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Abstract

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X-RAY STRUCTURAL STUDIES OF PEPSIN

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For the correct explanation of the functional properties of a protein, knowledge of its complete structure is necessary. The complete structure of a protein molecule, with indication of the spatial position of all atoms, can be established by only one method—the method of X-ray structural crystallography. Although its application involves great expenditures of labor, the brilliant results obtained in recent years in studies of the structure of hemoglobin and myoglobin give grounds to hope for the successful application of this method to determine the structure of other proteins as well.

The object of our investigations is the proteolytic enzyme pepsin. It has a molecular weight of 34,500 and, apparently, consists of a single polypeptide chain containing 327-343 amino-acid residues ([1,2]).

Pepsin was one of the first proteins obtained in crystalline form ([3]). Using pepsin as an example, Bernal and Crowfoot first showed that single crystals of proteins give good diffraction patterns ([4]). However, up to now this protein has not been the object of detailed X-ray structural investigations. Apparently, this is explained by the fact that the crystalline modification of pepsin described by Bernal and Crowfoot ([4]), and then by Perutz ([5]), belongs to the hexagonal system and contains 12 molecules in the unit cell. The large number of protein molecules in the unit cell considerably complicates the conduct of complete investigations.

We attempted to study another crystalline modification of pepsin, which was described by Northrop as "plate-needle-like" ([6]), in contrast to the "bipyramidal" ([3]) modification described by Bernal and Crowfoot. This modification is obtained by crystallizing pepsin from alcoholic solutions in an acidic medium.

We studied porcine pepsin. It was purified by the method described in the work of Stepanov and Greil'([7]). Crystallization was carried out by Northrop's method ([6]) from a 20% ethyl-alcohol solution at an optimal protein concentration of 0.8-1%. The alcoholic solution of pepsin was acidified with 2.5 N H_2SO_4 to pH 2, then filtered. The filtrate was placed in a desiccator and left at room temperature. Usually the crystals precipitated on the third day.

The largest plates obtained by this method were used for X-ray structural investigations. For this purpose the crystal was placed in a sealed capillary with wall thickness ~ 0.01 mm, in which the humidity necessary for preserving the crystal was maintained. The crystals were photographed on Lemazhikhin and Lebedev ([8]) fine-focus X-ray tubes in precision cameras using CuK_α radiation at 37 kV, 4 mA. The precision cameras were made through collaboration at the Institute of Inorganic Chemistry of the Slovak Academy of Sciences according to the designs of Dr. F. Hanic and I. Maďar. Precision cameras developed and manufactured at the Institute of Crystallography of the Academy of Sciences of the USSR were also used. Zero-layer Weissenberg photographs were obtained.

of the reciprocal lattice, the cell parameters were measured, and the extinctions of the space group were determined.

The following data were obtained:

$$a = 55.6 \pm 0.4 \text{ Å}; \qquad b = 36.5 \pm 0.3 \text{ Å};$$

$$c = 74.5 \pm 0.6 \text{ Å}; \qquad \beta = 74 \pm 1^\circ.$$

Space group $P2_1$. The volume of the unit cell is $V = 145\,000 \pm 4000$ Å^3.

As is known, the unit cell of a protein crystal always contains some amount of crystallization liquid, the loss of which leads to a sharp increase in the defectiveness of the crystal lattice. The density of dried and wet pepsin crystals was measured by the gradient-column method ([9]) (composition of the mixture: bromobenzene–benzene–xylene).

The density of dry crystals is $\rho_c = 1.287 \pm 0.003$ g/cm^3.

The density of wet crystals is $\rho_b = 1.15$–1.21 g/cm^3. The density of wet crystals is determined with a larger error, since, when liquid is removed from the surface of the crystals, part of the crystallization liquid is inevitably lost.

The density of the mother liquor is $d = 0.97$ g/cm^3.

These data make it possible to determine the weight content of protein and crystallization liquid in a pepsin crystal. The protein content is found to be 64–81%. Direct weighing of wet and dried crystals gives a value for this quantity of 65–76%.

Calculations show that the weight of the protein component in the unit cell lies within the limits 63 000–84 000. This permits the assertion that the unit cell contains two molecules. Thus, the molecules occupy general positions in the unit cell. Comparing this result with the chemical data, one may conclude that they possess no intrinsic symmetry.

Since the unit cell contains only two molecules, the monoclinic modification may prove to be a very convenient object for complete X-ray structural investigations. For the successful performance of these studies, it is necessary to obtain sufficiently large isomorphous crystals of pepsin derivatives with atoms of heavy metals.

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Note: Figure translations are in progress. See original paper for figures.