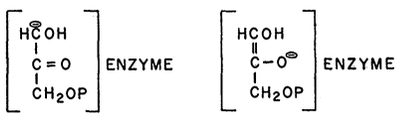
Spectrophotometric Evidence for Formation of a Dihydroxyacetone Phosphate-Aldolase Complex

Previous studies (*1, 2*) have shown that when dihydroxyacetone phosphate (DHAP) is incubated with muscle, aldolase labilization of a carbon-bound hydrogen occurs. On the basis of this observation the enzyme-DHAP complex was formulated as involving a carbanion or an enediol structure:



It was suspected that such structures would absorb in the ultraviolet region. For example, both dihydroxyacetone and D,L-glyceraldehyde in 1*N* sodium hydroxide show strong absorption in the ultraviolet with maxima at 220 mμ (*3*). The present investigation deals with the ultraviolet absorption characteristics of several combinations of protein and substrate.

Dihydroxyacetone phosphate, when it is dissolved in 0.002*M* phosphate buffer, *p*H 7.0, shows no absorption in the range 280 to 215 mμ. In the presence of muscle aldolase (Fig. 1, experiments 1 and 2), however, there is strong light absorption in the region between 250 and 233 mμ. The apparent decreases in optical density at lower wavelengths, as indicated by the broken lines, are artifacts caused by the fact that protein fluorescence contributes most of the light transmitted by the cuvettes (*4*). The absorption between 250 and 233 mμ increases with increasing concentration of both DHAP (experiment 1) and aldolase (experiment 2). With the highest concentration of DHAP and aldolase a tendency toward saturation is discernible. The shifting of the apparent peak in experiment 2 is an artifact caused by increased fluorescence consequent to increased protein concentration (*4*). The specificity of the enzyme-substrate combination was tested with substitutes for either the protein or DHAP. No absorption was observed in the system DHAP-albumin, or in the systems glucose-6-phosphate-aldolase and dihydroxyacetone-aldolase.

These results suggest a specific requirement for both DHAP and aldolase. D,L-Glyceraldehyde shows no absorption in the range 280 to 225 mμ in the presence of aldolase. This is consistent with the conclusion (*2*) that the aldehyde component of the condensation system is not activated by the enzyme in the absence of DHAP. Additional evidence that the absorption measured is produced by combination of the enzyme with its substrate is found in the obliteration of the absorption by the addition of D,L-glyceraldehyde (experiment 3, curve *J*). The presence of the aldehyde permits the catalysis of hexose-1-phosphate synthesis, and the equilibrium of this reaction leaves very little free DHAP to combine with the enzyme. The addition of excess DHAP (curve *K*) restores the absorption. These results indicate that the absorption observed in the low ultraviolet region is indeed a measure of an aldolase-DHAP complex, and they provide confirmation of earlier findings relating to the ability of muscle aldolase to activate DHAP in the absence of aldehyde and to the inability of the enzyme to activate the aldehyde acceptor.

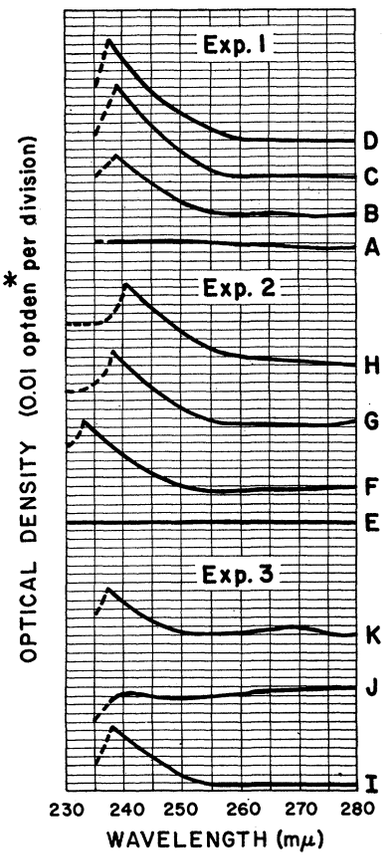


Fig. 1. Experiment 1: effect of DHAP concentration on the absorption spectrum of DHAP-aldolase. Curve *A* shows the absorption determined with 2 mg of crystalline aldolase in both test and reference cuvettes in 1.0 ml of 0.002*M* potassium phosphate buffer, *p*H 7.0. The test cell contained (curves *B, C,* and *D*), in addition, 0.02, 0.07 and 0.1 ml, respectively, of 0.01*M* DHAP, and the reference cell contained an equal volume of water. Experiment 2: effect of adolase concentration on the absorption by DHAP-aldolase. Curve *E* was obtained with 1.0 μmole of DHAP in 1.0 ml of 0.005*M* potassium phosphate buffer, *p*H 7.0. Curves *F, G* and *H* were obtained after the addition of 0.05, 0.15 and 0.25 ml of a 20-mg/ml aldolase solution to both the reference and test cells. Experiment 3: effect of D,L-glyceraldehyde on the absorption spectrum of DHAP-aldolase. Curve *I* was obtained when both cells contained about 2 mg of aldolase in 1.03 ml of water. The test cell contained, in addition, 0.5 μmole of DHAP. Curve *J* was obtained after the addition of 1.0 μmole of D,L-glyceraldehyde in 0.01 ml to both cells. Curve *K* was obtained after the addition of 2.0 μmole of DHAP in 0.12 ml to the test cell and the addition of an equal volume of water to the reference cell. In each experiment the successive curves have been displaced upward in order to simplify presentation. A Cary recording spectrophotometer, Model 14, was used for all measurements. Since use of the term *optical density unit* is technically incorrect, the term *optden,* defined as a unit of absorption measured in a 1-cm cuvette, is used. The optden has the dimension of log $(I_0/I) \times cm^{-1}$.

YALE J. TOPPER
ALAN H. MEHLER
BEN BLOOM
National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland

References

1. I. A. Rose and S. V. Rieder, *J. Am. Chem. Soc.* 77, 5764 (1955).
2. B. Bloom and Y. J. Topper, *Science* 124, 982 (1956).
3. Y. J. Topper and B. Bloom, unpublished observations.
4. A. H. Mehler *et al., Science,* this issue.

6 September 1957

Separation and Detection of Trifluoroacetyl Amino Acids

The need for separating and identifying trifluoroacetyl amino acids has arisen because of increased use of trifluoroacetic anhydride as an acetylating agent for the amino group during synthesis and study of peptides and proteins. Trifluoroacetyl amino acids do not form color complexes with ninhydrin, nor are the usual tests for fluorine adaptable for small quantities of these derivatives. However, the acetylated amino acids will undergo hydrolysis at a *p*H greater than 10 (*1*) with the liberation of the free amino acid. The free acids may then be detected with ninhydrin. Separation of mixtures of the trifluoro derivatives and the free amino acids is readily accomplished by means of paper electrophoresis.

Samples containing a mixture of either trifluoroacetyl glycine and glycine or trifluoroacetyl alanine and alanine were separated on a Reco electrophoresis apparatus (model E-800-2); potassium acid phthalate—sodium hydroxide buffer of *p*H 6.0 and of ionic strength 0.045 was used. The electrophoretic determinations were conducted at 600 v for 1 hour. At the end of this time the paper strips were dried, dipped in 1 percent alcoholic sodium hydroxide (1 ml of saturated sodium hydroxide solution in 99 ml of 95 percent ethanol), and dried. The color