DECREASE IN THERMAL STABILITY OF FROG LIVER CARBAMYL PHOSPHATE SYNTHETASE BY SUBSTRATES AND COFACTORS*

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While testing the effect of temperature on citrulline synthesis with rat liver enzymes a sharp decrease in rate was noted beyond 36°-38° (Grisolia and Cohen, 1952).

A similar effect occurs with frog liver enzymes; for example, 1.0, 2.8, 3.3 and 2.3 micromoles of citrulline were formed when reaction mixtures were heated for 3.5 minutes at 26°, 36°, 46° and 52°, with the liberation of 2.3, 6.9, 8.8 and 5.8 micromoles of phosphate. In another experiment, 1.6, 0.9 and 0.3 micromoles of citrulline were formed with the liberation of 4.2, 2.2 and 0.6 micromoles of phosphate when the system was incubated at 40°, 52° and 56°. However, the enzyme is fairly stable up to 50°-56° (there are some variations in stability depending upon enzyme concentration). For example, when the enzyme was incubated in the absence of substrates for 3.5 minutes at 40°, 52° and 56° and then cooled and assayed at 38°, 4.2, 4.0 and 3.6 micromoles of citrulline were formed. A 5-10 minute preincubation of the components of the reaction mixture (without enzyme) up to 56° followed by cooling, does not decrease enzyme activity at 38°; thus the decrease in activity is not due to reversible inactivation or to heat effects on the nonprotein components of the reaction mixture.

Similar effects were noted when NH_4Cl was replaced by NH_2OH. For example, in the latter experiment above reported

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2.4, 2.1 and 0.8 micromoles of inorganic phosphate were liberated at 40°, 52° and 56°. As previously demonstrated, NH_2OH when replacing ammonia results in phosphate liberation without concomitant citrulline synthesis (Grisolia and Marshall, 1955). It appears then that the effect noted is the result (totally or in part) of thermal instability of carbamyl phosphate synthetase produced by substrates. Further, the noted effects are not due to decrease in activity of ornithine transcarbamylase; there was over a 5-10 fold excess of this enzyme after heating for 5 minutes at 58°, and also an additional 50-fold excess of purified ornithine transcarbamylase was added to samples incubated at and beyond 50°. Interaction of carbamyl phosphate synthetase with ornithine transcarbamylase cannot be excluded, however.

As shown in Table I the effect appears to be specific and

TABLE I

THE EFFECT OF AG, ATP AND Mg++ UPON THERMAL STABILITY OF FROG LIVER CARBAMYL PHOSPHATE SYNTHETASE

Micromoles added during preincubation				Preincubated at	
Tris-buffer	AG	ATP	Mg++	43°	57°
				µmoles citrulline formed	
25	-	-	-	3.37	1.87
25	1	-	-	3.33	0.12
25	1	-	10	3.33	0.04
25	10	-	10	3.21	0.00
25	-	2	-	-	1.47
25	-	8	-	-	0.42
25	-	8	10	-	2.05
25	1	2	-	-	0.03

0.4 mg of protein, frog liver acetone fraction (Marshall, Metzenberg and Cohen, 1958) were preincubated for 5 minutes in 1 ml. with the indicated components and temperatures. After cooling, each tube was made up to 2.0 ml. and to contain the following, ex-

pressed in micromoles; Tris buffer pH 7.4, 150; ATP, 16; $NaHCO_3$, 100; NH_4Cl, 50; $MgSO_4$, 20; ornithine, 20; AG, 10. The samples were then incubated for 20 minutes at 38°. Citrulline was estimated by the method of Archibald (1944).

it can be demonstrated at low concentrations of acetyl glutamic acid, AG.* It is of extreme interest that ATP at higher concentrations decreases also the thermal stability although when combined with Mg++ it is not inhibitory.

In addition to the possible significance of the above findings for the little understood catalytic mechanism of action of AG in citrulline biosynthesis, the effects shown appear to be unique in enzyme chemistry. To the knowledge of the authors there is no record of a finding similar to the one reported here. It is generally accepted that addition of substrates or components necessary for enzymatic activity have no effect or a protective effect upon thermal stability. Whether the noted effect is due to exchange of a component necessary to an active center with AG and/or ATP, or to a change in the geometry (coiling or folding of the protein) after interaction with AG and/or ATP remains to be investigated. It is apparent, however, that the effect may be related to the often observed but little understood protection by substrates. It appears likely that interaction with an active center may result in exposure or modification of other structures necessary for enzyme activity to thermal inactivation. Further physical and chemical studies on this phenomenon are in progress.

*The abbreviations used in this paper are: AG, acetyl glutamic acid; ATP, adenosine triphosphate; Tris, tris (hydroxymethyl)-aminomethane.

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