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Interaction of 2-Naphthalenesulfonate with β-Cyclodextrin: Studies with Calorimetry and Proton Nuclear Magnetic Resonance Spectroscopy

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The interaction of 2-naphthalenesulfonate (2-NS) with β-cyclodextrin (β-CD) was investigated in a 0.1 M phosphate buffer at pH 7.4 using an LKB 2277 microcalorimeter and a flow-mixed mode at 25 °C. The thermodynamic parameters for inclusion complex formation obtained were as follows: $\Delta G° = -14.7$ kJ/mol ($K = 381$ M^{-1}), $\Delta H° = -26.4$ kJ/mol, $\Delta S° = -39.1$ J/mol K. The formation constant K was determined as 428 M^{-1} at the same temperature from the change in chemical shift of the proton nuclear magnetic resonance spectra (^{1}H-NMR) in 0.1 M phosphate buffered deuterium oxide solution at pD 7.4. The main driving force for inclusion complex formation was considered to be the van der Waals–London dispersion force. From measurements of the ^{1}H-NMR and model building with Corey–Pauling–Koltun atomic models, the probable structure of the complex was determined to be: the naphthalene ring of 2-NS is included deeply at the head of 6-H and 7-H of 2-NS in the cavity of β-CD from the secondary hydroxyl group side, the sulfonate group being outside the cavity.

Key words 2-naphthalenesulfonate; β-cyclodextrin; flow-microcalorimeter; thermodynamic parameter; ^{1}H-NMR; 6-p-toluidinyl-2-naphthalenesulfonate

Cyclodextrins (CDs) are known to include a number of drugs in their hydrophobic cavities, and the inclusion complexes are increasingly used to of increase the stabilization and solubility of drugs, and in many other applications.[1] Also, it has been known that CDs are also regarded as a model for studies of enzyme–substrate interaction.[2] The quasi-enzyme behavior that CD exhibits in substrate binding suggests that the interaction is specific between a CD and some guest molecules. This makes it important to learn the inclusion modes of substrates within the hydrophobic cavities of these cycloamyloses in aqueous solution, correlating them with the thermodynamic parameters for inclusion complex formation.

We have already reported[3] the interaction of 8-anilino-1-naphthalenesulfonate (ANS) with β-CD. The interaction of 1-naphthalenesulfonate (1-NS), which has a naphthalene ring and sulfonate group but no benzene ring, with β-CD was investigated to determine how the thermodynamic parameters for inclusion complex formation and the inclusion modes are affected when the guest has or does not have a benzene ring.[4] We have also already reported[5] the interaction of 6-p-toluidinyl-2-naphthalenesulfonate (TNS) with β-CD and found that half the benzene ring and a large portion of but not the whole the naphthalene ring are included.

In the present study, the interaction of 2-NS of β-CD, structure of which has no toluidiny group but a hydrogen atom at position 6 of TNS, with β-CD was investigated in aqueous solution using direct calorimetric measurements, proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy, and Corey–Pauling–Koltun (CPK) atomic models. How the thermodynamic parameters and the inclusion are affected was discussed, depending on whether or not the guest has a toluidinyl group at position 6 of TNS.

Experimental

Materials Reagent grade β-CD supplied by Nakalai Tesque Co. was recrystallized twice from water and dried over P$_2$O$_5$ for 5 h at 110 °C in a vacuum before use. 2-NS supplied by Wako Pure Chemicals as a sodium salt was recrystallized twice from water, together with the activated charcoal to strip the impurities, then it was dried in a vacuum for 3 h at 130 °C. The

water used was obtained by distilling twice the ion-exchanged water. All other chemicals used were of reagent grade. The compounds Na$_2$DPO$_4$ and KD$_2$PO$_4$ were obtained by treating Na$_2$HPO$_4$ and KH$_2$PO$_4$, respectively, with deuterium oxide and vaporizing the solvent; the resulting compounds were used to adjust pD.

^{1}H-NMR Spectra ^{1}H-NMR experiments were all measured in 0.1 M phosphate buffered deuterium oxide solution at pD 7.4 at 25 °C. ^{1}H-NMR experiments were conducted on a Varian VXR-500 (499.8 MHz) spectrometer with tetramethylsilane (TMS) as an external reference. Two-dimensional nuclear Overhauser enhancement spectroscopy (NOESY) experiments were performed using the phase-sensitive method (hyper-complex method) with a mixing time of 600 ms. Two-dimensional rotating nuclear Overhauser effect spectroscopy (ROESY) experiments were performed in the phase-sensitive mode (hyper-complex method) with a spin-lock mixing pulse of 250 ms.

