Molecular Symmetry of Fructose-1,6-diphosphatase by X-ray Diffraction Analysis*

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Large single crystals of rabbit liver fructose-1,6-diphosphatase suitable for a high resolution structure analysis have been grown from polyethylene glycol. The space group of these crystals is 1222 with a = 75 Å, b = 81 Å, and c = 132 Å and there are 2 tetrameric molecules in the unit cell. These crystals have one protein subunit as the crystallographic asymmetric unit and establish point group symmetry 222 as the molecular symmetry.

Large single crystals of fructose-1,6-diphosphatase from chicken and turkey liver in both alkaline form (1) and neutral form (2) have been reported. In spite of the proteolytic cleavage that occurs in conversion of the neutral to the alkaline form of this enzyme (1), the diffraction patterns indicated that the two forms are virtually isomorphous. In both cases, the crystals, although quite large, gave diffraction patterns limited to approximately 3.0 Å resolution and suffered substantial radiation damage. In addition, the molecule crystallized with an entire tetramer of 144,000 daltons as the asymmetric unit. Using enzyme from the liver of rabbit, we have obtained a new crystal form of the protein which seems ideally suited for a high resolution structure analysis and, furthermore, reveals the precise symmetry arrangement of the protein subunits.

EXPERIMENTAL PROCEDURES

Materials—Polyethylene glycol was from Fisher Scientific Co. and was not further purified. All other chemicals were of reagent grade including the dioxane. The purified enzyme was provided by Drs. O. Teolas and B. L. Horecker of the Roche Institute of Molecular Biology, Nutley, N.J.

Methods—Crystallization: the enzyme was concentrated to 7 mg/ml and equilibrated with 0.01 M Tris/HCl at pH 7.6 by vacuum dialysis. Crystallization was effected by vapor diffusion of 20-μl droplets initially 3.5 mg/ml in protein and 5% polyethylene glycol 4000 against 0.5-ml reservoirs of 10% polyethylene glycol 4000 using the hanging drop method (see Ref. 3). The crystals, shown in Fig. 1, grew as thick rods having the cross-sections of a rhombus in from 2 days to a week.

Photography—Crystals were sealed in quartz capillaries by conven-
To understand the symmetry of fructose-1,6-diphosphatase, we need to consider the crystallographic unit cell. In this context, the 2 molecules of fructose-1,6-diphosphatase must be centered on points (0, 0, 0) and (l/2, l/2, l/2) in the unit cell and each contribute one protein subunit as a crystallographic asymmetric unit. This has two implications: (a) the molecule must have point group symmetry 222, i.e., have its four identical subunits related by three mutually perpendicular dyad axes; and (b) the space group of the crystals must be I222 and not I2,2,2.

The unit cell dimensions of a ~ b ~ c ~ 75 Å, the occurrence of this same approximate dimension of 75 Å in the turkey liver enzyme crystals (1), and packing considerations strongly suggest that the tetrameric molecule is roughly spherical with a diameter of approximately 75 Å.

The crystals diffract to a resolution of at least 2.5 Å and seem imminently suitable for a high resolution x-ray diffraction analysis which is now in progress.

It is perhaps interesting to note that these crystals of neutral, or uncleaved, fructose-1,6-diphosphatase are composed of highly symmetrical molecules having 222 point group symmetry, while crystals of the alkaline form of the enzyme (1) do not crystallographically demonstrate the presence of such symmetry. This suggests that the identity of the four subunits may be lost or that their symmetrical relationship is altered upon conversion from the neutral to alkaline form by proteolytic cleavage.

REFERENCES
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