Short Communications

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Preliminary X-ray data for two crystalline forms of di*iso*propylphosphoryl trypsin.*

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As part of an investigation of the structure of crystalline enzymes which is in progress in these Laboratories, di*iso*propylphosphoryl trypsin (DIP-trypsin) has been prepared and crystallized.

Trypsin (2 × crystallized, salt free, lyophilized) was purchased from the Worthington Biochemical Corporation. Di*iso*propyl phosphorylfluoridate (DFP) was obtained from the Aldrich Chemical Company, Inc. The inhibition of the enzyme was performed according to the procedure described by Cunningham (1954); the trypsin was allowed to react with an excess of DFP at pH 8·0 in the presence of calcium chloride, which was added to reduce autolysis of the enzyme. The solution was acidified and ammonium sulfate was added in amount sufficient to precipitate the calcium ions. The DIP-trypsin was precipitated twice by further addition of ammonium sulfate. The inhibited enzyme was then dialyzed until salt free and finally lyophilized. In a series of experiments, crystals suitable for X-ray study were obtained from solutions with pH ranging from 5 to 8 in which the concentration of the protein varied over the range from 1 to 3%, and the concentration of magnesium sulfate from 4·6 to 11·5% by weight. Two crystal forms (one a thick, trapezoidal or hexagonal tablet and the other a rod-like prism) were frequently observed in the same crystallizing solution. Growth of the tabular form was apparently favored at pH 5 and the upper range of magnesium sulfate concentration, whereas the rod-shaped form prevailed at higher pH and lower concentrations of magnesium sulfate. Both forms grew readily at room temperature, but very slowly or not at all at 0 to 5 °C.

The tabular crystals were usually well-formed, separate, individual crystals, up to 0·4 mm. in greatest dimension. From a solution having a protein concentration of 1% the crystals grew more slowly, but eventually became larger than those grown from a 3% protein solution. The rod-like crystals tended to grow in bundles or sheets; however, at pH 8 some single crystals were obtained which showed well-developed faces and were about 0·1 × 0·7 mm. in dimension.

For X-ray photography, all crystals were mounted wet in sealed capillaries and photographed with Weissenberg and precession cameras. The tabular crystals are trigonal (Laue symmetry $\bar{3}m$) with the three-fold axis perpendicular to the trapezoidal faces. Reflections $000l$ were found only if $l=3n$; the point group is thus 32. The two-fold axes are perpendicular to the $11\bar{2}0$, $1\bar{2}10$, and $\bar{2}110$ planes, so that the space group is either $P3_121$ or $P3_221$. Reflections extend to a minimum spacing of less than 2 Å. The rod-shaped crystals were found to be orthorhombic. The needle axis was designated as the a axis; the b and c axes make angles of about 45° with the normals to the principal faces. The only systematic absences were $h00$, $0k0$, and $00l$ with h, k, or l odd, indicating that the space group is $P2_12_12_1$. Reflections extend to a minimum spacing of 2 Å. Preliminary data for the two forms are summarized in Table 1. The number of molecules per unit cell was calculated on the assumption of a crystal density of 1·2 g.cm.$^{-3}$ (reasonable for a protein crystal containing 30 to 50% of water of crystallization) and a molecular weight of 23,000 to 24,000 for the anhydrous protein.

Attempts to obtain other forms of DIP-trypsin which might be even more favorable for investigation are in progress.

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Reference

Cunningham, L. (1954). *J. Biol. Chem.* **211**, 13.

Table 1. *X-ray data for two forms of* DIP-*trypsin*

Crystal habit	Crystal system	*a*	*b*	*c*	Space group	Molecules per unit cell	Volume of asymmetric unit
Tabular	Trigonal	55 Å	55 Å	109 Å	$P3_121$	6	47,600 Å^3
Needle	Orthorhombic	55	59	67	$P2_12_12_1$	4	54,300

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