Measurements of the Heat of Reaction An LKB 2277 microcalorimeter (thermal activity monitor) was used in the flow-mixed mode at 25 °C. At the beginning, the calibration of the calorimeter was carried out by giving a fixed amount of heat from the calorimeter heater while 0.1 M phosphate buffer was mixed at same flow rate as at the measurements of the sample. A solution of β-CD and 2-NS was pumped into the measuring cell using an LKB 2132 microperpex pump with a flow rate of 2.78×10^{-3} ml s^{-1} (10.0 ml h^{-1}). The heat flow was monitored with a Pharmacia LKB model REC 2 recorder potentiometer. All data were corrected for dilution heat effect.

Results

^{1}H-NMR spectra and a CPK model were used to estimate the structure of the inclusion complex between 2-NS and β-CD. In Fig. 1a a ^{1}H-NMR spectrum of 0.91×10^{-4} M β-CD in 0.1 M phosphate buffered deuterium oxide solution at 25 °C is shown. The spectrum is different from that (Fig. 1b) in the presence of 2.0×10^{-3} M 2-NS. Comparison of these spectra revealed that the signals due to protons of all types in the β-CD molecule shifted upfield in the presence of 2-NS. The shift of 5-H on the inner surface of the cavity at the primary hydroxyl group side was most prominent, followed by the 3-H lying on the inner surface of the secondary hydroxyl group side. Though 6-H lying on the inner surface of the primary hydroxyl group side shifted upfield, however the upfield shift was not as prominent as that of 3-H of β-CD. The upfield shifts of signals due to 1-H, 2-H, and 4-H which lie on the outer surface of the cavity were not pronounced and their magnitudes were similar. The magnitudes of the upfield

shifts of the signals due to β-CD in the presence of 2-NS at various concentrations are shown in Fig. 2a.

Fig. 3a shows a ^{1}H-NMR spectrum of 3.0×10^{-4} M 2-NS in 0.1 M phosphate buffered deuterium oxide solution at 25 °C. The assignments of the proton signals of 2-NS were undertaken on the basis of ^{1}H–^{1}H chemical shift correlation spectroscopy (COSY) and NOESY measurements. In the pres-

ence of β-CD, proton signals due to 2-NS shifted respectively though the magnitudes of the shifts were different depending on the position of 2-NS. When β-CD (1.0×10^{-3} M) was added to the 2-NS (3.0×10^{-4} M)(Fig. 3b), 1-H, 4-H, 8-H, and 5-H of 2-NS shifted upfield but 3-H, 7-H, and 6-H signals of 2-NS were not prominent. Also, the proton signals of 5-H, 6-H, and 7-H due to 2-NS became remarkably broader. If the higher concentrations of β-CD were added, 4-H and 8-H signals of 2-NS broadened followed by 3-H of 2-NS, and it was found that the broadening of 1-H signal due to 2-NS was most difficult. The magnitudes of shifts of proton signals due to 2-NS in the presence of β-CD at various concentrations are shown in Fig. 2b. In Fig. 2b, 1-H signal of 2-NS shifted upfield most remarkably, followed by 4-H, 8-H, and 5-H signals of 2-NS in this order. As the broadening of 5-H signal due to 2-NS was more prominent in the presence of the higher concentration of β-CD, it was impossible to measure
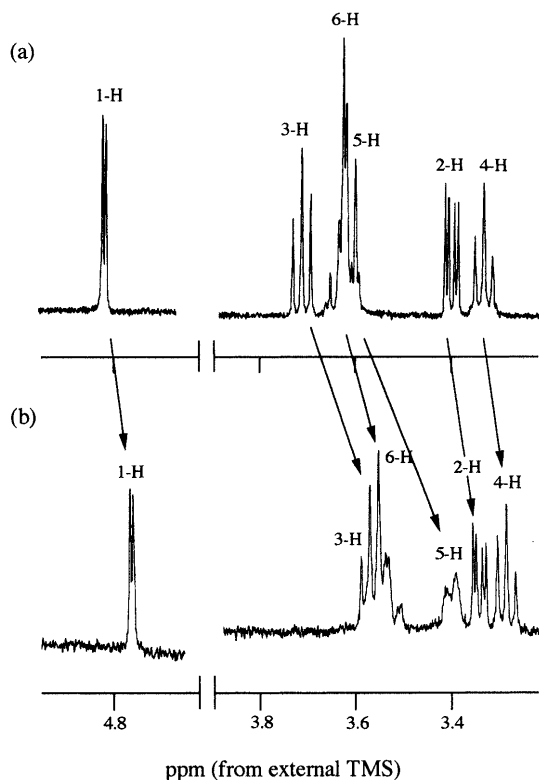

Fig. 1. ^{1}H-NMR Spectra of β-CD in the Presence of 2-NS in 0.1 M Phosphate Buffered Deuterium Oxide Solution (pD 7.4) at 25 °C

(a) β-CD alone (0.91×10^{-4} M), (b) β-CD (0.91×10^{-4} M)+2-NS (2.0×10^{-3} M).

