Hill, J. M. and V. W. Woodward, Assays for aspartate and ornithine transcarbamylase by means of the pH-stat.

Aspartate transcarbamylase (ATCase) activity has been determined by a calorimetric assay for carbamyl aspartate (Davis and Woodward 1962 Genetics 47: 1075). The colorimetric assay can be adapted to ornithine transcarbamylase activity by assaying far citrulline. These same enzymes can be assayed by titration of hydrogen ion released in a pH-stat. The following equation describes the reaction in question:

$$NH_2CO_2PO_3^{=} + COO^-CH_2CHNH_3^+COO^- \rightleftharpoons COO^-CH_2CHNHCOCH_2COO^- + HOPO_3^{=} + H^+ .$$

carbamyl phosphate aspartate carbamyl aspartate

Reagents: 0.005 M sulfamic acid (primary standard solution); 0.01 M NaOH, standardixed against sulfamic acid; 0.05 M L-aspartate, standard solution (0.665 g dissolved in 50 ml 0. 10 M NaOH and diluted to 100 ml); 0.005 M corbamyl phosphate (0.0204 g dissolved in 25 ml cold water and kept in ice bath); H_2O, boiled and copped 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 entrance of electrodes, thermocouple, thermometer, NaOH delivery tube, nitrogen delivery tube, and reaction deliver/ syringe,. 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 carbomyl 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 µmoles 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 carbomyl phosphate to convert to a carbamyl amino acid, and determining the number of pmoles H^+ released.

Starting with 2.00 ml 0.05 M aspartate, 0.180 units of enzyme, H20, 0.01 M NaOH, 0.50 ml 0.00425 M carbamyl phosphate in a total final volume off.85 ml, it was determined that the NaOH was 0.00947 M, and that the initial velocity of the reaction was 0.1843 µmole H^+ per minute. Subtracting carbomyl phosphate hydrolysis, 0.0057 µmoles H^+ per minute, leaves an initial velocity of 0.1786 µmoles H^+ per minute. Aliquots of this reaction mix were assayed colorimetrically, and the initial velocity was shown to be 0.180 µmoles carbamyl aspartate per minute. This close agreement speaks for the validity of the pH-stat assay method at this pH and under these conditions.

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