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A recipe for the crystallization of enzymes

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Abstract
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Jakoby, W. B. **A recipe for the crystallization of enzymes.** The crystallization of a protein is often a final step resulting in further purification and, hopefully, serving as one index of purity. Although confidence in equating such an esthetically appealing and ordered structure as a crystal with purity is often misplaced, more critical methods are available for that decision. More often the problem has been that of inducing crystallization.

During the past year we have enjoyed uniform success in attempting the crystallization of twelve enzymes by a technique of extracting highly purified protein precipitates with ammonium sulfate solutions of decreasing concentration. No claim for originality is made since purification by such methods dates to the early work of Warburg. Crystallization of an enzyme by this method was described by Pontremoli et al. (1961 Proc. Natl. Acad. Sci. U. S. 47: 1942) and several laboratories have used it successfully.

The routine which we have adopted for crystallization involves the complete precipitation of 10-20 mg protein with ammonium sulfate and the successive extraction of the resultant precipitate with 1 ml volumes of solutions of decreasing ammonium sulfate concentration; all such solutions are 0.05 M with respect to potassium phosphate at pH 7.0 although other buffers have been used also. All operations are conducted at or near 0°C. The precipitate is evenly suspended in the salt solution by triturating with a glass rod and then centrifuged for 10 minutes at 10,000 x g. The supernatant fluid is poured off and allowed to stand at room temperature while the residue is resuspended in a solution of lower salt concentration and the process is repeated. The first extraction is performed at salt concentrations somewhat higher than that expected from the solubility of the protein. Thus, for a protein known to precipitate at an ammonium sulfate concentration of 30% to 40% of saturation, extraction is performed sequentially with solutions which are 45%, 41%, 38%, 34% and 30% of saturation. Crystallization often begins within an hour, occasionally within minutes, of transfer after centrifugation from the cooled vessel to one at room temperature. The crystals obtained in this manner are usually about 1 μ in their largest axis and are difficult to distinguish without the aid of a dark-field condenser and an oil immersion lens. With dark-field optics, the highly refractive outline of the crystals easily allows differentiation from possible amorphous material. Crystal shape is characteristic of the individual system. Electron microscopic studies of three proteins by Labaw, using the technique of shadowed carbon replicas,(1964 J. Ultrastruct. Res. 10: 66) has confirmed the crystallinity of the products. After one or two recrystallizations, carried out in the same manner, a

silky sheen is usually observed when a suspension of the crystals is agitated; the silky appearance is usually not present on initial crystallization.

It is apparent that an enormous number of variations is possible in carrying out the described procedure. It is therefore of interest that each of the twelve systems with which we hove tried this method has allowed crystallization without recourse to changes in pH, temperature or other conditions except for the inclusion of a mercaptan where warranted. Our experience at this time includes dehydrogemses, decarboxylases, transferases and protein hormones and involves proteins usually sensitive to room temperature, proteins with high and low polysaccharide content and complexes of more than one protein. The molecular weight range has been between 30,000 and 3,500,000. The only requirement for crystallization appears to be that the preparation is at a stage of purity where the enzyme comprises more than 30% of the total protein. - - - National Institutes of Health, Bethesda, Maryland.