Reduction of Ninhydrin with Cyclodextrin-1,4-dihydronicotinamides as NADH Models

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Cyclodextrin-1,4-dihydronicotinamides (**1**) and (**2**) are more stable in aqueous solution and show a large rate enhancement (15 and 50 times greater respectively) in the reduction of ninhydrin compared with monomeric NADH.

Cyclodextrins, forming inclusion complexes with a wide variety of substrates in aqueous solution,[1] affect the rate of various chemical reactions and exhibit substrate selectivity,[2] so that they are often conveniently and successfully utilized as enzyme models.

α- and β-Cyclodextrin-1,4-dihydronicotinamide, (**1**) and (**2**), as NADH coenzyme models, were prepared from mono(toluene-*p*-sulphonated) cyclodextrins as starting materials,[3] *via* (at the C-6 of the glucose ring) iodination, substitution by nictotinamide, and reduction according to the general procedure of Haynes and Todd.[4]†

It has been established that NADH and its model compounds, 1,4-dihydronicotinamide derivatives, undergo structural alternation in aqueous solution (Scheme 1), resulting in a shift of the characteristic u.v. absorption band in the 340—360 nm region downward to around 290 nm.[5] The product absorbing at 290 nm was later confirmed to be 6-hydroxy-1,4,5,6-tetrahydronicotinamide by an *X*-ray study.[6] In our hydration experiments in phosphate buffer (pH 7.0), the absorption band for *N*-propyl-1,4-dihydronicotinamide (**3**) at 358 nm rapidly shifted downward to around 290 nm, whereas those for (**1**) or (**2**) did so slowly. A typical hydration with time is given in Figure 1. The hydration rate was obtained spectrometrically by measuring the intensity of the absorption band at 358 nm, under anaerobic conditions. The hydration rates of (**3**) were decreased by adding a cyclodextrin into the system. These results seem to be caused by the formation of a complex between cyclodextrin and the nicotinamide moiety in aqueous media. An *X*-ray study has also made clear the structure of the cyclodextrin inclusion complex with nicotinamide.[7] However, the inclusion phenomena in aqueous solution are not clear yet.

To stimulate dehydrogenase catalysis, a system was devised using (**1**) or (**2**) as a hydrogen donor and ninhydrin as a hydrogen acceptor, and the reduction rates were followed spectrometrically in aqueous media (pH 7.0) at 25 °C. The disappearance of the dihydronicotinamide unit of (**1**) or (**2**) was followed at 358 nm. ^{1}H N.m.r. spectroscopy revealed the formation of the product by observation of a multiplet at δ 9.5—8.0 for the oxidised (**1**) or (**2**).

Plots of the first-order rate constants k_{obs} of (**1**) and (**2**) *vs.* ninhydrin concentration are shown in Figure 2. Such saturation behaviour may be generally regarded as evidence for complex formation between (**1**) or (**2**) and ninhydrin. Since

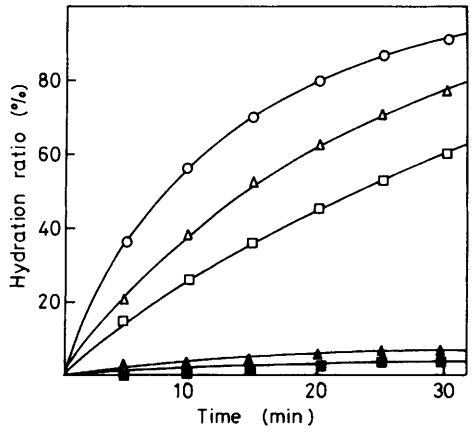
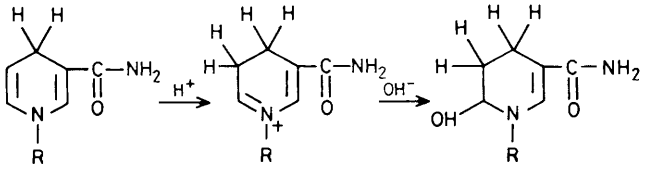


Figure 1. Hydration ratio of dihydronicotinamide compounds *vs.* time (min) in phosphate buffer (pH 7.0) at 25 °C, [cyclodextrin (α-, β-)] = 4 × 10^{-4} M; [(**1**)], [(**2**)], and [(**3**)] = 2 × 10^{-4} M; (**1**) = ▲; (**2**) = ■; (**3**) = ○; (**3**)/α-cyclodextrin = △; (**3**)/β-cyclodextrin = □.



(**1**) R = α-cyclodextrin
(**2**) R = β-cyclodextrin
(**3**) R = Pr^n

Scheme 1

$$C + S \underset{}{\overset{K_m}{\rightleftharpoons}} C{\cdot}S \xrightarrow{k_{cat}} C' + P$$

Scheme 2

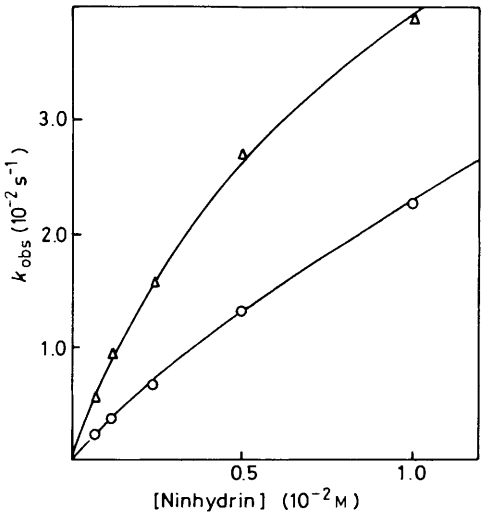


Figure 2. Reduction of ninhydrin with (**1**) and (**2**) in phosphate buffer (pH 7.0) at 25 °C, [(**1**)] and [(**2**)] = 2 × 10^{-4} M; (**1**) = ○; (**2**) = △.

† Compounds (**1**) and (**2**) were purified using column chromatography until a single peak was obtained on h.p.l.c.

Table 1. Kinetic parameters for the reduction of ninhydrin.

	K_m (10^{-3} M)	k_{cat} (10^{-2} s^{-1})	k_{cat}/K_m (mol^{-1} dm^3 s^{-1})
(1)	35	10	2.9
(2)	4.3	4.8	11
NADH	—	—	0.21

these reductions are of the Michaelis–Menten type (coenzyme-like), rate constants k_{cat} and dissociation constants K_m were determined, assuming Scheme 2.

C [(**1**) or (**2**)] and S (ninhydrin) reversibly form an aggregate of C·S (complex) to produce C′ [oxidized form of (**1**) or (**2**)] and P (product). k_{cat}, K_m, and second-order rate constants k_{cat}/K_m can be evaluated from Eadie–Hofstee plots.[8] The k_{cat}/K_m values, as shown in Table 1, indicate a large rate enhancement (15- and 50-fold, respectively) for (**1**) and (**2**) compared with monomeric NADH. Under equimolar conditions (**2**) is more reactive than (**1**), which may be caused by the difference in their cavity size. The values of K_m also suggest that (**2**) forms the complex more easily than (**1**).

Based on the above investigation it can be concluded that (**1**) and (**2**) are stable in aqueous solution since they have a hydrophobic cavity and can reduce a substrate included in the cavity in the same way as an enzyme. Thus, these artifical enzymes provide direct mechanistic evidence in dehydrogenase catalysis for binding effects and rate enhancement, and may be used in the investigation of NADH-dependent enzymes.

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