Possible interpretation and extrapolation of heating experiments with enzymes in the presence of substrates or cofactors

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THE phenomenon of increase of thermal stability of enzymes by substrates has often been observed and used to advantage for enzyme purification. In general, the increase in thermal stability by substrates is believed to be due to protection of an active centre in the enzyme. We have shown recently,[1] however, that under certain conditions substrates or cofactors remarkably decrease the thermal stability of frog liver carbamyl phosphate synthetase. It has occurred to us that this may be a relatively common phenomenon to which little attention has been paid. Increased inactivation of enzymes in the presence of substrate may have occurred often and escaped attention, as it had with us during enzyme purification studies. We have found recently,[2] in testing some twenty enzymes for thermal inactivation, that about half are not affected or are protected by substrates, while the other half are more susceptible to heat in the presence of certain substrates or cofactors. A brief example of the influence of substrate on the thermal stability of glutamine synthetase is given in Table 1. As previously

TABLE 1. THE EFFECT OF SUBSTRATES AND Mg^{2+} ON THERMAL STABILITY OF BRAIN GLUTAMINE SYNTHETASE

Additives (μmoles)		Percentage of initial activity left	
Tris*		Exp. 1	Exp. 2
50	None	52	64
50	5 ATP	20	23
50	20 Mg^{2+}	44	67
50	20 NH_4Cl	47	—
50	20 NH_2OH	—	12
50	20 glutamate	66	80

For experiments 1 and 2, 2·4 and 1·6 units, respectively, of partially-purified beef-brain glutamine synthetase[5, 6] were incubated for 5 min at 60° in 0·5 ml with the indicated components. After cooling, each tube was made up to 1·2 and 4·5 ml for experiments 1 and 2, respectively, and to contain the following, expressed in micromoles (figures in parenthesis are for experiment 2); Tris buffer, pH 7·2, 50 (500); ATP, 15 (50); $MgSO_4$, 20 (200); HN_4Cl or NH_2OH, 50 or (200); glutamate, 100 (500); cysteine (200). The tubes were then incubated for 20 min at 37 °C (30 °C). In experiment 1, NH_4Cl was used, while for experiment 2 the NH_4Cl was replaced by NH_2OH. The extent of the reaction was estimated as described by Elliot[5, 6]. For experiment 2 a succinic anhydride standard was used.

* Tris = Tris(hydroxymethyl)aminomethane.

shown,[1, 2] substrates or combinations thereof may change enzyme stability. It is seen in the table that ATP and hydroxylamine (although the effect by the latter substrate may not be specific) decrease the stability of the enzyme, while glutamate protects. In the experiments shown in the table, ATP when combined with Mg^{2+}, or with Mg^{2+} and glutamate, or with Mg^{2+} and NH_4Cl, provided protection. Recently Inagaki[3] has shown that glutamic dehydrogenase is less stable to heat in the presence of reduced diphosphopyridine nucleotide than in its absence, and Jenkins *et al.*[4] have shown a similar effect with glutamic-aspartic transaminase and aspartate. However, these workers have not commented on the possible broad biological significance of this phenomenon or on its possible use as a tool for enzymic studies. Substrate interaction may protect an active centre or, changing the geometry of the enzyme, may make other groups more susceptible to inactivation by physical agents. Since the decrease in enzyme stability to heat with substrates is a relatively common phenomenon and since

decreased enzymic activity induced by substrates has been noted by us with X-rays, ultraviolet light and ultrasonic waves (to be presented elsewhere), it seems possible that this may reflect a general effect which can provide a working hypothesis for certain aspects of poorly-understood problems such as tachyphylaxis, drug habituation, chemotherapy, fever and allergic phenomena. The administration of a drug, or of a substrate in a large excess, may result in increased destruction of an enzyme particularly at high temperatures and if other necessary components are not present at concentrations which will protect the enzyme. Under these conditions, either after resynthesis of the enzyme(s) or after removal of end products, the drug will be effective again (tachyphylaxis). Either enzyme binding, or destruction, or both, or slow resynthesis of the enzyme, may require higher doses to produce similar effects due to affinity considerations (drug habituation). It is well known that many drugs, for example, streptomycin, penicillin, certain sulphonamides and isoinazid can be more effective at lower temperatures. Fever might influence biological systems in an unsuspected manner. Examples in immunology possibly related to the phenomena under considerations, are the cold agglutinins and the higher precipitate obtained at 0 °C than at 37 °C in cross reactions.

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Enzymic oxidation of psilocine and other hydroxyindoles

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IT has recently been shown that the gill plates of *Mytilus edulis* L. contain an oxidase that acts on 5-hydroxyindoles, e.g. 5-hydroxytryptamine, bufotenine and 5-hydroxytryptophan; in the oxidation of these substances yellowish-brown pigments are formed.[1]

We have recently been able to compare the enzymic oxidation of bufotenine with that of its 4-hydroxy and 6-hydroxy analogues. The 4-hydroxy analogue of bufotenine is psilocine, a psychotropic amine naturally occurring in the fungus *Psilocybe mexicana* Heim,[2] where it is found together with its phosphate ester, psilocybine.

It was found that both hydroxyindoles were oxidized by the *Mytilus* preparation. The rate of oxidation of the 6-hydroxy derivative was similar to that of bufotenine; a faint yellowish-orange colour appeared during the reaction. With psilocine as substrate, oxygen uptake was more rapid than with bufotenine or with 5-hydroxytryptamine, and a blue colour appeared in the reaction. The rapid phase of the oxidation was over when about one atom of oxygen per molecule of added psilocine had been consumed. The contents of the manometer flask then showed a deep blue colour, with an absorption maximum at 625 mμ

We also had an opportunity of studying the *N*-1-methyl derivatives of both bufotenine and of psilocine. The 5-hydroxy compound was oxidized at about the same rate as bufotenine. The *N*-1-methyl derivative of psilocine was oxidized more slowly than psilocine itself, and the development of the blue colour was less rapid. These observations show that the substrates of the *Mytilus* oxidase