The Use of Mixed Solvents in the Study of Enzyme Kinetics

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Possible advantages to be obtained through the use of aqueous-organic solvent mixtures in the study of enzyme kinetics are twofold: the influence of the nature of the solvent on the reaction might give information on the mechanism of the reaction, as it has in the case of many non-enzymic reactions, and the resultant depression of the freezing point of the solution might be useful in extending the temperature range over which the reaction can be studied.

To determine the extent to which these possibilities may be realized, the effects of dioxan, ethanol, methanol, and glycerol on the enolase reaction have been studied in detail. To avoid the danger of artefacts, the solvent study was accompanied by careful investigation of all factors influencing the kinetic behavior. This led, for example, to the discovery that buffer ions played an unexpected role in the reaction, resulting in changes of apparent activation energy and in low-temperature inhibition.

The results of this investigation have shown that serious misinterpretations of data may be made by assuming that the same factors are involved in solvent effects on enzymic reactions as on nonenzymic reactions. Specifically, dielectric constant changes in the medium were found to have no appreciable effect on the reaction.

The use of organic solvents to lower the freezing point of the medium was found to be justified for the enolase reaction since it did not cause any change in the temperature dependance of the reaction; hence the reaction mechanism is apparently unaffected despite the lowering of the activity. Through this technique it has been found possible to lower the reaction rate sufficiently to investigate kinetic behavior at higher enzyme concentration than would otherwise be possible without the use of advanced electronic equipment, such as that used by Chance and others [1]. The use of high enzyme concentrations is desirable both to avoid certain types of artefacts (*e. g.* inactivation by surface denaturation or traces of metal ions) and to increase the information obtainable by kinetic means. Results of these investigations, including studies of "low temperature inhibition", will be presented.

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Ornithine Carbamyl Transferase

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The enzyme which catalyzes the formation of citrulline [1,2] was purified from rat liver mitochondria. The final preparation moved as a single compound during paper electrophoresis between pH 5 and 8.5. During ultra centrifugation an impurity amounting to *ca.* 5—8 % was detected.

The enzyme catalyzed the following stoichiometric reaction:

L-ornithine + carbamyl phosphate ⇌ L-citrulline + phosphate

Out of 26 tested amino acids only L-ornithine could accept the carbamyl group of carbamyl phosphate. The name ornithine carbamyl transferase is proposed for the enzyme. The reversibility of the reaction was demonstrated with the aid of isotopic substrates and the equilibrium constant was found to be about 10^5. The liver enzyme described by Krebs *et al.*[3] which catalyzed the phosphorolysis or arsenolysis of citrulline was found to be identical with ornithine carbamyl transferase.

The reaction is strongly inhibited by *p*-chloromercuribenzoate. Ornithine and carbamyl phosphate can to some extent protect the enzyme from inhibition. Isotope exchange experiments gave no indication for the formation of a carbamylated enzyme. The reaction mechanism will be discussed.

Carbamyl aspartate was formed from citrulline + aspartate in the presence of phosphate by the combined action of ornithine carbamyl transferase and aspartate carbamyl transferase [4].

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2. Jones, M. E., Spector, L. and Lipmann, F. *J. Am. Chem. Soc.* **77** (1955) 819.
3. Krebs, H. A., Eggleston, L. V. and Knivett, V. A. *Biochem. J. London* **59** (1955) 185.
4. Reichard, P. and Hanshoff, G. *Acta Chem. Scand.* **10** (1956) 548.