ON THE MECHANISM OF THE TRANSHYDROGENASE REACTION CATALYZED BY A BEEF HEART FLAVOPROTEIN[1,2]

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The symmetrical transhydrogenase reactions,

$$DPNH + DPN^+ \rightleftharpoons DPN^+ + DPNH$$

$$TPNH + TPN^+ \rightleftharpoons TPN^+ + TPNH$$

were shown to be catalyzed by flavoproteins by Weber and Kaplan (1957) using the acetylpyridine analogues of DPN and TPN, respectively. In a study of the reaction in deuterated water catalyzed by the Straub (1939) diaphorase, Weber et al. (1957) demonstrated that the reaction proceeded by electron transfer and that the catalytic mechanism involved enzyme reduction, since this enzyme effected an exchange of DPNH hydrogen with deuterated water in the absence of an acceptor. Since a resolved TPNH-cytochrome C reductase (Horecker, 1950) was found to catalyze the transhydrogenase reaction, Weber et al. (1957) proposed that a protein function, other than FAD, was reduced in the reaction. In this communication we wish to present evidence for the possible dithiol nature of this group.

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We have studied the transhydrogenase reaction of a flavoprotein isolated from beef heart mitochondria and purified by methods similar to those reported by Massey (1958, 1960). In addition to the DPNH-DPN transhydrogenase activity, this enzyme exhibits strong lipoic dehydrogenase and menadione reductase activity and catalyzes a slower diaphorase reaction with 2,6-dichlorophenol indophenol. Searls and Sanadi (1960) have demonstrated that preincubation of diaphorase with DPNH and arsenite or cadmium lead to inhibition of the lipoic dehydrogenase activity. The data in Fig. 1 indicate a similar effect on the transhydrogenase reaction when the beef heart flavoprotein is incubated with cadmium in the presence of DPNH. Furthermore, BAL or

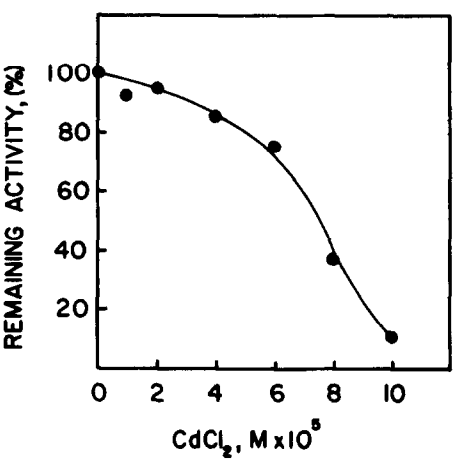

Fig. 1

Inhibition of the reduced flavoprotein by cadmium chloride. 0.01 ml of enzyme were preincubated 10 minutes at 30° with 0.01 ml of the following additions: 1 M sodium phosphate (recrystallized from EDTA), pH 7.5, 0.01 M KCN, $CdCl_2$ to the final concentration indicated and 0.1 M buffer as above or DPNH, 1 mg/ml diluted with the same buffer. The reactions were stopped by chilling and dilution to 0.5 ml. 0.02 ml of the reactions mixtures were assayed in the transhydrogenase reaction using the thionicotinamide analogue of DPN as described by Stein et al (1959). The data are expressed as the percentage remaining activity in the samples with DPNH compared to the controls without DPNH. The control reactions had a rate of about 0.42± 0.026 optical density units at 400 mμ per minute over the entire concentration range of cadmium.

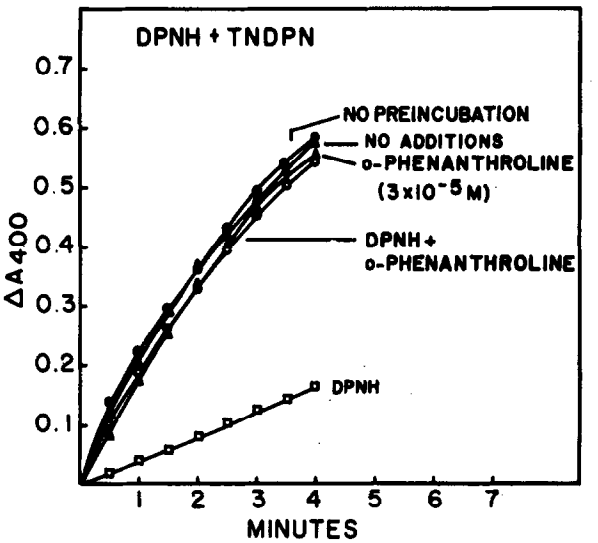

Fig. 2

Inhibition of the flavoprotein by DPNH. The enzyme was preincubated with various components of the transhydrogenase reaction mixture in the cuvette for 2.5 minutes, the remaining components added and the reaction was started by adding the thionicotinamide analogue of DPN. (●) - no preincubation, (△) - enzyme preincubated in buffer only, (▲) - enzyme preincubated with buffer and o-phenanthroline (3×10^{-5} M), (□) - enzyme preincubated with buffer and DPNH, (○) - enzyme preincubated with buffer, DPNH and o-phenanthroline (3×10^{-5} M).

1,2-dimercaptopropane (10^{-3} M) reverse the cadmium inhibition, whereas glutathione, at the same concentration has no effect. KCN is included in the reaction mixture, since we have observed an inhibition of the transhydrogenase reaction when the enzyme is preincubated with DPNH alone. Fig. 2 shows this effect, and its prevention, in this case by o-phenanthroline.

These observations may be explained most simply by the sequence,

$$\text{DPNH} + \text{H}^+ + \begin{matrix} \text{S} \\ | \\ \text{S} \end{matrix}\!\!\diagdown\!\!\diagup \text{Enz-FAD} \rightleftharpoons \text{DPN}^+ + \begin{matrix} \text{HS} \\ \diagdown \\ \diagup \\ \text{HS} \end{matrix}\!\!\diagdown\!\!\diagup \text{Enz-FAD}$$

where the dithiol group is the first group reduced by DPNH; FAD may not participate in the reaction. Since the lipoic dehydro-

genase and transhydrogenase reactions are inhibited and reactivated under the same conditions, it is reasonable to assume that both pyridine nucleotides and lipoic acids interact with the same postulated dithiol group on the enzyme. Dye reduction could, then, result from either direct interaction of DPNH and enzyme FAD, as discussed by Searls and Sanadi (1960), or electron transfer from the reduced dithiol function to FAD.

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