NMR Spectroscopic Analysis on the Chiral Recognition of Noradrenaline by β-Cyclodextrin (β-CD) and Carboxymethyl-β-cyclodextrin (CM-β-CD)

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β-CD and CM-β-CD as chiral NMR shift agents were used to resolve the enantiomers of noradrenaline (NA). The stoichiometry of each complex formed between the CDs and the enantiomers of NA was found to be 1 : 1 through the continuous variation plots. The binding constants (K) of the complexes were determined from ^{1}H NMR titration curves. This result indicated that both β-CD and CM-β-CD formed the complexes with the *S* (+)-NA more preferentially than its *R*(−)-enantiomer. The K values for the complexes with β-CD ($K_{S(+)}$ = 537 M^{-1} and $K_{R(-)}$ = 516 M^{-1}) was larger than those with CM-β-CD ($K_{S(+)}$ = 435 M^{-1} and $K_{R(-)}$ = 313 M^{-1}), however, enantioselectivity (α) of *S*(+)- and *R*(−)-NA to CM-β-CD (α = 1.38) was larger than that to β-CD (α = 1.04), indicating that CM-β-CD was the better chiral NMR solvating agents for the recognition of the enantiomers of NA. Two dimensional rotating frame nuclear Overhauser enhancement spectroscopy (ROESY) experiments were also performed to explain the binding properties in terms of spatial fitting of the NA molecule into the macrocyclic cavities.

Key Words : β-Cyclodextrin (β-CD), Carboxymethyl-β-cyclodextrin (CM-β-CD), Chiral NMR shift agent, Noradrenaline (NA), NMR spectroscopic analysis

Introduction

NMR chiral shift reagents have been often employed to resolve mixtures of enantiomers.[1-3] Among them, CDs have been extensively used for the discrimination and separation of enantiomers, because of its easy availability.[4,5] CDs are cyclic (α-1,4)-linked oligosaccharides of α-D-glucopyranose with a hydrophilic outer surface and a relatively hydrophobic central cavity which provides a microenvironment into which hydrophobic molecules may enter and be included.[6] Native and derivatized CDs have been used to resolve enantiomers in HPLC[7] and CE[8] and also act as efficient chiral solvating agents for NMR spectroscopy.[9,26] Recently, molecular modeling study has been also reported on the chiral recognition of some enantiomers by CDs.[27,28] However, little was reported for the exact mechanism of interaction between the CDs and enantiomers in aqueous solution.

NMR spectroscopy is one of the powerful experimental techniques for the investigation of intermolecular interactions. This technique provided the very fast evidence of the inclusion complex formation by CDs in the liquid phase[10] and could be also used for the elucidation of chiral recognition mechanisms using chiral selectors.[2,11]

Noradrenaline (NA) is one of the neurotransmitters in mammalians, which exhibits vasoconstriction and blood pressure elevation. NA is produced from dopamine by dopamine β-hydroxylase. As only the *R*(−)-enantiomer of NA shows potent biological activities, *R*(−)-enanatiomer is usually used in pharmaceutical preparations. Although the enantiomeric separations of NA were performed in HPLC with β-CD type chiral stationary phase[12] and in capillary electrophoresis (CE) on a micromachined device using CM-β-CD[13] as a chiral additive, no reports have been made on the mechanisms of chiral recognitions by β-CD or CM-β-CD in the molecular level.

Here, we investigated the complexation processes of β-CD or CM-β-CD with NA enantiomers, the stability of the complexes formed and their possible structures in aqueous solution by various NMR experiments.

Experimental Section

Chemicals. β-CD was from Sigma Chemical Co. (St. Louis, MO, USA) and CM-β-CD with an averaged degree of substitution (D.S.) of 3.5 was obtained from Wacker Chemie (Munich, Germany). Racemic noradrenaline · HCl were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and (*S*)-(+)-noradrenaline L-bitartrate and (*R*)-(−)-noradrenaline L-bitartrate monohydrate from Aldrich Chemical Co. (Milwaukee, MI, USA). The chemical structures of β-CD, CM-β-CD and NA used in this study are shown in Figure 1.