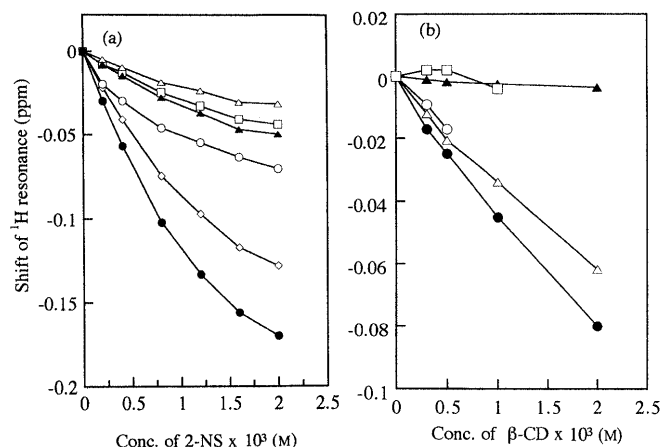
Fig. 2. (a) Induced ^{1}H-NMR Chemical Shifts of (a) β-CD (0.91×10^{-3} M) in the Presence of 2-NS and (b) 2-NS (3.0×10^{-4} M) in the Presence of β-CD

(a) △, 4-H; □, 1-H; ▲, 2-H; ○, 6-H; ◇, 3-H; ●, 5-H. (b) □, 6-H, 7-H; ▲, 3-H; ○, 5-H; △, 4-H, 8-H; ●, 1-H.
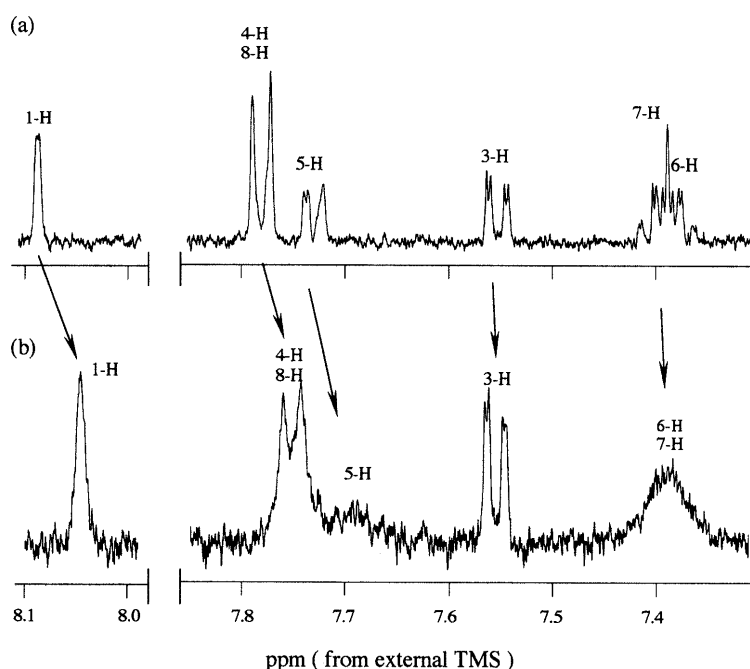

Fig. 3. ^{1}H-NMR Spectra of 2-NS in the Presence of β-CD in 0.1 M Phosphate Buffered Deuterium Oxide Solution (pD 7.4) at 25 °C

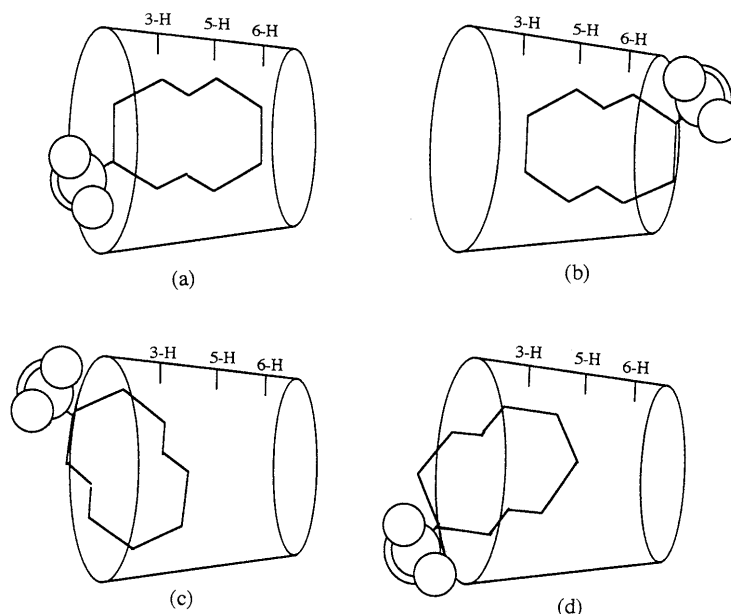(a) 2-NS alone (3.0×10^{-4} M), (b) 2-NS (3.0×10^{-4} M)+β-CD (1.0×10^{-3} M).

