Short Communications and Preliminary Notes

THE SPECTROPHOTOMETRIC MEASUREMENT OF ENZYMICALLY PRODUCED OXALOACETIC ACID

by

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Recent publications[1,2] of methods for measuring the oxaloacetic acid produced enzymically by the glutamic-aspartic transamination reaction have used spectrophotometric measurements of the change in optical density of the entire reaction mixture at 280 mμ. These procedures are designed for the measurement of the kinetics of a purified enzyme system and the amount of protein added is so small as to permit ultraviolet spectrophotometry without its removal.

For an enzyme assay procedure the use of whole homogenates is desirable, but in this case the direct spectrophotometric measurement is not possible as the amount of homogenate required to produce a measurable quantity of oxaloacetate contains too much protein for spectrophotometry without its prior removal. Methods are available for assaying transaminase activity in whole homogenates[3,4] and these depend on the measurement of the CO_2 or pyruvate resulting from the catalytic decarboxylation of the oxaloacetate produced. Neither method is entirely satisfactory owing to the high blanks[3], lack of sensitivity[3,4] and working losses in the extraction[4]. The new method described here incorporates the use of whole tissue homogenates and the manifold advantages of spectrophotometry.

The removal of interfering protein was effected by trichloracetic acid since this performed the additional function of stopping the enzyme action and providing a strongly acid medium in which oxaloacetic acid is stable[5]. At this low pH the U.V. absorption of oxaloacetic acid is minimal and the sensitivity of measurement correspondingly low. However, the absorption increases progressively with the rise in pH and in alkaline solutions the stability is also increased. It is, therefore, desirable that measurements of enzymically produced oxaloacetate should be conducted at pH values well above 7, and in this work a pH of 9.2 has been found to be satisfactory. To prevent the spontaneous decomposition of oxaloacetic acid a rapid change from strongly acid to alkaline pH was effected by the addition of the calculated quantity of NaOH to the acid supernatant. When, as a further precaution, borate buffer pH 9.2 was added to this mixture a further increment in absorption was found. That this was a specific effect of borate and not of pH was demonstrated by the addition of borate to a solution of oxaloacetate already at pH 9.2 (see Fig. 1).

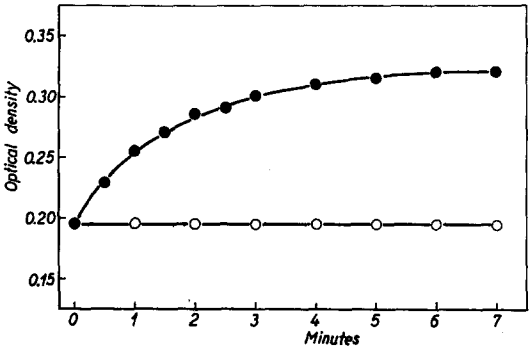

Fig. 1. Changes in optical density of a solution of oxaloacetate after the addition of borate. Open circles: 0.2 μmols oxaloacetate/ml at pH 9.2. Black dots: 0.2 μmols oxaloacetate/ml at pH 9.2, 0.5 *M* borate added at time 0.

The additional absorption suggested complex formation between the borate and oxaloacetate. Complexes between borate and *cis*-diol structures have been described[6]. The enol form of oxaloacetate would provide such a *cis*-diol configuration, and there are suggestions[7] that the increase in absorption of oxaloacetic acid with increasing pH can be ascribed to an increase in the enol form. It would appear, therefore, that in our experiments complex formation is occurring between the borate and the enol form of oxaloacetic acid. Further experiments (to be published elsewhere) indicate that the carbonyl group and the adjacent carboxyl group are both involved in the complex.

The new method reported here makes use of the conditions described by CAMMARATA AND COHEN[1] for their purified enzyme system. We have used the same concentration of substrate and periods of pre-incubation as given by these authors except that our homogenates required no pyridoxal

phosphate supplementation and that instead of the continuous measurement of oxaloacetate production we have incubated the enzyme plus substrate for a finite period of 10 minutes.

The reaction was carried out in 10 ml centrifuge tubes at 37° C. Buffered homogenate (0.05 to 0.15 ml of a 1:10 in 0.01 *M* phosphate buffer, pH 7.4) was pre-incubated for 20 minutes and aspartate then added. After a further 10 minutes α-ketoglutarate was added and the enzyme reaction allowed to proceed for exactly 10 minutes. The reaction was stopped by the addition of 0.5 ml 30% (w/v) trichloracetic acid and the tubes cooled. After centrifugation the supernatant was decanted into tubes graduated at 10 ml and previously charged with 1.0 ml of *N* NaOH. 2.0 ml of 0.5 *M* borate buffer, pH 9.2, were added and the volume made up to 10 ml. The optical density of this solution was then measured at 280 mμ. Further tubes, at each enzyme level, containing all the reactants were submitted to the same procedure except that the trichloracetic acid was added immediately prior to the α-ketoglutaric acid, and these served as blanks. The oxaloacetate concentration was determined by reference to a standard curve constructed by measuring the optical density of different amounts of oxaloacetate treated in the same way.

Using the method described here the enzyme assay is linear over a range of 5–15 mg of tissue (fresh weight). The sensitivity of the estimation may be seen from Fig. 2 in which the optical density of oxaloacetic acid solutions treated as above is expressed as a function of the concentration. For comparison, a similar curve, calculated from the data of NISONOFF, HENRY AND BARNES[2] is included.

Errors due to the decarboxylation, both spontaneous and catalysed, of the oxaloacetic acid produced enzymically are negligible, since we have calculated from the data of NISONOFF, HENRY AND BARNES[2] that this could not exceed 2.5% of the total oxaloacetate produced.

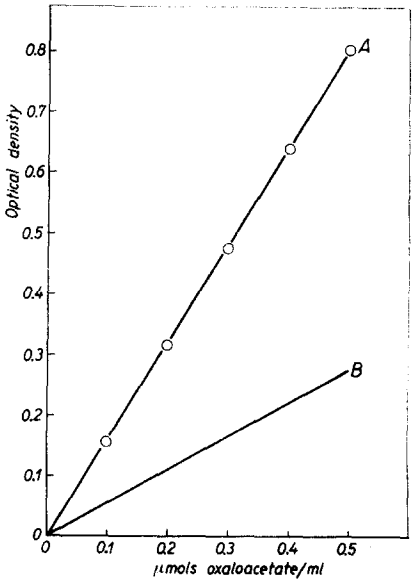

Fig. 2. Relation between oxaloacetic acid concentration and optical density. Curve A represents the relationship obtained using the method described above. Curve B is a similar curve calculated from the data of NISONOFF, HENRY AND BARNES[2].

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DISSIMILATION OF DL-ALANINE-1-^{14}C BY RAT BRAIN HOMOGENATES*

by

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We would like to report data which indicate that the brain can dissimilate amino acids other than glutamic acid to an appreciable extent. Adult rats of Spray Doli Strain weighing around 150–200 grams were used. After sacrificing these animals by decapitation, the entire brain (1.5 g) was im-

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