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ELECTRON MICROSCOPY OF CRYSTALLINE EDESTIN

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Existing electron microscopes, when properly adjusted, are capable of resolutions of about 20 Å or better, thus permitting the direct observation of spherical protein particles having molecular weights of about 4000. However, adequate instrumental resolution alone does not insure that the information recorded will be useful; the images of randomly disposed minute particles which are commonly observed in protein preparations are likely to be of dubious value unless they can be identified and characterized. The value and reliability of electron microscope observations are much increased if molecules can be observed in crystalline configurations in which they produce identifiable and accurately measurable patterns. Although the macromolecular units of virus crystals have been successfully observed by Wyckoff (1) and others, none of the non-virus proteins of smaller molecular dimensions has hitherto been studied in the crystalline state by electron microscopy. Edestin crystals were chosen for the initial experiments because of their relatively high stability and because direct quantitative evidence concerning the dimensions of the edestin molecule is of interest, as it bears on numerous previous experiments on the same protein by indirect methods.

EXPERIMENTAL

Methods—Crystalline edestin was extracted from hemp seed according to the method described by Bailey (2) and recrystallized up to six times. Specimens for electron microscopy were prepared by dissolving the crystalline protein in 2.5 per cent NaCl at 55° and applying 1 drop of the warm solution to a conventional electron microscope specimen screen with collodion film. Since the solubility of edestin decreases rapidly under about 35°, crystals form in a few minutes on the supporting film as the drop cools. In order to remove salt and uncrystallized protein, the specimen was flushed quickly with a stream of water from a medicine dropper, washed with 75 per cent alcohol, and dried.

Crystalline globulin from squash seed (Hubbard squash) was prepared according to a method described by Vickery et al. (3). Crystals for electron microscopy were obtained at room temperature by adding water slowly to a solution of the protein in 10 per cent NaCl until a visible precipitate formed. 1 drop of the crystallizing suspension was then applied to a conventional specimen mount, washed, and dried.
In preliminary experiments, specimens were shadowed and observed directly, but electron scattering by the underlying protein was too great to permit satisfactory observation of the metallized surface structures. The difficulty was overcome by evaporating a thin film of SiO at normal incidence over the shadowed surface and subsequently removing the collodion film with acetone and the protein by immersing the specimen in 0.05 N HCl for 1 or 2 minutes. The metal always adheres to the SiO film when the underlying material is removed. Although the original collodion film is between the SiO and the metal grid, the transfer film is never loosened from its support, probably because acetone does not penetrate much beyond the open areas. Metals such as U, Ni, Cr, and Pt were used for shadowing, usually with a shadow to height ratio of 3:1 and in calculated weights of about $2.5 \times 10^{-6}$ gm. per sq. cm. or less in the plane of the specimen. SiO was applied in weights of between 1.0 and $1.5 \times 10^{-6}$ gm. per sq. cm. The rigidity of SiO and the absence of manipulative procedures on unsupported thin films are deemed advantages of the method. It is noteworthy that the method may also be used for the examination of frozen aqueous substances (4), which suggests the possibility of examining protein crystal surfaces without removing water of crystallization.

Electron Microscope Results—Edestin crystals, grown on a collodion film in this manner, appear most commonly as equilateral triangles with truncated corners, as shown in Fig. 1, although occasionally twinned forms and crystals with hexagonal outline occur. Significant portions showing molecular nets on the crystals of Fig. 1 have been circumscribed and reproduced at higher magnification below the micrograph. Although there is considerable disorder in parts of the triangular faces, due either to distortion produced by drying or to non-crystalline surface deposits, areas are always visible showing well defined hexagonal nets with rows of adjacent molecules oriented parallel to the triangle edges as shown in Figs. 1, a and c. On sloping faces at truncated corners the net is rectangular, as shown in the enlarged portion, Fig. 1, b. So far as can be determined from the heights of steps as estimated from shadow lengths and from examination of individual particles, the molecules appear to be approximately spherical, about 80 Å in diameter. From a study of these features, it has been concluded, as has been previously reported in brief (5), that the observed structures closely approximate orthographic projections of a face-centered cubic lattice, with the triangular faces representing (111) planes and the rectangular nets at the truncated corners representing contiguous (100) planes. A photograph of a model of a crystal as deduced from the electron microscope observations is shown in Fig. 2.