Fig. 4. Possible Structures of the Inclusion Complex of 2-NS with β-CD in Aqueous Solution Based on CPK Space-Filling Models

the chemical shift of this signal. The signals of 6-H and 7-H due to 2-NS shifted downfield slightly and then the shift turned upfield with the higher concentration of β-CD, though the shifts were small. Because of the broadening of the signals, it was impossible to measure the chemical shifts of 6-H and 7-H of 2-NS in the presence of a higher concentration of β-CD. The 3-H signal of 2-NS barely shifted in the presence of higher concentration of β-CD. Signals due to protons which do not come into close contact with the atoms on the inner surface of β-CD tend to shift upfield as a result of the C–C bond anisotropy effect, and so on.[6—8] Therefore, it is presumed that 1-H, 4-H, and 8-H signals due to 2-NS are included rather loosely in the cavity of β-CD. It is further presumed that 3-H of 2-NS exists outside the cavity, because the 3-H signal of 2-NS hardly shifts and the broadening of this signal is only slight in the presence of β-CD. Since the broadening of the 5-H, 6-H and 7-H signals of 2-NS are very prominent, it is estimated that 5-H , 6-H and 7-H of 2-NS are included in the cavity of the narrow primary hydroxyl group side of β-CD and hence the motions of these protons are restricted.

Judging from the investigation using the CPK model and taking into account that -SO$_3^-$ group is hydrophilic, the four kinds of structures described in Fig. 4 are considered as possible structures for the inclusion complex. These are described as follows: a) the naphthalene ring of 2-NS is included deeply at the head of 6-H and 7-H of 2-NS in the cavity of β-CD from the secondary hydroxyl group side, the sulfonate group again being outside the cavity; b) the naphthalene ring is deeply enclosed at the head of 6-H and 7-H of 2-NS in the cavity of β-CD from the primary hydroxyl group side, sulfonate group being outside the cavity; c) the naphthalene ring is shallowly enclosed at the head of 4-H and 5-H of 2-NS from the secondary hydroxyl group side, so as to the latter being positioned at the center of the cavity, and the short axis of the naphthalene ring inclines to the molecular axis of β-CD; d) the naphthalene ring is shallowly enclosed at the head of 7-H of 2-NS from the secondary hydroxyl group side of β-CD, the short axis of the naphthalene ring

strongly inclines to the molecular axis of β-CD to a great extent and the sulfonate group is left outside the cavity. Upfield shifts of proton signals lying on the inner surface of β-CD are believed to result mainly from the magnetic anisotropy of the naphthalene ring of the 2-NS molecule. Therefore, the results that the magnitude of the upfield shifts of the signals due to the protons on the inner surface of β-CD is in the order of 5-H$>$3-H$>$6-H (Fig. 2a) suggests that the structure shown in Fig. 4b is not preferential, because there the upfield shift of the 6-H signal of β-CD should be more prominent than that of the 3-H of β-CD, although it can be explained that the upfield shift of 5-H of β-CD is most prominent in Fig. 4b. Also, in Figs. 4c and 4d, the upfield shift of 3-H of β-CD would be more prominent than that of 5-H. Therefore, neither the structure shown in Fig.4c or 4d are not preferential.

