Although cross-reactivity of chymotrypsin and trypsin to certain substrates is not without parallel, it was not anticipated that mμg amounts of trypsin would necessarily produce a rapid hydrolysis of CT*p*NP. However, the rate curve obtained with trypsin was comparable to that observed with chymotrypsin and velocity was proportional to the trypsin concentration (Fig. 1, B). The maximum rate of hydrolysis occurred at about pH 8. As was found for chymotrypsin, tryptic activity toward CT*p*NP was completely inhibited by diiso propylphosphorofluoridate.

Thus, the use of CT*p*NP permits direct and rapid measurements of reaction rates utilizing quantities of chymotrypsin as low as 3 mμg/ml and amounts of trypsin as low as 7 mμg/ml.

This work has been supported in part by research grants A-727 and RG 4624 from the United States Public Health Service.

Biochemistry Department, School of Medicine, University of Pittsburgh, Pittsburgh, Pa. (U.S.A.)

CHARLES J. MARTIN
JULIUS GOLUBOW
A. E. AXELROD

[1] C. J. MARTIN AND A. E. AXELROD, *Biochim. Biophys. Acta*, 27 (1958) 52.
[2] C. J. MARTIN, *Federation Proc.*, 15 (1956) 455.
[3] W. J. DREYER, R. D. WADE, AND H. NEURATH, *Arch. Biochem. Biophys.*, 59 (1955) 145.
[4] N. M. GREEN AND H. NEURATH, *J. Biol. Chem.*, 204 (1953) 379.
[5] N. M. GREEN AND H. NEURATH, in H. NEURATH AND K. BAILEY, *The Proteins*, Vol. II, Part B, Academic Press, Inc., New York, 1954, p. 1155.

Received October 14th, 1957

The dissociation of glutamic dehydrogenase by reduced diphosphopyridine nucleotide (DPNH)

In 1952, OLSON AND ANFINSEN[1] showed that crystalline glutamic dehydrogenase exhibited an anomalous sedimentation behavior in the ultracentrifuge. The authors attributed this behavior to either dissociation or unfolding of the enzyme at low concentrations. It has now been shown that dissociation of the enzyme may be obtained by the addition of a substrate for the enzyme, DPNH.

Crystalline glutamic dehydrogenase was prepared from beef liver by the method of STRECKER[2] and recrystallized twice with saturated sodium sulfate at 5°[1]. At pH 7.4 in 0.05 *M* phosphate buffer, the enzyme shows a single peak in the ultracentrifuge. In all experiments, the Spinco Model E analytic ultracentrifuge was used*. The pattern, shown in Fig. 1a, has a hypersharp leading boundary, and is slightly skewed on the trailing edge, similar to that obtained by OLSON AND ANFINSEN. The addition of DPNH to this enzyme solution causes the single peak to split into two distinct peaks as shown in Fig. 1b. In this particular experiment, the molar ratio of DPNH to enzyme was approximately 200:1. Increasing amounts of DPNH decrease the amount

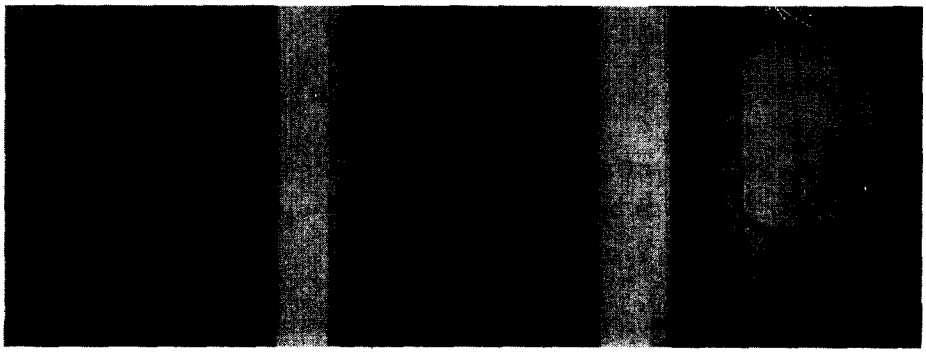

Fig. 1. Sedimentation patterns of glutamic dehydrogenase in 0.05 *M* phosphate buffer, pH 7.4 at 59,780 r.p.m. (a) alone after 660 sec, (b) in the presence of $1.3 \cdot 10^{-3}$ *M* DPNH after 660 sec, (c) in the presence of $5 \cdot 10^{-3}$ *M* *o*-phenanthroline after 1590 sec. The protein concentration was approximately 4.5 mg/ml. Sedimentation is from left to right.