On the assumption that the lattice is face-centered cubic, it is possible to calculate the dimensions of the unit cell from measurements on the
The perpendicular distance between rows of molecules may be obtained with fair accuracy by measuring the distance across several adjacent rows and dividing by the total number. In over thirty such measurements from different crystals the distance varied between 68 and 72 A, with an average of 69.7 A. It is readily calculated from this that $a_o = 114$ A. The distance between the prominent rows in the (100) face, as shown in the rectangular inset in Fig. 1, is about 80 A, which is, of course, consistent. According to the simplified model shown in Fig. 2, the diameter of the molecules in the dry state is close to 80 A, but perfect sphericity in the actual crystals is unlikely.

From the dimension of the unit cell, together with a value for the crystal density, the dry molecular weight of edestin may be calculated. Published values for the density of dry edestin crystals vary between about 1.30 and 1.35 (6), according to the methods used, yielding values for the molecular weight of 290,000 and 300,000, respectively, which are in excellent agreement with the value of 310,000 reported by Svedberg and Pedersen (7) from ultracentrifugal data.

An interesting feature, apparent in Fig. 1, is the tendency for a monolayer to grow out over the background from triangular crystal edges. The structure is two-dimensional, approximating hexagonal packing close to the crystal edge and tending gradually toward a rectangular pattern away from the crystal. The general background is mostly non-crystalline although occasionally small areas exhibit hexagonal patterns, evidently where crystals are beginning to form.

The crystal structure of the similar globulin from squash seed (3) is identical with that described for edestin, as far as the electron microscope can show. The perpendicular distance between rows of molecules on the (111) faces varied from 62 to 79 A, with an average of 69.4 A.

**X-Ray Results**—X-ray powder patterns of edestin crystals from the same preparation used for electron microscopy were made by Dr. R. S. Bear using a small angle camera. Crystals were dispersed in 2.5 per cent NaCl, sealed in a glass capillary about 1 mm. in diameter with a 25 μ wall, and exposed to copper radiation ($\lambda = 1.54$ A) through a nickel filter. A magnified reproduction of the inner part of a pattern taken at 9.9 cm. from the specimen is shown in Fig. 3. The first three relatively intense rings are visible in the reproduction and several others, mostly of lower intensity, have been observed at wider angles on the original films. Results of measurements on the first four rings are listed in Table I, where the final column shows the calculated value of $a_o$ for each of the rings, on the assumption that the lattice is cubic and that the appropriate indices are those listed in the fourth column. Values for $a_o$ are consistent within limits of experimental error and, since sets of indices are all odd or all
even, the results suggest a face-centered cubic lattice. The average value for \( a \) is 140 Å. Other rings, not listed, are too faint or broad to allow unequivocal assignment of indices, but in all instances, indices consistent with the above results may be assigned within experimental error.

The difference between the x-ray and electron microscope values for the unit cell dimensions is attributed to hydration. The water content of wet edestin crystals has been measured by Bailey (2), whose results show that wet crystals contain about 39 per cent water by weight. If the density of the dry protein is assumed to be 1.35 and the cell dimension for the perfectly dry crystals is 114 Å, it is readily calculated that this quantity of water would increase the dimension to 140 Å. In the preparative procedure for electron microscopy, the crystals were placed in a vacuum and probably heated slightly by radiant heat from the evaporation filaments. There is no assurance that the crystals were completely dehydrated, but the close correspondence between the wet cell dimension calculated from electron microscope data and that determined directly by x-ray diffraction indicates that the value of 114 Å applies to the protein in a state of thorough dehydration.

