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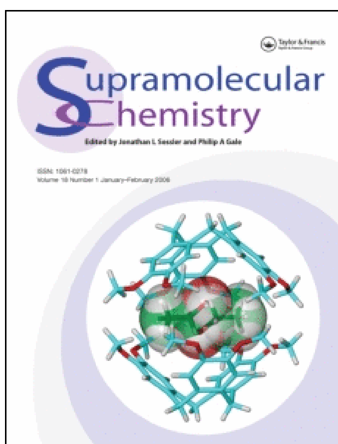

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Synthesis and characterization of α-, β- and γ-cyclodextrin-nicotinamide

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Synthesis and characterization of α-, β- and γ-cyclodextrin-nicotinamide

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α-, β- and γ-cyclodextrin-nicotinamide (α-, β- and γ-CDNA) were synthesized as NADH coenzyme models, and the binding abilities were investigated. CDNA binds a negatively charged guest stronger than unmodified cyclodextrin because of the electrostatic interaction between the nicotinamide residue and the guest molecule. Different binding abilities were measured and were dependent on cavity size.

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INTRODUCTION

Cyclodextrins have been extensively studied for enzyme models because cyclodextrin has a hydrophobic cavity which acts like a binding site of an enzyme.[1] We have already reported on α-chymotrypsin models using modified cyclodextrins[2] and NADH coenzyme models using α-and β-cyclodextrin-nicotinamide (α- and β-CDNA).[3] We synthesized γ-CDNA and expected to obtain different properties as an enzyme model due to the difference of the cavity size and the difference in conformation between the cavity and the nicotinamide residue (Fig 1). In this paper we describe the synthesis of γ-CDNA and the binding abilities of α-, β- and γ-CDNA.

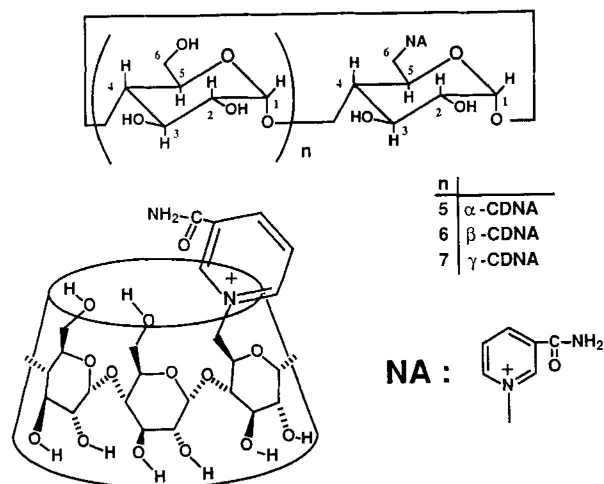

Figure 1 Structures of α-, β- and γ-*CDNA*.

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RESULTS AND DISCUSSION

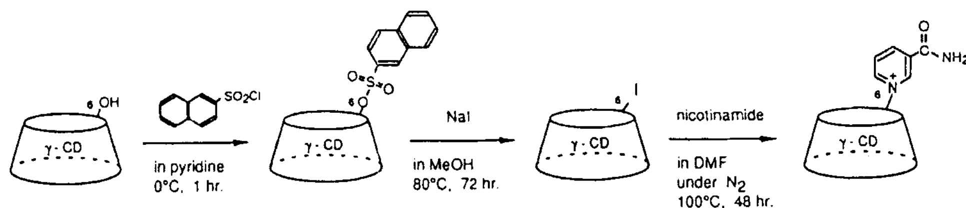
1. Synthesis of γ-CDNA

Scheme 1 shows the synthetic methods for γ-CDNA. In the case of α- or β-CDNA, mono-tosylated α- or β-cyclodextrin was used as a starting material.[3] For γ-CDNA, mono-naphthylated γ-cyclodextrin[4] was used, because it was easier to purify mono-naphthylated γ-cyclodextrin using column chromatography on high porosity polystyrene gel DIAION HP-20 than mono-tosylated γ-cyclodextrin.

Figure 2 shows the ^{1}H-NMR spectra of α-, β- and γ-CDNA in D_2O. They gave similar spectra. The peaks in the region 8.1–9.2 ppm are protons of the nicotinamide residue and those in the region 2.6–5.4 ppm are protons of cyclodextrin. Shifts in the peaks caused by modification of the nicotinamide residue were observed. From the ratio of the integral intensity between the peaks of the nicotinamide residue and the anomeric protons of the cyclodextrin, it was confirmed that α-, β- and γ-cyclodextrin were each modified by one nicotinamide residue.

