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THE CYCLODEXTRIN-NICOTINAMIDE COMPOUND AS A DEHYDROGENASE MODEL SIMULATING APOENZYME-COENZYME-SUBSTRATE TERNARY COMPLEX SYSTEM

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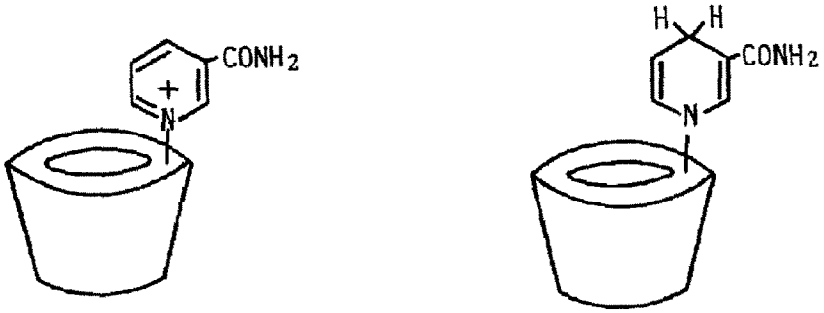
Abstract: The cyclodextrin-dihydronicotinamde had a dihydronicotinamde group at the open side of cyclodextrin cavity, and showed a large rate enhancement in the reduction of substrate upon complexation comparing with NADH.

In the lines of studies to simulate enzyme action using cyclodextrins[1], the molecular design of the coenzyme NADH-dependent enzyme catalysis[2-4] may be set up with a dihydronicotinamide(coenzyme) attached to a cyclodextrin(apoenzyme) and a complexed substrate in the aqueous system. This paper describes the first synthesis of β-cyclodextrin-dihydronicotinamide(Id) which has a nicotinamide moiety on a cyclodextrin molecule and can form a complex with a substrate and reduce it. Id also reveals a large rate enhancement for the reduction of a substrate comparing with monomeric NADH. This is the first approach for the model reaction of compulsory ordered mechanisms[5] in which the pyridine nucleotide compulsorily binds first.

We have already reported the selective modification of the secondary hydroxyl groups of cyclodextrins by functional groups.[6,7] The key step of the preparation was the selective tosylation of one secondary hydroxyl group, which occured by the reaction of β-cyclodextrin with 5 times molar quantity of *p*-tolylsulfonyl chloride in pH 12.5 aqueous alkaline solution at room temperature for one hour.[7] Partial hydrolysis of β-cyclodextrin tosylate in an acidic solution gave glucose and 3-tosyl glucose which was determined by paper chromatography. Here, one of the secondary hydroxide anion at C-3 position on glucose ring of β-cyclodextrin can attack the sulfur atom of *p*-tolylsulfonyl chloride included in its cavity, giving C-3 monotosyl β-cyclodextrin. Then, the cyclodextrin tosylate was allowed to react with 10 time molar quantity of nicotinamide in DMF at 110°C for 2 days.[8] The product was precipitated into acetone, followed by gel chromatography with high porous polystylene gel. The product was applied to a column (DIAION HP-20; Φ3 x 70 cm) and eluted with water, 5% aqueous methanol and then 10% aqueous methanol. The 10% aqueous methanol eluate was evaporated to dryness, giving β-cyclodextrin-nicotinamide (I) which had carbamoyl pyridinium moiety attached to β-cyclodextrin. These chromatographic procedures were repeated until t.l.c. indicated this was pure (R_f 0.05 with the developing solvent; *n*-butanol-DMF-water 2:1:1). Yield was 10% based on the starting cyclodextrin tosylate.

Nmr spectrum of I in D_2O, refered to TMP (3-(trimethylsilyl)-tetradeuteriopropionic acid sodium salt), showed absorption at δ 4.86 assigned to C_1H of glucose ring. Also it showed multiplet peaks at δ 9.5-8.0 ppm due to nicotinamide, and qualtet at δ 7.7-7.1 ppm due to *p*-tolylsulfate anion which may form the ion-pair with quaternary pyridinium ion. Anal. Calc. for $C_{55}H_{82}N_2O_{38}S\cdot 3H_2O$: C, 46.11; H, 5.88; N, 1.96; S, 2.24. Found; C, 46.07; H, 5.67; N, 2.11 S, 2.53.

Cyclodextrin-dihydronicotinamide (Id) was prepared by the reduction at C-4 position of nicotinamide moiety of I according to a general procedure of Haynes and Todd.[9] After chromatography with HP-20, the fractions eluted with 33% aqueous methanol gave the purified Id. Yield was *ca.* 50%. The structure of Id was confirmed by UV absorption λ_{max} 355 nm (ε_{max} 2100) due to the reduced form of pyridine ring. The schematical structures of these newly prepared cyclodextrin-nicotinamide compounds I and Id was shown as below.