### DISCUSSION

Although the molecular weight of edestin calculated from electron microscopy agrees with that determined from ultracentrifugal data, the conclusion that the edestin molecule is approximately spherical is at variance with deductions regarding its shape based on measurements of

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**TABLE I**

<table>
<thead>
<tr>
<th>Intensity</th>
<th>( \frac{2 \sin \theta}{\lambda} )</th>
<th>( h + k + l )</th>
<th>( (hkl) )</th>
<th>( a_0 = \frac{\lambda}{2 \sin \theta} \sqrt{h^2 + k^2 + l^2} )</th>
<th>Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very strong</td>
<td>0.0124</td>
<td>3</td>
<td>(111)</td>
<td>140</td>
<td></td>
</tr>
<tr>
<td>Strong</td>
<td>0.0201</td>
<td>8</td>
<td>(220)</td>
<td>141</td>
<td></td>
</tr>
<tr>
<td>Weak</td>
<td>0.0310</td>
<td>19</td>
<td>(331)</td>
<td>141</td>
<td></td>
</tr>
<tr>
<td>Very weak</td>
<td>0.0406</td>
<td>32</td>
<td>(440)</td>
<td>139</td>
<td></td>
</tr>
</tbody>
</table>

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**Fig. 1.** Electron micrograph showing surface structure of edestin crystals shadowed with nickel. \( \times 100,000 \). Circumscribed areas \( a, b, \) and \( c \) are shown below the micrograph at a magnification of \( \times 300,000 \).

**Fig. 2.** Model of edestin crystal with a face-centered cubic lattice, exposing a (111) face.

**Fig. 3.** Central portion of 9.6 cm. x-ray powder pattern of wet edestin crystals, enlarged 3 times. The inner ring corresponds to an 81 A spacing.
the dielectric dispersion of edestin solutions. Oncley (8) has reported that such data indicate the molecule has an axial ratio of 9:1 when dissolved in 2 M glycine. Also, frictional ratios obtained from sedimentation experiments may be interpreted as evidence of an asymmetry ratio as high as 4:1, depending on assumptions concerning hydration of the molecule (6). However, if a rather high degree of hydration is assumed for the molecule in solution, these latter data would be consistent with a nearly spherical molecule, in agreement with the electron microscope data. Electron microscope observations, either on edestin crystals or on the non-crystalline background material, are incompatible with a high degree of asymmetry. Conceivably the shape is different in solution, but such an explanation would not be acceptable without independent proof. It would be of considerable interest to establish the reason for the apparent discrepancy between the methods.

The conclusions concerning the structure of edestin crystals derived from electron micrographs are strengthened by the agreement with available x-ray data, when allowance is made for differences in hydration. It is to be noted, however, that the x-ray data are not so extensive or precise that they provide a unique interpretation by themselves. Since it is often the case that x-ray patterns of proteins are not well developed, the value of complementary evidence from electron microscopy in this type of investigation is apparent.

No previous small angle x-ray results have been reported on crystalline edestin. Crowfoot and Fankuchen (9) obtained powder patterns from air-dried crystals of a similar globulin from tobacco seed and concluded that the observed diffractions are consistent with a face-centered cubic lattice having $a_0 = 123$ A. In a later review, however, Fankuchen (10) expressed some dissatisfaction with the quality of the x-ray patterns on which the conclusions were based. Since the results on tobacco seed globulin as reported are close to those found for edestin and squash seed globulin, it seems probable that the three are similar in structure, but additional electron microscope or x-ray data on tobacco seed globulin would be desirable.

It is of interest to add that, following this initial investigation, the methods described have been applied to a number of other crystalline proteins. Electron micrographs have been obtained showing regular and extensive molecular patterns on the faces of crystalline canavalin, concanavalin B, and catalase, to cite examples which will be described later. The results indicate that the electron microscope is capable of providing precise information regarding the size and shape of molecules in protein crystals.
SUMMARY

1. Electron micrographs are described which show the molecular configuration at the surface of edestin crystals. It is concluded that the lattice is face-centered cubic with $a_o = 114$ Å in the dry state.

2. The edestin molecule appears as a particle about 80 Å in diameter. With an assumed density of 1.35, the molecular weight is calculated as 300,000 in the dry state, in good agreement with values deduced from ultracentrifugal data.

3. Measurements from x-ray powder patterns of wet crystals are presented, indicating a face-centered cubic lattice having $a_o = 140$ Å, which larger value is consistent with published data concerning the water content of hydrated edestin crystals.

4. As far as the electron micrographs can show, the structure of crystals of squash seed globulin (Hubbard squash) is identical with that of edestin.

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BIBLIOGRAPHY