2. Binding abilities of CDNA

When an achiral molecule was included in the chiral cavity of cyclodextrin, an induced circular dichroism (ICD) band was observed in the wavelength region of the absorption band. By adding cyclodextrin or modified cyclodextrin to the solution of *p*-nitrophenol (PNP), a positive ICD band was observed at around 400 nm. The formation of an inclusion complex between PNP and cyclodextrin or modified cyclodextrin was indicated. Figure 3 shows the dependence of the ICD intensity of PNP on the concentration of α-CDNA at 410 nm. The hyperbolic curve and limiting



Scheme 1

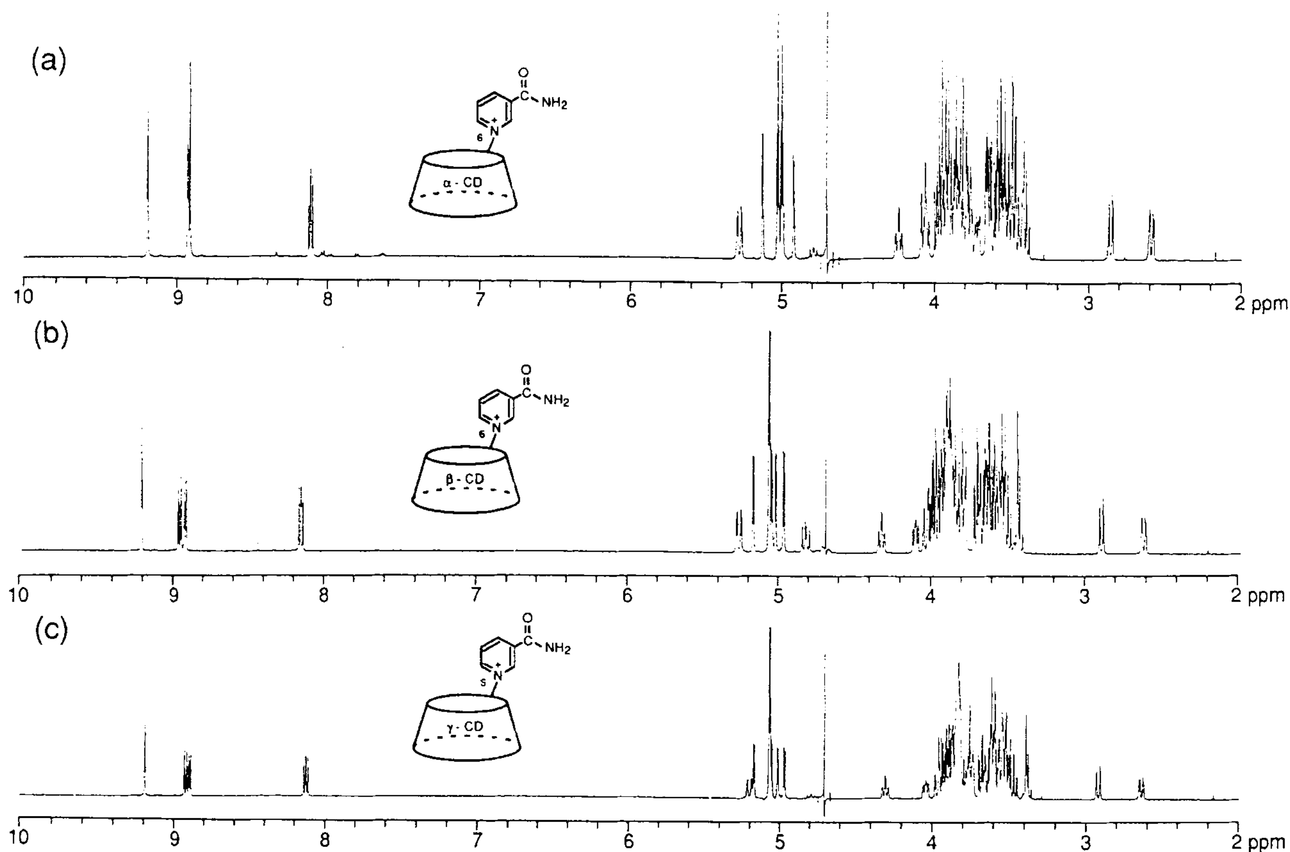


Figure 2 500-MHz ^{1}H-NMR spectra of (*a*) α-CDNA, (*b*) β-CDNA, and (c) γ-CDNA in D_2O.