If the complex has the structure described in Fig. 4a, it would not be surprising that the upfield shift of the 5-H signal is most prominent, followed by those of the 3-H and the 6-H of β-CD in this order. Consequently, 2-NS is thought to be enclosed in β-CD in the manner illustrated in Fig. 4a. To confirm the structure of the inclusion complex, a ROESY spectrum of the solution containing β-CD (3.0×10^{-4} M) and 2-NS (1.0×10^{-3} M) was measured with a spin-lock mixing pulse of 250 ms (Fig. 5). In the ROESY spectrum, the cross-peak connecting two proton resonances indicate that those proton nuclei are in close proximity in the ground state of the molecule. The unambiguous cross peak was observed between the 5-H of β-CD and the 5-H of 2-NS. Also, the cross peaks were observed between 5-H of β-CD and 6-H, 7-H, and 8-H of 2-NS and between 3-H of β-CD and 4-H of 2-NS. These cross peaks support the structure shown in Fig. 4a. Further, judging from the facts that the cross peak is observed between 6-H of β-CD and the 5-H of 2-NS, the broadening of the signals of 5-H, 6-H, and 7-H due to 2-N are pronounced, and -SO$_3^-$ group is hydrophilic, the structure shown in Fig. 6 is believed to be a highly possible one, in which SO$_3^-$ group lies a little toward the center of the cavity.
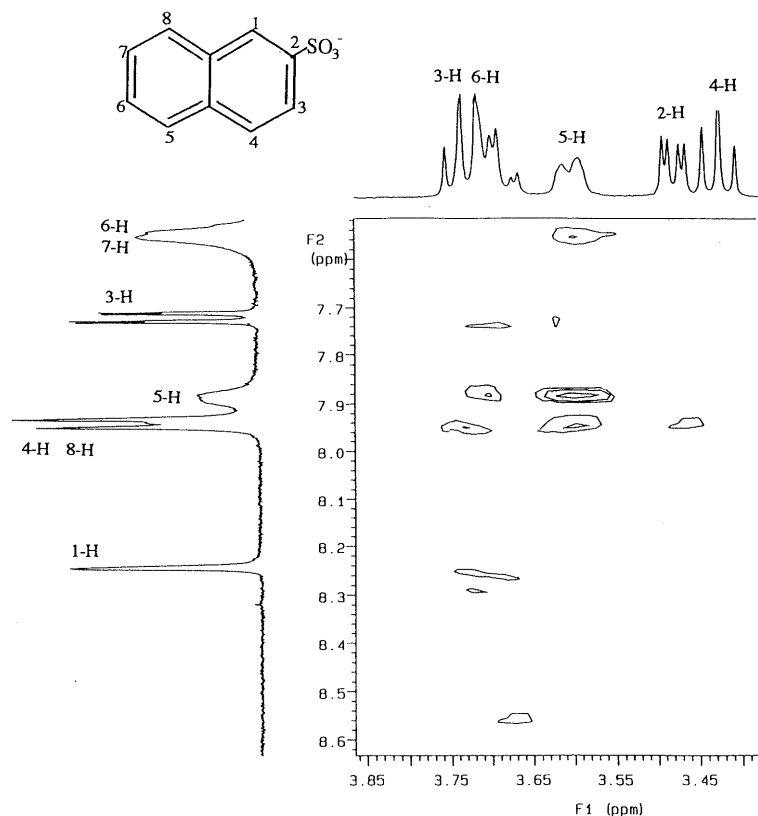
Harata and Uedaira reported[9] that the structure of the 2-

Fig. 5. ROESY Spectrum of Solution Containing 2-NS (1.0×10^{-3} M) and β-CD (3.0×10^{-4} M) in the 0.1 M Phosphate Buffered Deuterium Oxide
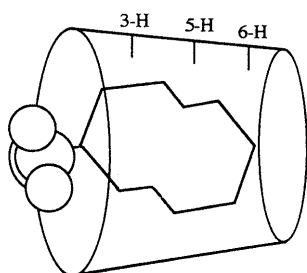

Fig. 6. A Highly Possible Structure of the Inclusion Complex of 2-NS with β-CD

substituted naphthalene complex is estimated to be an axial inclusion, namely, naphthalene ring is included at the head of 6-H and 7-H of naphthalene ring in the cavity of β-CD, by the circular dichroism spectra and by calculation of the rotational strength. The reported inclusion mode is consistent with the present one, although it was not reported which direction of β-CD the geust is included from.

Thermodynamic parameters for inclusion complex formation were determined by direct calorimetric measurements. Assuming a 1 : 1 complex ,the formation constant K is represented in Eq. 2

$$2\text{-NS}+\beta\text{-CD} \tag{1}$$

$$K=\frac{X}{(\text{NS}_0-X)(\text{CD}_0-X)} \tag{2}$$

where NS_0, CD_0 and X represent the total concentration of 2-NS, total concentration of β-CD, and the concentration of 2-NS–β-CD complex, respectively. Here, the concentration is molar concentration.

The heat of mixture of 2-NS and β-CD solution, ΔH_m is obtained from Eq. 3

$$\Delta H_m=\Delta H_{obs}-(\Delta H_{NS}+\Delta H_{CD}) \tag{3}$$

where ΔH_{obs}, ΔH_{NS}, and ΔH_{CD} represent the heat of mixture actualy observed, the heat of dilution of 2-NS solution, and the heat of dilution of β-CD solution, respectively.

Now, if ΔH_m and V, the volume of the solution are used, then ΔH°, the change in enthalpy for complex formation, is represented in Eq. 4.

$$\Delta H^\circ=\frac{\Delta H_m}{XV} \tag{4}$$

From Eqs. (2) and (4), the heat of mixture, ΔH_m is represented in Eq. 5.

$$\Delta H_m=\frac{\Delta H^\circ V\left\{\left(\text{NS}_0+\text{CD}_0+\frac{1}{K}\right)-\sqrt{\left(\text{NS}_0+\text{CD}_0+\frac{1}{K}\right)^2-4\text{NS}_0\text{CD}_0}\right\}}{2} \tag{5}$$

if $R=\text{CD}_0/\text{NS}_0$ and $A=\text{NS}_0\cdot K$, Eq. (6) applies.[10]

$$\Delta H_m=\frac{\Delta H^\circ V\,\text{NS}_0\left\{(A+AR+1)-\sqrt{(A+AR+1)^2-4A^2R}\right\}}{2A} \tag{6}$$