NMR measurements. The ^{1}H, ^{13}C NMR and ROESY experiments were carried out on a Brucker AMX 500 MHz instruments in D_2O (pD 7.0) solution at 25 °C. ^{1}H NMR chemical shifts of the NA, β-CD, CM-β-CD and complexes were obtained using the residual HOD signal at δ 4.8 ppm taken as an internal reference. For an assignment of the resonances of β-CD, CM-β-CD, NA and the complexes, homonuclear (H-H) correlation spectroscopy (H-H COSY), destortionless enhancement polarization transfer (DEPT) and hetero single-quantum correlation spectroscopy (HSQC) were also performed. ROESY spectra were recorded with 64 scans per FID, using a pulse train to achieve a spin-lock field

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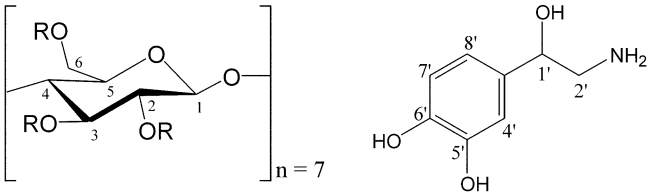

R = H β-CD
R = H or CH_2COO^- CM-β-CD

Figure 1. Chemical structures of *β*-CD, CM-*β*-CD (left) and NA (right).

with mixing time of 200 ms for the complex with *β*-CD and 250 ms for CM-*β*-CD. All ROESY experiments were performed with molar ratio of 1 : 1 (*β*-CD or CM-*β*-CD: NA). The volume of intramolecular cross-peak between the H1 and H4 protons of NA was used as an internal reference. All the cross-peak volumes related to intermolecular NOEs were then normalized by using the reference peak.

Stoichiometry and binding constant (*K*). The stoichiometry of the complexes of the CDs with the NA enantiomers was determined by the continuous variation method.[14,15] The total concentration of the NA enantiomers with the *β*-CD or CM-*β*-CD was kept constant at 10 mM and the molar fraction of the NA was varied from 0.2 to 0.8. For the determination of the binding constants, *S*(+)- and *R*(−)-NA were dissolved to give the concentration of 1 mM in D_2O. The NA solutions were successively added to the solutions of *β*-CD and CM-*β*-CD with varying concentrations. The apparent binding constants (*K*) of the NA enantiomers with *β*-CD or CM-*β*-CD were calculated on the basis of Scotts modification[16] of Benesi-Hildebrand equation.[17] In Scotts equation,

$$[CD]_t/\Delta\delta_{obs} = [CD]_t/\Delta\delta_c + 1/K\Delta\delta_c$$

$[CD]_t$ is the molar concentration of the CDs, $\Delta\delta_{obs}$ is the observed chemical shift difference for a given $[CD]_t$ concentration, $\Delta\delta_c$ is the chemical shifts difference between a pure sample of complex and the free component at the saturation. The slope of the plot of [CD] is thus equal to $1/\Delta\delta_c$ and the intercept with the vertical axis to $1/K\Delta\delta_c$, allowing the estimation of *K*.

Results and Discussion

^{1}H and ^{13}C NMR chemical shift changes of the complexes. The NMR signals observed in binary selector-solute solutions are time-averaged signals of both the complexed and uncomplexed substances. These can give rise to the shift displacements coupled with shift nonequivalence. These phenomena were observed on the ^{1}H and ^{13}C NMR spectra when *β*-CD or CM-*β*-CD was complexed with the NA enantiomers. The observed chemical shift changes are first indicative of the interactions of *β*-CD or CM-*β*-CD with NA (Figure 2). Generally, upfield chemical shifts were observed in complexed-NA compared with NA in free state (Figure