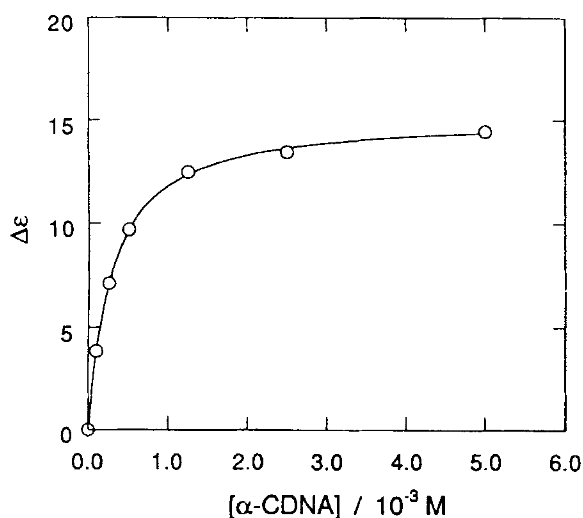


Figure 3 Dependence of ICD intensity of PNP on the concentration of α-CDNA.

value of Δε indicated that only a 1:1 complex was formed. α-Cyclodextrin, β-cyclodextrin, α-CDNA and β-CDNA gave similar results. However, in the case of γ-cyclodextrin, no ICD band was observed. This seemed to be caused by the large cavity of γ-cyclodextrin.

ICD intensities of the β-cyclodextrin–PNP and the β-CDNA–PNP complexes at around 400 nm decreased upon addition of adamantanecarboxylic acid (ACA) or adamantanol (AOH). This shows there was an inhibitory effect of ACA and AOH on the association of β-cyclodextrin or β-CDNA with PNP. In the case of the α-cyclodextrin–PNP complex, the change of ICD intensity could not be detected by the addition of ACA. For the α-CDNA–PNP complex, upon addition of ACA, the ICD intensity decreased slightly. For the α-cyclodextrin cavity, ACA might be too large to be bound more strongly than PNP, but a weak

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interaction between α-CDNA and ACA was detected. This suggested electrostatic interaction caused by the nicotinamide residue.

Tables 1 and 2 give binding constants for cyclodextrins and modified cyclodextrins with guests, estimated from these results. α- or β-CDNA could bind PNP or ACA more strongly than α- or β-cyclodextrin. This indicates the presence of electrostatic interactions between the nicotinamide residue and the negatively charged guests.

Bromocresol green (BCG) forms a strong complex with γ-cyclodextrin compared with α- or β-cyclodextrin.[5] Figure 4 shows the change in the absorption spectra of BCG upon adding various cyclodextrins. In the case of adding γ-CDNA, the largest increase in the absorption spectrum was observed at around 620 nm. On the other hand, the absorption band at around 620 nm was decreased by the addition of α- or β-cyclodextrin. These differences suggest a difference in the conformations of the complexes. Table 3 gives binding constants of γ-cyclodextrin and γ-CDNA with BCG. γ-CDNA can bind BCG 1.6 times more strongly than γ-cyclodextrin.

α-, β- and γ-CDNA have different binding abilities as mentioned above. The reduced forms of α- and β-CDNA were stable from hydration in aqueous solution because of the effect of the cavity and could therefore be used as reducing agents.[3a] γ-CDNA is expected to have different and specific properties as an NADH model compound.

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Table 1 Binding constants (M^{-1}) of various cyclodextrins with PNP

α-Cyclodextrin	*α-CDNA*	*β-Cyclodextrin*	*β-CNDA*
2080	3490	500	770

Borate buffer, with PNP, 25°C (pH 9.6).

Table 2 Binding constants (M^{-1}) of modified and unmodified β-cyclodextrins with ACA and AOH

	β-Cyclodextrin	*β-CDNA*
ACA	18,000	31,000
AOH	4700	5250

Borate buffer, 25°C (pH 9.6).

Table 3 Binding constants (M^{-1}) of modified and unmodified γ-cyclodextrins with BCG

γ-Cyclodextrin	*γ-CDNA*
1684	2625

Citrate buffer, with BCG, 25°C (pH 4.0).

