BIOCHEMICAL LETTERS

The rate-determining step in the liver alcohol dehydrogenase-catalysed reduction of acetaldehyde is an isomerization of the enzyme

The reaction catalysed by liver alcohol dehydrogenase involves an ordered ternary complex mechanism, with coenzyme binding first and product release preceding coenzyme release (Dalziel, 1975). Steady state kinetic studies (Theorell & Chance, 1951; Dalziel, 1963) showed that the rate-determining step for reduction of aliphatic aldehydes is the dissociation of the enzyme-NAD⁺ complex (step k'_{-1} in eqn. 1):

where Ald is the aldehyde and Alc the alcohol. This conclusion is supported by the close agreement between the values of k'_{-1} calculated from steady state kinetics (Dalziel, 1963) and those measured directly (De Traglia *et al.*, 1977; Kvassman & Pettersson, 1979). The following discussion indicates that the rate-determining step in acetaldehyde reduction is actually an isomerization of the enzyme during dissociation of NAD⁺.

Recently we used a pressure relaxation technique to study protein fluorescence changes associated with the NAD⁺ binding equilibrium (Coates *et al.*, 1977). The dependence of the observed relaxation time on NAD⁺ concentration required that the process consist of at least two steps (eqn. 2), with a binding step and an isomerization step:

The values of k_a (k_{-2} of Coates *et al.*, 1977) determined at three pH values are shown in Fig. 1, together with Kvassman & Pettersson's (1979) estimates of the NAD⁺ dissociation rate constant,

k'_{-1} . The agreement between the two sets of data is close, particularly in view of the errors inherent in the determinations of k_a (see, for example, Fig. 2 of Coates *et al.*, 1977). Furthermore, k'_{-1} and k_a have similar pH dependence, in each case consistent with a pK_a of about 7.6 (Coates *et al.*, 1977; Kvassman & Pettersson, 1979). This strongly suggests that the same enzymic step controls both k'_{-1} and k_a .

The simplest explanation is that the two-step mechanism of eqn. (2) also applies to the displacement experiments of Kvassman & Pettersson (1979). If k_b is much faster than the isomerization rate constants, k_a and k_c , the observed rate constant for NAD⁺ dissociation in the displacement experiments (k'_{-1}) will be equal to k_a . This interpretation is

Fig. 1. Comparison of the rate constants for NAD⁺ dissociation (k'_{-1}) and isomerization of the enzyme-NAD⁺ complex (k_a)

Values of k'_{-1} (O) are from Kvassman & Pettersson (1979) and k_a values (●) are from Coates *et al.* (1977). The dissociation curve was calculated for a maximum value of 160 s^{-1} and a pK_a of 7.6.

supported by the low value of the association rate constant k'_1 ($1.0 \mu\text{M}^{-1} \cdot \text{s}^{-1}$ at pH 7; Kvassman & Pettersson, 1979); for the two step mechanism of eqn. (2), $k'_1 = (k_c \cdot k_d)/(k_b + k_c)$, giving an observed rate constant lower than the true rate constant for binding, k_d (Fersht, 1977, pp. 118–121).

The preceding evidence shows that the rate of NAD^+ dissociation is controlled by the rate of isomerization of the enzyme- NAD^+ complex (k_a) and this isomerization is consequently the rate-determining step for the liver alcohol dehydrogenase-catalysed reduction of acetaldehyde. This isomerization involves a change in protein fluorescence, which is probably caused by a conformational change in the enzyme (Laws & Shore, 1978). If this is the case, the evidence presented here represents one of the first direct demonstrations that a conformational change is rate-determining in a dehydrogenase-catalysed reaction (see Fersht, 1977, p. 131; Dalziel, 1975).

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(Received 17 February 1981)

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