 2. Part of an octahedral face seen at a higher magnification. The edge between two faces is visible at the top of the photograph. 75,000 ×.

microscope they seem moderately well-formed though with faces that are rather rough and with edges that are rounded. They were not improved by recrystallization. The different but still isotropic crystals that form when only 1 % of $CdSO_4$ is present are flat plates bounded by very poorly developed faces. Both types were examined.

The preparations for electron microscopy were the evaporated carbon replicas previously described[2]. Crystals to be replicated were deposited on collodion-covered grids by allowing microdrops of a suspension to stand on them for a few seconds. These deposits were then very lightly shadowed with palladium and covered with a vertically evaporated layer of graphite about 100 Å thick. After dissolution of the collodion and of the crystals this combined metal-carbon layer was ready for examination.

RESULTS

At electron microscopic magnifications, the irregularity of all the faces on ferritin crystals is very apparent. They are everywhere badly pitted and large areas are overlaid with disordered molecular deposits. Nevertheless, it is not hard to find regions of order and to see the molecular arrangement that prevails in both types of crystal. A typical octahedral crystal is shown in Fig. 1; part of a face on such a crystal appears at a somewhat higher magnification in Fig. 2. Its molecules form a close packed hexagonal net with lines of the net parallel to the triangular edges of the face. Measurements of the particle separation on many of these crystals has

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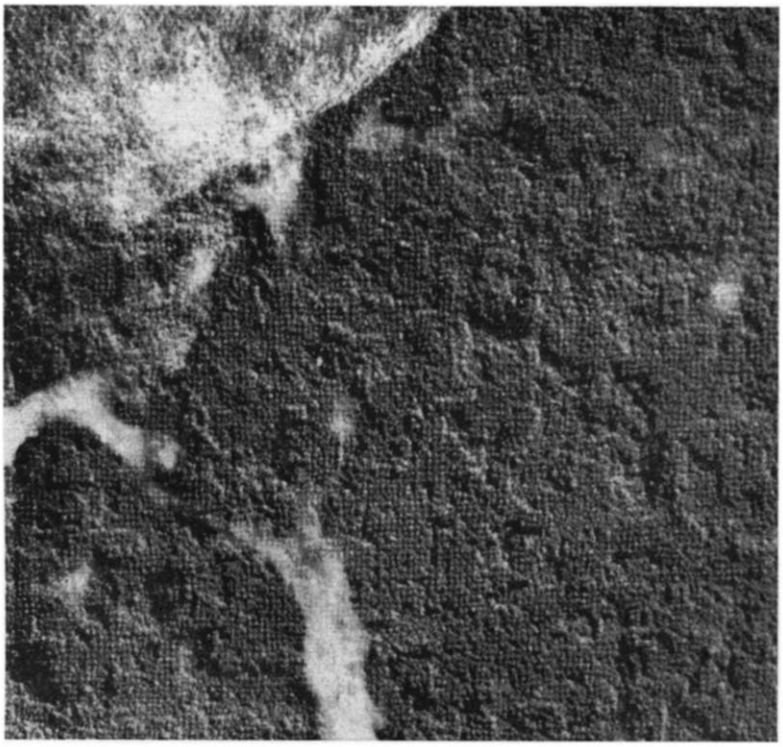

Fig. 3. Part of one of the large faces that bound the flat plates from 1 % $CdSO_4$ showing its square molecular net. 75,000 ×.

Fig. 4. Part of a dodecahedral face of a ferritin crystal showing several regions of unobscured molecular order. 75,000 ×.

led to a value of 109 A $\pm$ *ca.* 4 A. This is the distribution to be expected if the molecules are in a cubic close packed array throughout the crystal, the length of edge of the tetramolecular unit cube being 154 A.

Crystals of other proteins showing this octahedral habit commonly have their corners replaced by small cubic faces but such (100) faces have been sought without success on these ferritin crystals. Examination of the flat crystals grown from 1% $CdSO_4$, however, shows that the molecules on their surfaces are arranged in the square net characteristic of such (100) faces (Fig. 3). The separations along the edges of the net as measured on a number of these crystals is the same as that found on the octahedral crystals; this fact combined with the optically isotropic character of both the plates and the octahedra makes it probable that they represent two crystalline habits of the same molecular arrangement. Octahedral faces do not occur on the flat crystals but occasionally a face has been seen which has the molecular distribution shown in Fig. 4. It consists of parallel rows of contacting molecules separated from one another by an appreciable distance. As measured on photographs such as this the contacting molecules are, as before, 110 A apart while the distance between rows proves to be 154 A. This molecular distribution is that to be expected on the dodecahedral, (110), face of a crystal composed of cubic close packed molecules and the inter-row distance is in accord with this $-154\text{ A} = \sqrt{2} \times 110\text{ A}$. The molecular diameter corresponding to this separation is, as might be expected, near to but somewhat larger than the best measurements on electron micrographs of individual molecules[3].

The foregoing electron microscopic observations agree well with the X-ray diffraction measurements that have been carried out. Early studies[4] made the octahedral crystals cubic close packed (as here) with $a_0 = 163$ A for dry and 186 A for wet crystals. More recently[5] they have been reported as orthorhombic with the practically cubic dimensions $a_0 = b_0 = 112$ A, $c_0 = 158.5$ A for the dry and $a_0 = b_0 = 131.5$ A, $c_0 = 186$ A for the crystals when wet. The present electron microscopic observations demonstrate a conformity between crystal outlines and molecular order that proves the molecular arrangement to be the same in the wet and dry crystals. They also show that any orthorhombic departure from the cubic close packing which exists is below the limit of accuracy of the electron microscopic measurements.

SUMMARY

Electron microscopic observations on carbon replicas of dried crystals of the protein ferritin show that, within the limit of measurement, their molecules are in a cubic close packing with $a_0 = 154$ A. The same arrangement has been found for the octahedra crystallized from 5% $CdSO_4$ and the flat plates from 1% $CdSO_4$.

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