Figure 7 shows the plots of ΔH_m, the heat of mixture produced, to the R, ratio of concentration of β-CD to that of 2-NS, when 2-NS and β-CD are mixed, the concentration of 2-NS being constant (5.0×10^{-4} M) and the concentration of β-CD being changed (5.0×10^{-4}—5×10^{-3} M). Formation con-
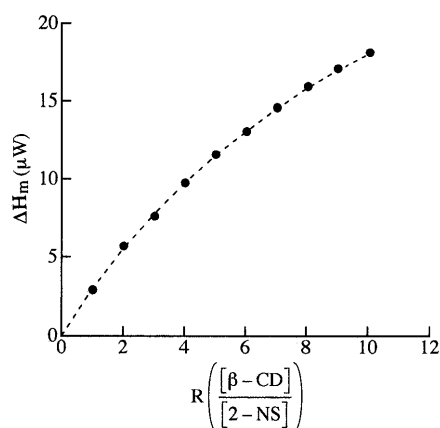
Fig. 7. Dependence of Heat Flow, Corresponding to Complex Formation on the Molar Concentration Ratio of β-CD to 2-NS (R) at 25 °C

●, observed heat flow, which is the mean of 3 repeated runs; ------, theoretical curve, which was obtained from Eq. 6 using the calculated parameters.

Table 1. Thermodynamic Parameters for Inclusion Complex Formation of 2-NS with β-CD at 25 °C

	K (M^{-1})	$\Delta G°$ (kJ/mol)	$\Delta H°$ (kJ/mol)	$\Delta S°$ (J/mol K)
	381 ± 25	-14.7	-26.4 ± 1.4	-39.1 ± 3.8
Literature[13]	234000	-30.7	-29.3	0

stant K and the enthalpy change for complex formation $\Delta H°$ can be estimated from Eq. 6 using the nonlinear least squares program MULTI.[11] The values obtained at 25 °C are shown in Table 1. Also, Fig. 7 shows the binding curve for β-CD with 2-NS, together with the theoretical curves obtained using the calculated parameters. The change in entropy ($\Delta S°$) accompanying the complexation was determined in the usual way using Eq. $\Delta G° = \Delta H° - T\Delta S°$.[12] The results obtained are illustrated in Table 1. Further, the thermodynamic parameters reported earlier,[13] which were determined by calorimetric titration in buffered aqueous solution at pH 7.20 (0.1 M sodium phosphate), are also shown in the Table. The present formation constant was found to be abnormally smaller than the formation constant in the literature. Because of this excessively small size, another determination of the formation constant was carried out from the change in the chemical shifts of 1-H due to 2-NS in the presence of β-CD using Eq. 7.[14] To equalize the experimental conditions with those carried out using our calorimeter, β-CD ranging in concentration from 5.0×10^{-4} M to 5.0×10^{-3} M was added to the constant concentration of 2-NS (5.0×10^{-4} M)

$$\Delta\omega_{obd} = \frac{\omega_0\left(1+R+\dfrac{1}{a}-\sqrt{\left(1+R+\dfrac{1}{a}\right)^2-4R}\right)}{2} \quad (7)$$

where $R=CD_0/NS_0$, $a=K\cdot CD_0$. Also, $\Delta\omega_{obd}$ is the change in the chemical observed, and $\Delta\omega_0$ is the complexation shift. Figure 8 shows the plots of the $\Delta\omega_{obd}$ of 1-H due to 2-NS , to the R, ratio of concentration of β-CD, to that of 2-NS. Formation constant K and complexation shift $\Delta\omega_0$ can be estimated from Eq. 7 using the nonlinear least squares program MULTI,[11] and K was 428 ± 22 M^{-1} ($\Delta\omega_0=-0.22\pm0.01$
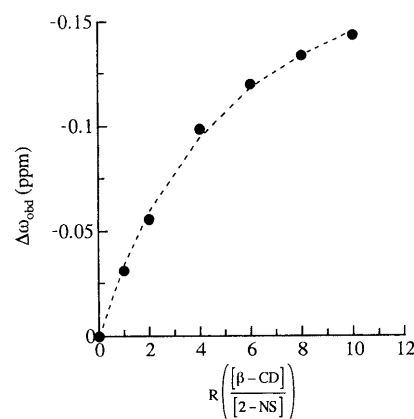

Fig. 8. Plots of $\Delta\omega_{obd}$ Values of 1-H signals due to 2-NS versus the Molar Concentration Ratio of β-CD at 25 °C

●, observed value, which is the mean of repeated runs; ------, theoretical curve, which was obtained from Eq. 7 using the calculated parameters.

ppm). In this way, the formation constant obtained from the microcalorimeter was consistent with that from the change in the proton chemical shift. Now, quite recently the formation constant was determined by Hamai and Watanabe,[15] at pH 7.3 (0.033 M phosphate buffer) and at 25 °C based on the capillary electrophoretic and spectrophotometric methods to be 480 ± 20 and 220 ± 30 M^{-1}, respectively. Therefore, our K values are comparable to those reported by them, especially that obtained by the electrophoretic method.

