Non-enzymatic Models of NADH Action—Importance of Hydrogen-bonding in the Enzyme–Coenzyme–Substrate Complex

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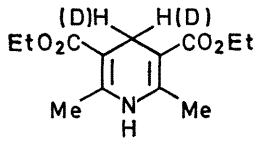
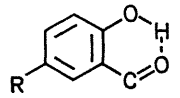
Summary Hydrogen-bonding in *o*-hydroxybenzaldehyde activates the carbonyl function towards reduction by Hantzsch ester (I); the reaction represents a model for the mechanism of NADH action.

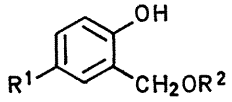
THE MECHANISM of NADH-mediated enzymatic reductions has received a great deal of attention through model studies involving potential substrates and 1,4-dihydropyridine derivatives.[1] No model reaction has been reported which satisfactorily parallels the enzymic transformation. Kosower[2] has suggested that the transition-state complex in the reduction of acetaldehyde with alcohol dehydrogenase (ADH) and NADH may involve polarization of the carbonyl function by hydrogen-bonding with an ammonium group located at the 'active site'. This feature of the transition state has been ignored in the studies made thus far. We present a non-enzymatic model, which emphasizes the importance of hydrogen-bonding in the mechanism of NADH action.



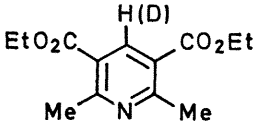
(I)



(II) R = H
(IV) R = NO_2



(III) R^1 = H R^2 = CH_2CH_2OH
(IV) R^1 = NO_2 R^2 = H



(VI)

An analogy may be drawn between the aldehyde–ADH–NADH (substrate–enzyme–coenzyme) complex, containing a hydrogen-bonded carbonyl group, and the transition state for the reaction of suitable hydroxy or amino (protonated) aldehydes or ketones with 1,4-dihydropyridine derivatives. The efficiency of such a model would depend upon the degree of orbital steering[3] attained by the reacting centres. As a simple example of the proposed model we have examined the reaction of suitably substituted benzaldehydes with Hantzsch ester (I).

The carbonyl group of benzaldehyde or *m*-nitrobenzaldehyde was found to be inert towards reaction with (I), under a wide range of reaction conditions. When a mixture of salicylaldehyde (II) and ester (I) was refluxed in ethylene glycol, small amounts of reduction product (III) could be identified (n.m.r., g.l.c.). This result clearly indicates the importance of hydrogen-bonding, *via* the phenolic hydroxy-group, in lowering the energy of the transition state for reduction of the carbonyl function. Since an increase in the acidity of the phenolic proton would enhance its hydrogen-bonding ability; introduction of an electron-withdrawing group in the aromatic ring, *ortho* or *para* to the –OH, should favourably influence the reduction process. This was shown by reaction of (I) with nitrosalicylaldehyde (IV). Heating of (IV) with the Hantzsch ester, in refluxing dioxan, converted it exclusively into the corresponding alcohol (V), which was identified by comparison with an authentic sample. A similar reduction was also observed in aqueous ethanol (1 : 1) at 40°. The direct transfer of hydrogen (in the dioxan reaction) from the Hantzsch ester to the carbonyl group, was established by the reaction of [4,4-$^2H_2$]-3,5-diethoxycarbonyl-1,4-dihydro-2,6-dimethylpyridine with aldehyde (IV). The n.m.r. spectrum of the corresponding alcohol (V) revealed that *one* deuterium atom had been incorporated in the benzyl methylene group. An equivalent amount of [4-^{2}H]-3,5-diethoxycarbonyl-2,6-dimethylpyridine (VI) was also formed during the reaction. The reaction between (I) and (IV) in dioxan was followed spectrometrically (n.m.r.). After accounting for a slow concomitant disproportionation[4] of (I), the reaction was found to be first order with respect to ester (I) and aldehyde (IV) with a bimolecular rate constant of 2×10^{-4} mol^{-1} s^{-1}.

The study of more sophisticated models closely paralleling the enzyme–coenzyme-substrate complex is currently in progress.

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[1] T. C. Bruice and S. Benkovic, 'Bioorganic Mechanisms,' Benjamin, 1966, vol. 2, pp. 343—349.
[2] E. M. Kosower, *Biochem. Biophys. Acta*, 1962, **56**, 474.
[3] D. R. Storm and D. E. Koshland, *Nat. Acad. Sci. U.S.A.*, 1970, **66**, 445.
[4] This reaction will be discussed in detail elsewhere.