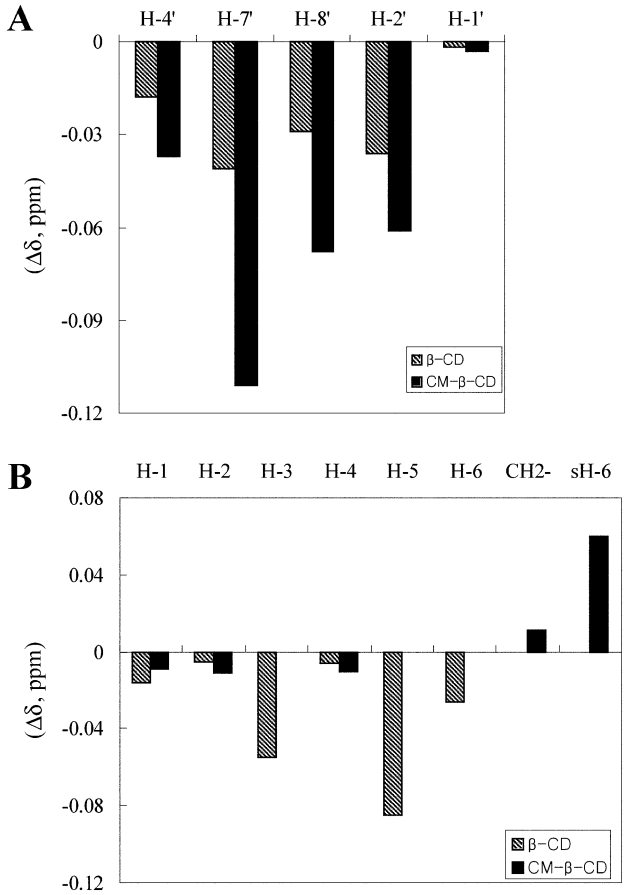

Figure 2. Changes ($\Delta\delta = \delta_{comlex} - \delta_{free}$, ppm) in the ^{1}H NMR chemical shifts of NA (A) *β*-CD and CM-*β*-CD (B) upon the complexation. Chemical shifts of the H-3, H-5 and H-6 protons of CM-*β*-CD were not measured due to overlap.

2A and Figure 3). Theses results indicated that the protons of NA were surrounded by electron density of *β*-CD or CM-*β*-CD after the complexation.[18,19] The changes in the chemical shifts of the protons of *β*-CD or CM-*β*-CD upon the complexation with NA were also observed as shown in Figure 2B. The resonance signals of all the protons of *β*-CD shifted to higher magnetic fields, indicating that these upfield shifts were due to the anisotropic shielding by ring current of NA included into its cavity upon the complexation with NA. Especially, the chemical shift of the H-5 proton of *β*-CD was the most pronounced followed by the H-3 proton. In the case of CM-*β*-CD, only the chemical shifts of the H-1, H-2, H-4, substituted H6 (sH-6) and methylene protons ($-CH_2-$) of carboxymethyl group of CM-*β*-CD were evaluated, while H-3, H-5 and H-6 protons were not due to severe overlap. Based on the significant upfield shifts of the NA protons, however, it was assumed that the H-3, H-5 and H-6 protons of CM-*β*-CD would also experience upfield shifts. From the above results, it could be characterized that the ring moiety of NA was embedded into *β*-CD or CM-*β*-CD cavity, and $\alpha(1\rightarrow 4)$ linkage oxygen of the CDs carried electron densities to ring moieties of NA. Figure 3 shows the partial ^{1}H NMR spectra of nonracemic NA (*R*(–)/*S*(+) = 1 : 2) in the absence or presence of *β*-CD and CM-*β*-CD. The signals of

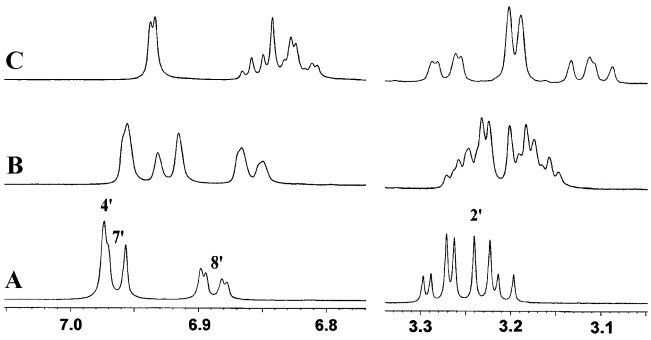

Figure 3. Partial ^{1}H NMR spectra of 10 mM nonracemic NA [*R*(–)/*S*(+) = 1 : 2] enantiomers in the absence (A) or presence of 10 mM *β*-CD (B) and 10 mM CM-*β*-CD (C).