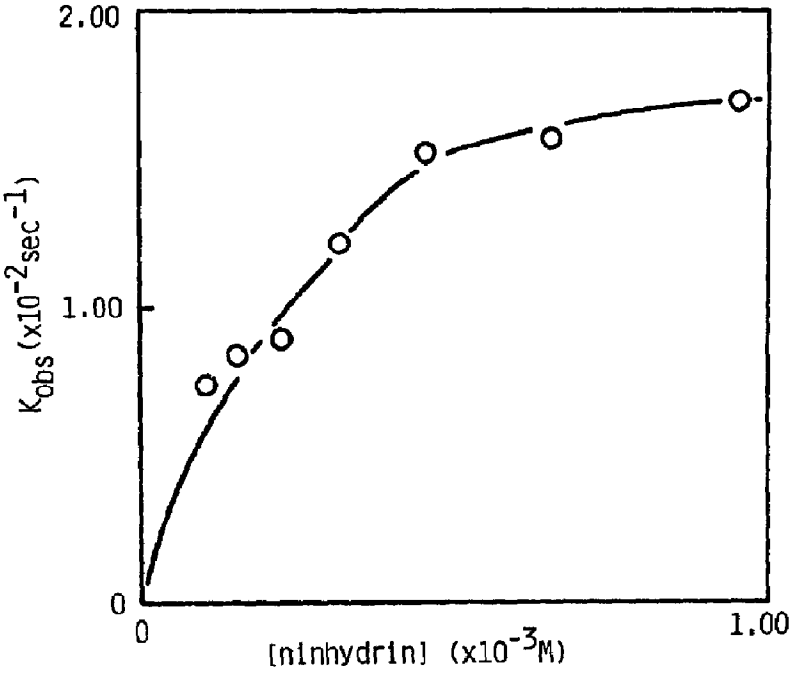


I Id

Half-wave potentials of I, NAD^+ and ninhydrin (hydrogen acceptor) were determined by polarography as to be -1.05, -1.00[10] and -0.95 volts respectively in pH 7.0 buffer solutions [0.2N-KH_2PO_4-0.2N-NaOH-0.1N-KCl] refered to saturated calomel electrode. Because half-wave potentials can be paralleled with redox potentials,[11] the reduction of ninhydrin with Id seemed to be reasonably possible.

The redox reactions of Id or NADH with ninhydrin were carried out in aqueous media(pH 7.0) and rates of reduction were followed spectrometrically. The disappearence of dihydronicotinamide was followed at 350 nm by a usual method at 25.0°C. Id was so stable in pH 7.0 aqueous solution at room temperature that the self-decomposition of Id was negligible. By the measurements of nmr, Id showed no peaks at δ 9.5-8.0 ppm, but after the reaction was over multiplet peaks at δ 9.5-8.0 ppm due to nicotinamide could be observed, which indicated that Id was completely oxidized to I. In the condition of excess ninhydrin concentrations, pseudo-first order rate constants k_{obs} were evaluated by Guggenheim plots.[12] Plotting k_{obs} versus ninhydrin concentrations showed increasing curvature with increasing ninhydrin concentrations as in Figure 1. Such saturation behavior in the presence of Id is generally regarded as a manifestation of complex formation between Id and ninhydrin. The kinetics of this reduction fit equations analogous to those of enzyme kinetics. The rate constant k for fully complexed ninhydrin and the complex dissociation constant K_d were determined, assuming the following schem

Figure 1. Reduction of Ninhydrin ($1.00\text{-}0.12 \times 10^{-2}$ M) with Id (1.00×10^{-4} M) in the pH 7.0 Buffer Solution at 25.0°C.



$$S + C \underset{}{\overset{K_d}{\rightleftharpoons}} [C \cdot S] \xrightarrow{k} P + C'$$

where S is ninhydrin which can be included by Id (as expressed in C) and reduced to give product P with rate constant k, and C′ is I. The well-known Eadie expression[13] for treatment of enzyme kinetic data is presented in the following equation.

$$k_{obs} = k - K_d \cdot k_{obs} / [S]$$

Therefore k can be obtained from the intercepts of Eadie plots and K_d from the slopes as were shown in Table 1. In the redox reaction of Id or NADH with ninhydrin in equimolar condition, second order rate constant k_{II} were evaluated from half-life method, which are also shown in Table 1.

Table 1. Reduction of Ninhydrin with Id and NADH

compound	$k^{a)}$ $\times 10^{-2}sec^{-1}$	$K_d^{b)}$ $\times 10^{-5}M$	$k_{II}^{c)}$ sec^{-1}
Id	2.0	2.1	12.6
NADH			0.31

a) First order rate constant determined by Eadie plots.

b) Dissociation constant determined by Eadie plots; Data from Figure 1.

c) Second order rate constant determined by half-life method at pH 7.0, 25.0°C, [Id] = 1.0×10^{-4} M, [NADH] = 1.0×10^{-4} M and [ninhydrin] = 1.0×10^{-4} M.

According to the k_{II} values, Id indicated large rate enhancement up to 40 times comparing with monomeric coenzyme NADH. This result suggested the importance of proximity effect[14] caused by complex formation between two compounds. The value of K_d (2.1×10^{-5} M) also suggested relatively tight complex formation between Id and ninhydrin.

Based on the above investigations it can be concluded that the newly prepared compound (Id) has a dihydropyridine ring at the open side of β-cyclodextrin troidal and can reduce a substrate included in the cavity in the same mode of reactions as enzyme reactions. Thus, this artificial enzyme has a direct mechanistic evidence for the dehydrogenase catalysis in several points such as tight binding, rate enhancement and proximity effect which were caused by the ternary complex formation.

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