Assays for aspartate and ornithine transcarbamylase by means of the pH-stat

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Abstract
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Aspartate transcarbamylase (ATCase) activity has been determined by a calorimetric assay for carbamyl aspartate (Davis and Woodward 1962 Genetics 47: 1075). The calorimetric assay can be adapted to ornithine carbamyltransferase by means of the pH-stat. The following equation describes the reaction in question:

\[
\text{NH}_2\text{CO}_2\text{PO}_3^- + \text{COO}^-\text{CH}_2\text{CH}_2\text{NH}_3^+ + \text{COO}^- = \text{COO}^-\text{CH}_2\text{CH}_2\text{NHCO}_2\text{H} + \text{HOPO}_3^- + \text{H}^+.
\]

carbamyl phosphate aspartate carbamyl aspartate

**Reagents:** 0.005 M sulfamic acid (primary standard solution); 0.01 M NaOH, standardized against sulfamic acid; 0.05 M L-aspartate, standard solution; 10 M NaOH and water; 0.005 M carbamyl phosphate (0.0204 g dissolved in 25 ml cold water and kept in ice bath); H$_2$O, boiled and cooled to exclude CO$_2$; protein solution adjusted to pH 8.5 with NaOH.

**Apparatus:** Sargent pH-stat equipped as follows: 2.5 ml barrel and plunger; 10 ml reaction container, with stopper permitting free entrance of electrodes, thermocouple, thermometer, NaOH delivery tube, nitrogen delivery tube, and reaction delivery syringes. Nitrogen cylinder and passage for delivery of nitrogen through 0.5 M NaOH.

**Procedure:** The pH-stat is calibrated against standard pH 8.0 buffer at 30°C. The barrel is filled with 0.01 M NaOH and the system is flushed with nitrogen. Water, enzyme, and aspartate are introduced into the reaction vessel and the mixture is titrated to pH 8.5. The reaction is begun with the addition of carbamyl phosphate. At the end of the assay an aliquot of 0.005 M sulfamic acid is titrated under the conditions of the assay to standardize the NaOH. From this titration the number of umoles of H$^+$ released during the assay can be calculated. Also, carbamyl phosphate can be assayed by letting the reaction go to completion, i.e., by permitting all the carbamyl phosphate to convert to carbamyl amino acid, and determining the number of umoles of H$^+$ released.

Starting with 2.00 ml 0.05 M aspartate, 0.180 units of enzyme, H2O, 0.01 M NaOH, 0.50 ml 0.00425 M carbamyl phosphate in a total final volume of 8.5 ml, it was determined that the NaOH was 0.00947 M, and that the initial velocity of the reaction was 0.1843 umoles H$^+$ per minute. Subtracting carbamyl phosphate hydrolysis, 0.0057 umoles H$^+$ per minute, leaves an initial velocity of 0.1786 umoles H$^+$ per minute. Aliquots of this reaction mix were assayed calorimetrically, and the initial velocity was shown to be 0.180 umoles carbamyl aspartate per minute. This close agreement speaks for the validity of the pH-stat assay method at this pH and under these conditions.

This work was performed under USPHS Grant GM-10206-03. Biology Department, Rice University, Houston, Texas.

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 a protein precipitate with ammonium sulfate solutions of decreasing concentration. No claim for originality is made since such methods date 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. All operations are conducted at or near 0°C. The precipitate is evenly suspended in the salt solution by trituration 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 few salt concentrations are 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 leave a fraction 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

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