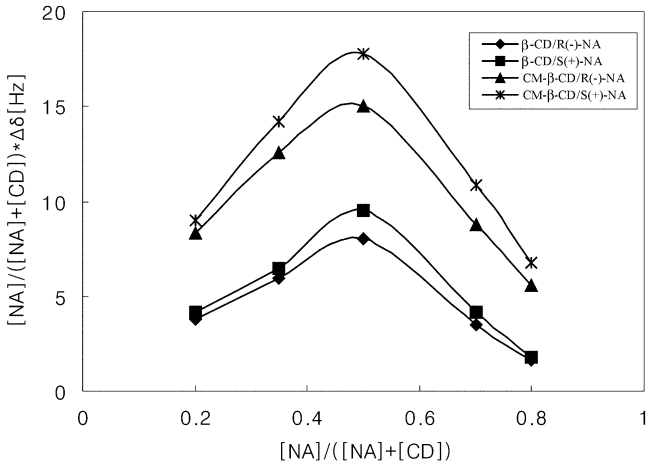

Figure 5. Job plots for the complexes of racemic NA with *β*-CD or CM-*β*-CD.

the ring protons (H-4', H-7' and H-8' protons) of NA significantly shifted upfield with upon the complexation with the *β*-CD or CM-*β*-CD. The signals of the H-7' and H-8' protons of NA were clearly split with the significant chemical shift displacements by the complexation with CM-*β*-CD, while the H-2' proton was only split by *β*-CD. Especially, the H-7' proton of NA experienced a remarkable shift ($\Delta\delta$ = -0.11) and was split with a larger complexation-induced shift in *S*(+)-NA comparing with that in *R*(–)-NA, by the complexation with CM-*β*-CD (Figure 3). Remarkable differences between the complexes with *β*-CD or CM-*β*-CD were also observed in the ^{13}C NMR spectra (Figure 4). In the complex with *β*-CD, the C-2', C-3', C-4', C-6' and C-8' carbons were split, while all the carbons of the NA-complexd with CM-*β*-CD were split except for the C-1'. In all cases, the chemical shifts of the carbons of NA-complexed with CM-*β*-CD were larger than those with *β*-CD. Especially, the complexation of NA with the CDs led to both diastereotopic and enantiomeric discrimination of the C-5' and C-6' carbons. However, the enantiomeric discrimination was much more pronounced for the complexed-NA with CM-*β*-CD rather than that with *β*-CD. Based on the complexation-induced chemical shift patterns in the ^{1}H or ^{13}C NMR spectra, it could be well correlated with the observed elution order of the NA enantiomers in HPLC with a *β*-CD type chiral stationary phase[12] or capillary electrophoresis (CE) using CM-*β*-CD[13] or *β*-CD[20] as a chiral additive.

Determination of stoichiometry of the complexes. A continuous variation method was applied to determine the stoichiometry of the complexes of NA and *β*-CD or CM-*β*-CD. The 1 : 1 complexes of each enantiomer of NA with *β*-CD or CM-*β*-CD was predominantly suggested from the Job's plot constructed for the chemical shift changes based on the resonance signals of the H-2' (for *β*-CD) and H-7' (for CM-*β*-CD) protons (Figure 5).

Determination of binding constants (*K*) of the complexes. The *K* values for complexation of NA with *β*-CD or CM-*β*-CD were determined from the ^{1}H NMR titration curves. All binding parameters for *β*-CD were calculated on the basis of the H-2' proton of NA enantiomers and for CM-*β*-CD on the basis of the H-7' proton. Scott plots of the complexes of *S*(+)-NA and *R*(–)-NA with *β*-CD or CM-*β*-CD were given in Figure 6. In all cases, the *K* values for the complexes of the *S*(+)-NA are larger than those of the *R*(–)-NA. The complexes of CM-*β*-CD ($K_{S(+)}$ = 435 M^{-1} and $K_{R(-)}$ = 313 M^{-1}) are less stable than those of *β*-CD ($K_{S(+)}$ = 537 M^{-1} and $K_{R(-)}$ = 516 M^{-1}). However, higher enantioselectivity (α = 1.38) of *S*(+)- and *R*(–)-NA to CM-*β*-CD was obtained than that to *β*-CD (α = 1.04). These results were summarized