* The author is indebted to Miss CARMELITA LOWRY for valuable technical assistance.

of enzyme in the fast peak with a corresponding increase in the concentration of the slow moving component. The splitting of the enzyme with DPNH may be completely reversed upon removal of the DPNH by dialysis. In addition to the influence of DPNH concentration, the pH of the solution appears to affect the extent of dissociation in the presence of DPNH, although the amount of dissociation of enzyme alone does not appear to be influenced by these same pH changes. No splitting of the enzyme is obtained by using *p*-chloromercuribenzoate or α-ketoglutarate, another substrate of the enzyme.

In view of the fact that Zn has been reported as a constituent of glutamic dehydrogenase[3] and of the apparent involvement of Zn in some enzymes which use DPNH as substrate[4], *o*-phenanthroline was added to the enzyme. The sedimentation diagram which resulted is shown in Fig. 1c. This enzyme has a sedimentation constant of slightly greater than half of the original material and as may be seen, the pattern is symmetrical as contrasted to Fig. 1a. The concentration of the *o*-phenanthroline used completely inhibited the enzymic reaction.

OLSON AND ANFINSEN have reported a molecular weight, from sedimentation and diffusion studies, for the enzyme of one million. Determinations of the molecular weight of *o*-phenanthroline-treated enzyme were made by the method of BALDWIN[5]. These determinations indicated that the protein may exist as a polymer of four enzyme subunits. The tetramer may be split either into dimers or monomers depending on the concentration of either *o*-phenanthroline or DPNH. As a result of splitting, the diffusion constant increases and the axial ratio decreases. The splitting caused by *o*-phenanthroline, a potent Zn binding reagent, is evidence for the role of Zn as an important component in the binding of the subunits. That both *o*-phenanthroline and DPNH cause dissociation of the enzyme may indicate the importance of Zn in the binding of DPNH.

So far as is known, glutamic dehydrogenase is the only enzyme which is affected in such a way by one of its substrates. The enzyme phosphorylase may also be dissociated into monomers, but only in the presence of *p*-chloromercuribenzoate[6]. In this case, the involvement of sulfhydryl groups is indicated in the combination of the four phosphorylase subunits.

Such a change in the molecular size and shape of glutamic dehydrogenase by a substrate for the enzyme offers a method by which further studies of the mechanism of the reaction may be made. Such investigations of the molecular and kinetic properties of the enzyme are under way in this laboratory.

CARL FRIEDEN

Department of Biological Chemistry, Washington University School of Medicine, St. Louis, Mo. (U.S.A.)

1 J. A. OLSON AND C. B. ANFINSEN, *J. Biol. Chem.*, 197 (1952) 67.
2 H. J. STRECKER, *Arch. Biochem. Biophys.*, 46 (1953) 128.
3 B. L. VALLEE, S. J. ADELSTEIN AND J. A. OLSON, *J. Am. Chem. Soc.*, 77 (1955) 5196.
4 B. L. VALLEE, *Advances in Protein Chem.*, 10 (1955) 317.
5 R. L. BALDWIN, *Biochem. J.*, 65 (1957) 503.
6 N. B. MADSEN AND C. F. CORI, *J. Biol. Chem.*, 223 (1956) 1055.

Received October 15th, 1957

Addendum (added in proof, December 11th, 1957)

The degree of splitting of the enzyme by DPNH, but not by *o*-phenanthroline, has been found to be dependent on the specific activity of the enzyme.

A study of the synthesis of catalase in liver of tumor-bearing mice by means of radioactive iron

It is well known that the liver catalase activity is sharply lowered in tumor-bearing animals. It has not yet been ascertained, however, whether this decrease is due to an actual decrease in the enzyme concentration or to the presence of some inhibitor which interferes in its determination[1]. According to PRICE AND GREENFIELD[2], less catalase may be prepared from livers of tumor-bearing rats than from the healthy control animals. However, their technique, although accurate, is a preparative one, and therefore involves the possibility of losses; furthermore, it clearly cannot distinguish between the catalase already present at the moment of tumor implantation and that formed thereafter. In an attempt to give an answer to this problem, we studied by means of radioactive iron the synthesis of liver catalase after tumor implantation.

Male albino mice of an inbred strain (ALAL) were implanted with S.180 and divided into two groups. Respectively 5 and 10 days after the implantation, the tumor-bearing mice and the