# EVIDENCE FOR A VICINAL DITHIOL IN DIHYDROTHIOCTYL DEHYDROGENASE*

Robert L. Searls and D. R. Sanadi

Gerontology Branch, National Heart Institute,
National Institutes of Health, PHS, D.H.E.&W.,
Bethesda, and the Baltimore City Hospitals,
Baltimore, Maryland

Received March 1, 1960

Recent work from several laboratories (Massey, 1958; Koike and Reed, 1959; Searls and Sanadi, 1959; Notani and Gunsalus, 1959) has shown that dihydrothioctyl (dihydrolipoyl) dehydrogenase is a flavoprotein closely related to Straub's diaphorase (Straub, 1939). The activities associated with the enzyme are shown in Equations 1 and 2 where $TS_2$ stands for a thioctyl derivative. Evidence for the possible involvement of a vicinal dithiol,

$$DPNH + H^+ + TS_2 \rightleftharpoons DPN^+ + T(SH)_2 \qquad (1)$$

$$DPNH + H^+ + Dye \longrightarrow DPN^+ + Dye\ H_2 \qquad (2)$$

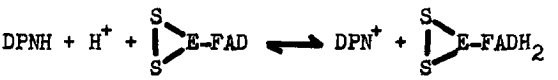
in addition to FAD, in Reaction 1 is presented in this communication.

The data in Table I show that dihydrothioctyl dehydrogenase forms a complex with arsenite or $Cd^{++}$ in the presence of DPNH. The interaction results in loss of activity in the reduction of thioctamide (Reaction 1) and a concomitant increase in the diaphorase activity (Reaction 2). DPN does not replace DPNH in the system. The effect of arsenite is largely reversed by a dithiol compound (2,3-dimercaptopropanol, BAL); however, a monothiol (cysteine), even at much higher concentrations is considerably less effective.

The results may be explained on the assumption that the reduction of thioctamide by DPNH proceeds through the mediation first of FAD and then of an enzyme-bound disulfide group (Reactions 3 to 5). The reduction of

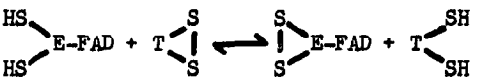
*This work was supported in part by a grant-in-aid from the American Heart Association, Inc.

2,6-dichlorophenol indophenol requires only FAD (Reaction 3 plus 6). The enzyme dithiol formed in the presence of DPNH could bind arsenite and $Cd^{++}$

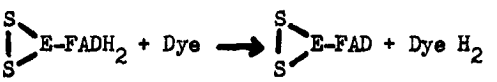



(3)


(4)




(5)




(6)

and result in inhibition of thioctamide reduction which consists of the sequence of Reactions 3-5. On the other hand, elimination of Reaction 4 would favor Reaction 6 since they are in competition for the $FADH_2$ formed in Reaction 3.

Reaction 5 may involve a disulfide interchange as postulated earlier (Sanadi, Langley and Searls, 1959) on the basis of the kinetic properties of the dihydrothioctyl dehydrogenase reaction. Since the purified flavoprotein has no detectable thioctic acid (Searls and Sanadi, in press), the vicinal dithiol may be associated with the enzyme protein as in the case of aldehyde dehydrogenases studied by Jakoby (1958). The possibility that it is a part of an unknown small molecule similar to thioctic acid cannot be excluded. There is a significant difference between these two systems. A monothiol was necessary for producing the inhibition by arsenite in the case of the aldehyde dehydrogenase; however, BAL or cysteine did not replace DPNH in the experiments shown in Table I. The specific requirement of DPNH favors the assignment of an oxidation-reduction role to the enzyme-disulfide group in dihydrothioctyl dehydrogenase.

The effectiveness of arsenite in the activation of the diaphorase reaction may depend on the particular electron acceptor used and how effectively it can compete with the disulfide for $FADH_2$. This type of competition

Table I

Effect of Arsenite and $Cd^{++}$ on Dihydrothioctyl Dehydrogenase

| Experiment | Preincubation Conditions | μm/min./mg. Thioctyl Dehydrogenase | μm/min./mg. Diaphorase |
|---|---|---|---|
| 1 | 1 — | 114 | 0.69 |
| | 2 DPNH | 107 | 0.58 |
| | 3 Arsenite | 112 | 0.58 |
| | 4 DPNH + Arsenite | 19.9 | 1.54 |
| | 5 DPNH + Arsenite + BAL | 74 | |
| | 6 DPNH + Arsenite + Cysteine | 45 | |
| 2 | 1 — | 94 | 0.60 |
| | 2 DPNH | 93 | 0.58 |
| | 3 $Cd^{++}$ $5 \times 10^{-5}$ M | 89 | 0.52 |
| | 4 DPNH + $Cd^{++}$ | 7.4 | 2.00 |

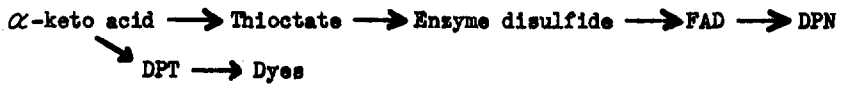
Dihydrothioctyl dehydrogenase: To solutions containing 2.6 μg. of dihydrothioctyl dehydrogenase, 0.4 mg. bovine serum albumin and 25 μmoles of phosphate buffer at pH 7.2 were added, as indicated, 0.03 μmole DPNH, 0.1 μmole sodium arsenite or 0.01 μmole cadmium chloride. Then 0.3 μmole BAL and 2 μmoles cysteine, each in 0.01 ml., were added as shown. The final volume in each preincubation mixture held in an ice bath was 0.2 ml. Dihydrothioctyl dehydrogenase activity was measured by adding 0.02 ml. of the preincubated enzyme solution to a reaction mixture containing 50 μmoles phosphate, pH 6.8, 0.18 μmole DPNH and 0.75 μmole DL-thioctamide in 0.98 ml. at 30°.

Diaphorase: 52 μg. of dihydrothioctyl dehydrogenase were preincubated as above, except the final volume was 0.4 ml. Then 0.02 ml. was added to 150 μmoles phosphate, pH 7.3, and 0.3 μmole DPNH in 2.95 ml. at 30°. The reaction was initiated by the addition of 0.03 ml. of 1% 2,6-dichlorophenol indophenol.

may be one reason for the large differences in the rates of reduction of artificial acceptors in various electron transport systems.

The oxidation of α-keto acids by DPN is a relatively simple segment of the more complex respiratory chain ending with oxygen as the terminal acceptor. Even so, the electron transfer sequence may involve at least three

intermediate carriers as shown:




The oxidation of the α-keto acid by artificial acceptors like ferricyanide or dyes may involve diphosphothiamine in a different sequence (Searls and Sanadi).

## References

Jakoby, W. B., J. Biol. Chem. 232, 89 (1958).

Koike, M. and Reed, L. J., J. Amer. Chem. Soc. 81, 505 (1959).

Massey, V., Biochim. Biophys. Acta 30, 205 (1958).

Notani, G. W. and Gunsalus, I. C., Fed. Proc. 18, 295 (1959).

Sanadi, D. R., Langley, M., and Searls, R., J. Biol. Chem. 234, 178 (1959).

Searls, R. L. and Sanadi, D. R., Proc. Nat. Acad. Sci. 18, 320 (1959).

Searls, R. L. and Sanadi, D. R., J. Biol. Chem., (in press).

Searls, R. L. and Sanadi, D. R., Biochem. Biophys. Res. Comm., this issue, p. 2

Straub, F. B., Biochem. J. 33, 787 (1939).