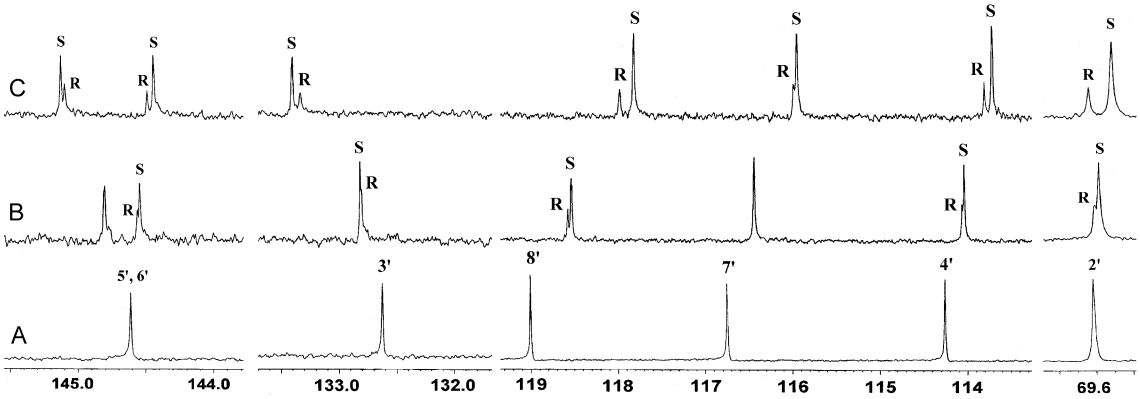

Figure 4. Partial ^{13}C NMR spectra of 10 mM nonracemic NA [*R*(–)/*S*(+) = 1 : 2] enantiomers in the absence (A) or presence of 10 mM *β*-CD (B) and 10 mM CM-*β*-CD (C).

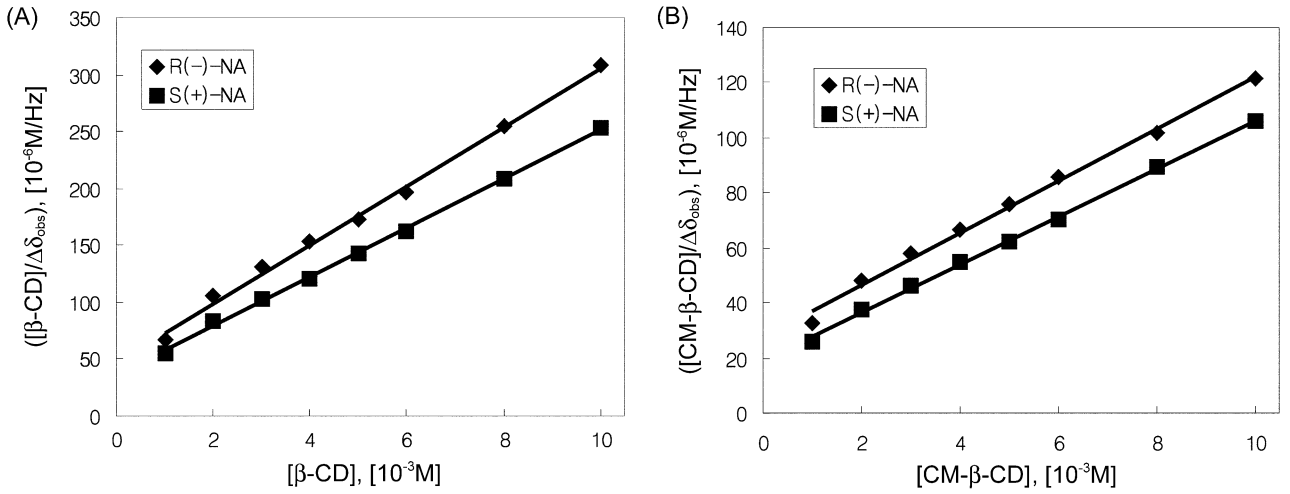

Figure 6. Scott's plots for the complexes of NA enantiomers with *β*-CD (A) or CM-*β*-CD (B).

in Table 1. These data are also in good accordance with the reported experimental data in HPLC[12] or CE.[13,20] It supports that CM-*β*-CD is a better chiral selector for NA enantiomers comparing with *β*-CD.