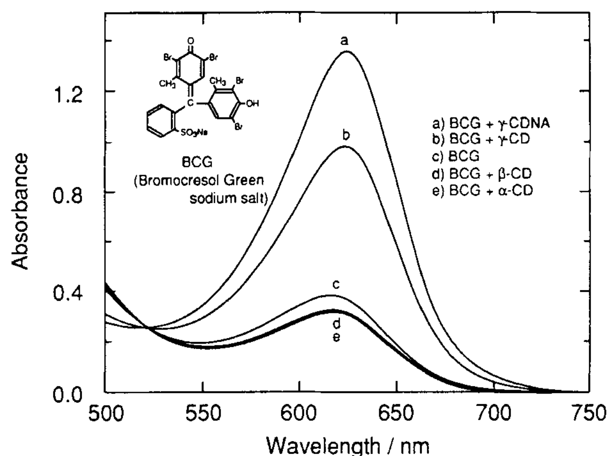

Figure 4 Absorption spectra of 0.05 mM BCG alone and in the presence of 5 mM α-, β- and γ-cyclodextrin, and γ-CDNA in a 10 mM citrate buffer (pH 4.0).

EXPERIMENTAL SECTION

1. Synthesis of α-, β- and γ-CDNA

α- and β-CDNA used in this work were prepared as previously reported.[3] To improve the purification methods, column chromatography with CM-Sephadex C-25 (Na^+ charged) and high porosity polystyrene gel DIAION HP-20 (elution with H_2O) were used.

γ-CDNA was synthesized as follows. Mono-naphthylated γ-cyclodextrin was prepared by the method of Ueno *et al.*[4] To a solution of mono-naphthylated γ-cyclodextrin (9.0 g) in dry methanol (200 ml), sodium iodide (9.0 g) was added. The resulting mixture was stirred at 80°C for 72 h. The reaction mixture was poured into vigorously stirred acetone (1 l) to give a white precipitate. After filtration, the residue was washed with acetone and dried under vacuum. The product was isolated by column chromatography on high porosity polystyrene gel DIAION HP-20 (elution with $H_2O/MeOH = 6:4$; yield 100%). To a solution of γ-cyclodextrin mono-iodide (1.5 g) in dry DMF (25 ml), nicotinamide (0.7 g) was added. The resulting mixture was stirred at 100°C for 48 h under nitrogen. The reaction mixture was poured into vigorously stirred acetone (1 l) to give a yellow precipitate. The product was isolated by column chromatography in a similar way to α- and β-CDNA except for the eluent for HP-20 ($H_2O/MeOH = 95:5$), and Sephadex G-15 was used instead of G-10 (yield 10%). The product was characterized by TLC, ^{1}H-NMR, and FAB-MS ($M^{+1} = 1401$).

2. Determination of binding constants of α-, β- and γ-CDNA

Binding constants of α-cyclodextrin, β-cyclodextrin, α-CDNA and β-CDNA with PNP were determined

as follows. Various concentrations of α-cyclodextrin, β-cyclodextrin, α-CDNA and β-CDNA were added to borate buffer (pH 9.6) containing PNP (5×10^{-5} M). The changes in the ICD spectra were measured at 25°C in the wavelength region of the absorption band for PNP, around 400 nm, using a JASCO J-600 spectropolarimeter.

Binding constants of β-cyclodextrin and β-CDNA with ACA or AOH were determined as follows. Various concentrations of ACA and AOH were added to borate buffer (pH 9.6) containing PNP (5×10^{-5} M). The changes in the ICD spectra caused by the inhibitory effect of ACA or AOH on the association of β-cyclodextrin or β-CDNA with PNP were measured at 25°C in the wavelength region of the absorption band for PNP, around 400 nm, using a JASCO J-600 spectropolarimeter.

Binding constants of γ-cyclodextrin and γ-CDNA with BCG were determined as follows. Various concentrations of γ-cyclodextrin or γ-CDNA were added to citrate buffer (pH 4) containing BCG (5×10^{-5} M). The changes in the absorption spectra were measured at 620 nm using a SHIMADZU UV-3100 spectrophotometer at 25°C.

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CONCLUSIONS

γ-CDNA was synthesized for the first time. Compared with unmodified cyclodextrins, the binding abilities of α-, β- and γ-CDNA with charged guest compounds were enhanced by the nicotinamide residue. On the other hand, their binding abilities were all different and dependent on their cavity size. The studies of their complex conformations and the effects of the conformations on their binding abilities and on enzymatic abilities are now in progress, α-, β- and γ-CDNA are expected to have specific properties as NADH model compounds.

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