The authors earlier reported the thermodynamic parameters for inclusion complex of TNS,[5] the structure of which has a anilino group at position 6 of 2-NS, with β-CD, as determined by fluorescence spectrophotometry. However, thermodynamic parameters obtained from direct measurement by calorimetry are known to be more reliable than those obtained from the slope of log K versus $1/T$ correlation. Thus, the thermodynamic parameters for inclusion complex formation of TNS with β-CD were measured by calorimetry in order to compare with those for 2-NS-β-CD complex formation. β-CD forms two kinds of complexes with TNS, having molar ratios of $1:1$ and $2:1$ complex (molar ratio of β-CD to TNS$=1:1$ and $2:1$). Formation constant K_1 and K_2 for the $1:1$ and $2:1$ complexes are shown by Eqs. 8 and 9, respectively,

$$K_1 = \frac{(CD-TNS)}{(CD)(TNS)} \quad (8)$$

$$K_2 = \frac{(CD)_2-TNS}{(CD)(CD-TNS)} \quad (9)$$

where CD, TNS, CD–TNS, and $(CD)_2$–TNS represent the concentrations of CD, TNS, $1:1$ complex, and $2:1$ complex, respectively. Here the concentration is a molar concentration. The ΔH_m, the heat produced when 2-NS and β-CD are mixed, represented in Eq. 10.

$$\Delta H_m = \Delta H_1 C_1 V + \Delta H_2 C_2 V \quad (10)$$

where ΔH_1, ΔH_2, C_1, C_2, and V represent the enthalpy change for $1:1$ complex formation, the enthalpy change for $2:1$ complex formation, the concentration of $1:1$, the concentration of $2:1$ and the volume of the solution, respectively. The total concentration of TNS, T_0 is represented in Eq. 11,
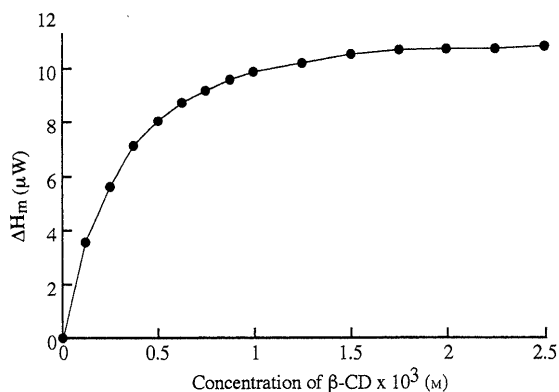
Fig. 9. Dependence of Heat Flow, Corresponding to Complex Formation of β-CD with TNS on the Molar Concentration of β-CD

●, observed heat flow, which is the mean of 3 repeated runs; ——, theoretical curve, which was obtained from Eq. 12 using the calculated parameters. The flow rate used was $5.55 \times 10^{-3}\,\mathrm{ml\,s^{-1}}$ ($20.0\,\mathrm{ml\,h^{-1}}$).

$$T_0 = \text{TNS} + \text{CD}-\text{TNS} + (\text{CD})_2-\text{TNS} \tag{11}$$

when total concentration of CD, C_0 is much larger than that of TNS, $C_0 \gg T_0$

$$\Delta H_\mathrm{m} = \Delta H_1 K_1 C_0 V \left\{ \frac{T_0}{1 + K_1 C_0 + K_1 K_2 C_0^2} \right\}$$

$$+ \Delta H_2 K_1 K_2 C_0^2 V \left\{ \frac{T_0}{1 + K_1 C_0 + K_1 K_2 C_0^2} \right\} \tag{12}$$

Eq. 12 is valid. Figure 9 shows the plots of ΔH_m, the heat produced, to the total concentration of β-CD added, the concentrations of TNS being constant (5.0×10^{-5} M). Formation constants K_1, K_2, and enthalpy changes, ΔH_1 and ΔH_2 for complex formation, can be estimated from Eq. 12 using the nonlinear least squares program MULTI.[11]

The thermodynamic parameters were determined as follows: $K_1 = 2750 \pm 150\,\mathrm{M^{-1}}$ ($3240\,\mathrm{M^{-1}}$) at 25 °C, $K_2 = 61 \pm 5\,\mathrm{M^{-1}}$ (11.9) at 25 °C, $\Delta H_1° = -25.3 \pm 2.0$ kJ/mol (-19.6 kJ/mol), $\Delta H_2° = 0 \pm 0.2$ kJ/mol (-2.80 kJ/mol), $S_1° = -18.9 \pm 1.8$ J/ mol K, (-1.7 J/mol K), $\Delta S_2(=34=3$ J/mol K (16.7 J/mol K). The values shown in parentheses represent those obtained by fluorescence spectroscopy.[5] The difference between values obtained by calorimetry and fluorescence spectroscopy was particularly remarkable with respect to the changes in entropy $\Delta S°$. Because the thermodynamic parameters for obtained by the calorimetry are certainly more reliable, the values were adopted in the following argument.