ROESY experiments of the complexes. The ROESY experiment first induced by Bothner-By *et al*.[21] has been found to be useful for the elucidation of the geometrical solution structure of CD-substrate complexes. Figure 7 showed the expansion of the ROESY contour plots showing the intermolecular transverse NOEs of the complexes of *β*-CD or CM-*β*-CD with racemic NA. In the case of the complex with *β*-CD, the cross peaks between the inner protons (H-3 and H-5) and the ring protons (H-4', H-7' and H-8') indicated a direct evidence that the ring moiety were included in the cavity of *β*-CD (Figure 7A). No cross-peaks were detected between protons of *β*-CD and the aliphatic protons (H-1' and H-2') of NA. The observed intermolecular NOEs between the ring protons of NA and all the protons of

Table 1. Complexation-induced chemical shift differences at saturation ($\Delta\delta_c$) and apparent binding constants (K [M^{-1}]) of NA with *β*-CD or CM-*β*-CD

CDs	$\Delta\delta_c$ (Hz)		K (M^{-1})		α^a
	$\Delta\delta_{c\ S(+)}$	$\Delta\delta_{c\ R(-)}$	$K_{S(+)}$	$K_{R(-)}$	
β-CD	43.5	38.7	537	516	1.04
CM-β-CD	114.9	106.3	435	313	1.38

[a] $\alpha = K_{S(+)} / K_{R(-)}$.

β-CD except for the H-1 and H-2 protons obviously suggest the possible coexistence of two different geometries in aqueous solution, of which the ring moiety of NA is included from both rim sides. The ^{1}H chemical shift changes of *β*-CD induced by the complexation suggested that the insertion of the ring moiety of NA from the primary rim was more predominant than that from secondary rim. This structure was further confirmed through volume integration

