The Mechanism of Alcohol Dehydrogenase Action

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The preparation of horse liver alcohol dehydrogenase (ADH) in a pure and crystalline state [1] has enabled us to carry out an extensive investigation on the reaction velocities and equilibria in the system:

$$DPN + C_2H_5OH \overset{ADH}{\rightleftharpoons} DPNH + H^+ + CH_3CHO$$

It was observed that the absorption band at 340 mμ in the free DPNH moved down to 325 mμ when DPNH was bound to ADH [2]. This phenomenon made a much more detailed study of the enzyme mechanism possible than in any DPN-enzyme system before.

Spectrophotometric measurements revealed that two molecules of DPNH were bound to one molecule of ADH (M = 73 000) at pH 7—9. The bonds are loosened at higher pH, so that around one molecule of DPNH is bound per mole of ADH at pH 10.

The molar concentration of ADH is so high in the liver that it approaches the DPN concentration. Our studies on the reaction equilibria with varied pH and [ADH] showed that the ratio between the dissociation constants of the DPN · ADH and DPNH · ADH complexes varied from 200, at pH 7, to a value slightly above 1 at pH 10. The redox potentials of the ADH-coferment complex were calculated from the equilibrium values to $E_0' = -0.196$ V at pH 6.4; -0.208 V at pH 7; -0.244 V at pH 8; -0.302 V at pH 9; and -0.351 V at pH 10. These potentials are much higher than for the free DPN—DPNH, and approach the potential level of the ethanol-acetaldehyde. This effect greatly facilitates the oxidation of ethanol in the ADH-system.

The slopes $\frac{d E_0'}{d pH}$ for the ADH-coferment complex indicate that an acid group had a pK' of 10 in the reduced, and pK' 7.8 in the oxidized complex.

This acid group was identified as sulfhydryl, linked to the reduced pyridine ring of DPNH, probably its ring nitrogen atom.

G. Wald in a recent, personal communication to us pointed out that the ADH activity was inhibited by *p*-chloromercurobenzoic acid. The inhibition was reversed by glutathione. We found that the absorption maximum of ADH-bound DPNH immediately shifts from 325 to 340 mμ on the addition of 0.003 *M* *p*-chloromercurobenzoic acid. Our conclusion is that this shift is caused by a bond between ADH—SH and pyridine being broken. This bond is necessary for the enzyme activity.

To our knowledge this is the first observation of SH-groups in apoenzymes interacting with coferments.

Determinations of the association and dissociation velocity constants of ADH · DPNH were carried out by the aid of the band shift by one of us (H. T.) and B. Chance in Philadelphia. Equations were derived for the kinetics of the enzyme systems. All experimental observations hitherto seem to fit with this theory.

A detailed report of this work will be published in this journal.

1. Bonnichsen, R. K., and Wassén, A. M. *Arch. Biochem.* **18** (1948) 361.
2. Theorell, H. *8:e Conseil de Chimie de l'Institut International Solvay.* Bruxelles (1950) p. 395.

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