VARIATIONS IN HEAT STABILITY OF ENZYMES WITH SUBSTRATES AND COFACTORS*

Santiago Grisolia and Barbara K. Joyce

McIlvain Laboratories
University of Kansas Medical Center
Kansas City, Kansas

Received October 26, 1959

The decrease in enzyme stability demonstrated when frog liver carbamyl phosphate synthetase was heated in the presence of substrates (Caravaca and Grisolia, 1959), led to the proposal (Grisolia, 1959) that, decrease in enzymatic stability by substrates might be a relatively common phenomenon.

We have found either protection or no action by substrates on thermal inactivation of yeast enolase, yeast and rice germ phosphoglyceric acid mutase, ornithine transcarbamylase and carbamate kinase; while wheat germ phosphoglyceric acid mutase, mammalian carbamyl phosphate synthetase, brain glutamine synthetase and muscle phosphoenol pyruvate kinase are more susceptible to heat in the presence of certain substrates or cofactors. As a further example, the influence of substrate on the thermal stability of four dehydrogenases (Sigma Chemical Company, crystalline or highest purity) is given in Table I. Also, it has been shown (Inagaki, 1959) that

*Some of the experiments mentioned have been done by Miss J. Caravaca and by Miss M. Fernandez. This work has been supported by grants from the National Institutes of Health and the American and Kansas Heart Association.

TABLE I

INFLUENCE OF SUBSTRATES AND COFACTORS
UPON THE THERMAL STABILITY OF DEHYDROGENASES

Substrate or Cofactors Added During Preincubation	% Activity Found After Preincubation				
	Alcohol	Glyceraldehyde Phosphate	Malic	Malic*	Lactic
None	58	49	50	6	67
Diphosphopyridine nucleotide	17	97	100	47	132
Reduced Diphosphopyridine nucleotide	9	37	310	59	94
Pyruvate	-	-	-	-	70
Ethanol	64	-	-	-	-
Acetaldehyde	66	-	-	-	-
Malate	-	-	100	65	-
Oxalacetate	-	-	60	6	-
3-Phospho-D-Glyceraldehyde	-	70	-	-	-
Cysteine	-	28	-	-	-
Lactate	-	-	-	-	97

1520; 11; 40; 68* and 0.8 units each of alcohol, glyceraldehyde phosphate, malic, malic* (in the presence of 1.6 M ammonium sulfate at pH 7.0) and lactic dehydrogenases in 0.25 ml. of tris-(hydroxymethyl)-aminomethane buffer, pH 7.4, were heated for 5 minutes at 43°; 55°; 53°; 66°* and 40° respectively, with 0.5 micromoles of the substrates or cofactors indicated. Aliquots were assayed (Methods in Enzymology, 1955) by procedures number 61, 67, 79 and 123. For alcohol dehydrogenase, the recommended diphosphopyridine nucleotide and alcohol was increased 10 and 6 fold respectively.

glutamic dehydrogenase is less stable to heat in the presence of reduced diphosphopyridine nucleotide. Although this worker has not commented on the possible biological significance of this phenomenon, he postulates as we have (Caravaca and Grisolia, 1959) that the decrease in thermal stability is due to secondary

effects in the protein molecule, following specific interaction with substrates or coenzymes. Recently (Reiner, 1959) has considered the "freak" case of decrease in enzyme stability to heat by substrates. This author has also pointed out the relation of this phenomenon to the, at first glance, apparently opposite phenomenon of increase in thermal stability of enzymes with substrates. Inhibition by high substrate concentration is well known in bacteriology and in enzymology; high substrate concentration and/or temperature may be inhibitory to enzymes in the cell since enzymes *in vivo* most likely operate at low substrate concentrations.

From a cursory perusal of the literature and from comments from colleagues it appears possible that a number of observations are related to the phenomenon under consideration, e.g. the cold agglutinins and the larger precipitate obtained in cross reactions (Heidelberger, 1954) at 0° than at 37°, the decrease noted in phenylalanine hydroxylase by tyrosine feeding (Auerbach, Waisman and Wycoff, 1958), the depression in glutamine synthetase by glutamine in HeLa cells cultures (De Mars, 1958), the complete loss in biosynthetic ability for cholesterol (Curran, Personal Communication, 1959) with liver clusters at 39° while it proceeds maximally at 36-37°. It is a well-known clinical fact that serum cholesterol decreases with fever.

Although, as shown here, the stability of several enzymes is influenced greatly by substrate, many more enzymes and biological systems will have to be tested for effects by heat, X-rays, ultraviolet light, etc., before the significance of these findings can be properly evaluated.

References

V.H. Auerbach, H.A. Waisman and L.B. Wycoff, Nature, 182, 871 (1958).

J. Caravaca and S. Grisolia, Biochem. and Biophys. Research Communications, 1, 94 (1959).

G. Curran, Personal Communication (1959).

Methods in Enzymology, edited by Colowick and Kaplan, Vol. I, Academic Press Inc., procedures number 79;61;123;67 (1955).

R. De Mars, Biochim. Biophys. Acta., 27, 435 (1958).

S. Grisolia, in Simposio en Oxitocina, edited by R. Caldeyro-Barcia, Montevideo (in press).

M. Heidelberger, in W. McElroy and B. Glass, The Mechanism of Enzyme Action, Johns Hopkins Press, Baltimore, 138 (1954).

M. Inagaki, J. of Biochem., 46, 1001 (1959).

J.M. Reiner, Behavior of Enzyme Systems, Burgess Publishing Company, 283 (1959).