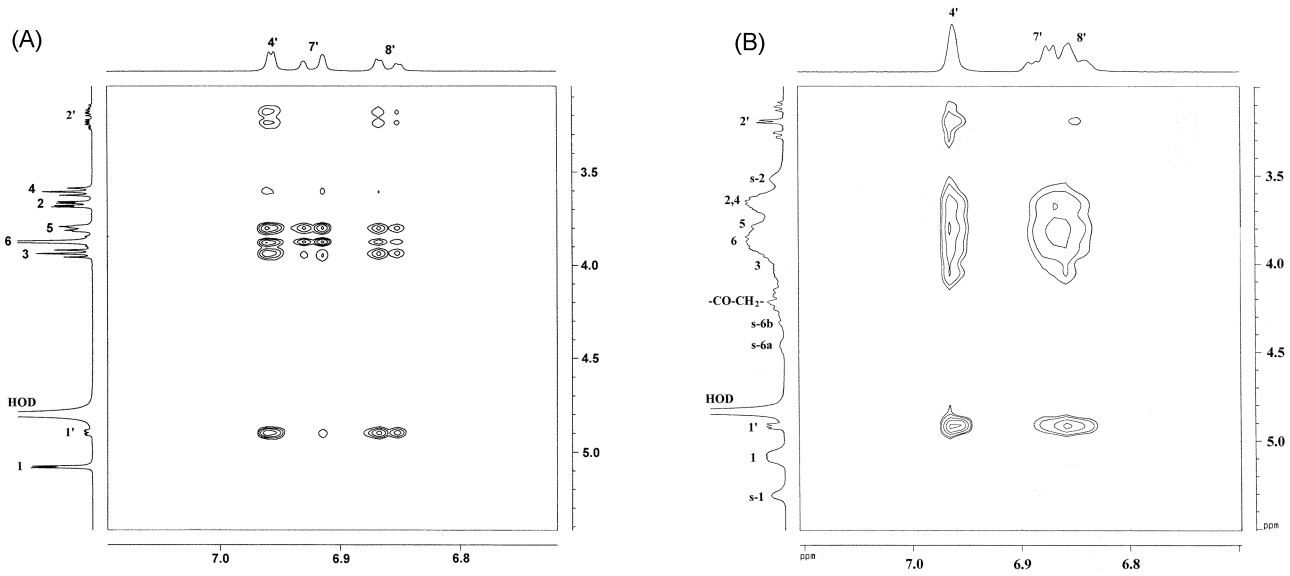

Figure 7. ROESY spectra of the complexes of equimolar racemic NA with 10 mM *β*-CD (A) or CM-*β*-CD (B) in D_2O at 25 °C. (s-1, s-2, s-6a and s-6b: the protons of CM-*β*-CD substituted with carboxymethyl group, -CO-CH_2-: methylene protons of carboxymethyl group).

Table 2. Volumes of intermolecular ROESY cross-peak[a] of the complex of β-CD and NA

β-CD protons	NA protons		
	H-4'	H-7'	H-8'
H-3	0.70	0.30	0.48
H-4	0.15	0.10	0.06
H-5	0.90	1.52	0.46
H-6	0.83	0.98	0.34

[a]Normalized to the volume of intramolecular H-1'/H-2' cross-peak.

of the significant cross-peaks of the ROESY spectra (Table 2). These bimodal structures have been reported for other β-CD-substrate complexes.[22,23] The insertion of NA from primary rim induced the ^{1}H chemical shift of the H-6 protons and the ROESY cross-peaks. In the case of the complexes with CM-β-CD, the observed ROESY spectrum is more complicated due to heterogeneity of the anionic CD. However, the cross-peaks was observed between the ring protons (H-4', H-7' and H-8') of NA and the H-2, H-3, H-4, H-5 and H-6 protons of CM-β-CD (Figure 7B). The correlation peaks with the aliphatic protons of NA were also detectable, of which no cross-peaks were observed in the complex with β-CD. The CM-β-CD could have slightly expanded structure at either side substituted with carboxymethyl groups compared with β-CD. Actually, in a commercial CM-β-CD, the substituent is positioned predominantly on the secondary rim. However, the substituent on the primary rim also is detectable.[24] Based on the 1 : 1 stoichiometry, ROESY spectrum for the complex of CM-β-CD and racemic NA provided the evidence on the bimodal complexation like as the case of the β-CD complex. This result suggested that the ring moiety of partially protonated NA in neutral pH was included at primary or secondary rim into the cavity of CM-β-CD and then simultaneous Coulombic interactions was induced between the amino groups of NA and the carboxyl groups of CM-β-CD. It was explained due to the ^{1}H chemical shift ($\Delta\delta$ = -0.06) of the H2' proton of NA and ROESY cross-peaks between the inner protons (H-3 and H-5) of CM-β-CD and the aliphatic protons (H-1' and H-2'). The chiral recognition of NA by CM-β-CD could thus occur through the cooperative effects of Coulombic and van der Waals interactions between the host and guest, based on the protonation of NA enantiomers at pD 7.0.

Here, we showed that the diastereomeric complexes of NA enantiomers with β-CD or CM-β-CD were characterized by ^{1}H, ^{13}C NMR spectra and ROESY. Each complex was shown to be predominantly 1 : 1 stoichiometry by the Job method. The complexes of CM-β-CD with NA ($K_{S(+)}$ = 435 and $K_{R(-)}$ = 313) were less stable than those of β-CD ($K_{S(+)}$ = 537 and $K_{R(-)}$ = 516). However, enantioselectivity (α = 1.38) of *S*(+)- and *R*(-)-NA to CM-β-CD was larger compared with that to β-CD (α = 1.04). These results suggest that stronger binding of a chiral solvating agent with enantiomers dose not necessary give higher enantioselectivity.[25]

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