Discussion

Next, the structure shown in Fig. 6 will be discussed from the standpoint of the induced chemical shifts of 2-NS in the presence of β-CD. The signals due to protons which do not come into close contact with the atoms on the inner surface of β-CD tend to shift upfield because of the C–C bond anisotropy effect and so on, as mentioned above. The signal due to the 1-H of 2-NS shifted upfield most prominently in the proton signals of 2-NS, followed by the 4-H and the 8-H of 2-NS in the presence of β-CD. The 1-H of 2-NS exists in the cavity without close contact with the atoms on the inner surface of the host molecule in the structure shown in Fig. 6. This might induce considerable upfield shifts of the signals

due to 1-H. Also, the 4-H and the 8-H of 2-NS exist in the cavity without close contact, but with weak contact in Fig. 6. Therefore, the upfield shifts of the signals due to the 4-H and the 8-H of 2-NS would not be as prominent as that of the 1-H of 2-NS. Also, the 5-H of 2-NS exists in the cavity of β-CD with closer contact than that of the 4-H and the 8-H resulting in a smaller upfield shift. The 6-H and the 7-H of 2-NS on the other hand, exist in the cavity but without effective contact because they are positioned near the center of the cavity and away from its inner surface. Thus, the upfield shift of the 6-H and the 7-H due to 2-NS would not be prominent. As described, the broadening of the 5-H, 6-H, and 7-H signals of 2-NS were very prominent in the presence of β-CD. These phenomena were thought to occur because the space which the 5-H, 6-H, and 7-H of 2-NS occupies in the cavity is so narrow that the motion of the hydrogen atoms might be restricted. From the investigation with the CPK model, 2-NS can enter the cavity of β-CD without close contact with inner surface atoms but the guest molecule cannot rotate freely around the molecular axis of β-CD in the structure shown in Fig. 6 owing to its contact with the inner surface atoms of β-CD. Therefore, the 2-NS molecule is believed to move, strongly rotating the 3-H and 1-H moiety of 2-NS lying in the wide cavity with the slight rotation of the 6-H, 7-H, and 5-H moiety lying in the narrow cavity. That is, it is assumed that the 2-NS molecule rotates in the cavity of β-CD in apparently the same mode as a top precession at the gravity field or Larmor precession and therefore, the rotaly motion of 6-H, 7-H, and 5-H would be restricted and the broadening of these signals induced. In addition to this speculation on broadening of the signals, another possibility for the broadening of the signal could be presumed, as shown hereafter. Since 5-H, 6-H, and 7-H of 2-NS are included more deeply in the cavity than the other protons of 2-NS, they remain longer in the cavity at the exchange process of β-CD–2-NS complex. Therefore, they experience different magnetic environments at the time scale of ¹H-NMR, resulting in a broadening of the signals.

In the complexation in aqueous solution, various driving forces have been reported to play a big part in the interaction[16] However, according to Tabushi et al.,[17] the van der Waals London dispersion forces and hydrophibic interaction are the major driving forces for the inclusion complexation between cyclodextrin and a guest molecule. In the present study of the interaction of 2-NS with β-CD, the complexation was accompanied by negative changes in entropy and in enthalpy. These thermodynamic parameters suggest that the van der Waals London dispersion force is the main contribution to inclusion complex formation and that the contribution of the hydrophobic interaction is minor.

When the thermodynamic parameters for 2-NS–β-CD complex were compared with those for TNS–β-CD complex (1 : 1), it was found that the formation constant for 2-NS–β-CD complex is smaller than that for TNS–β-CD complex (1 : 1). The change in enthalpy $\Delta H°$ for 2-NS–β-CD complex formation was more negative than that for TNS–β-CD complex (1 : 1). This might be attributable to the facts that the whole naphthalene ring is included in the 2-NS–β-CD complex, but approximately half of the benzene ring and four-fifths of naphthalene ring are enclosed in the TNS–β-CD complex (1 : 1). Therefore, because in the 2-NS–β-CD com-

plex 2-NS is enclosed more tightly and more rigidly, and the space of the cavity is less than in the case of TNS–β-CD complex (1 : 1), the change in enthalpy $\Delta H°$ of 2-NS–β-CD complex (1 : 1) would take a larger negative value than that of TNS β-CD complex (1 : 1). These are reflected on the values of the changes in entropy $\Delta S°$. Though the changes in entropy for the 2-NS–β-CD complex and TNS–β-CD complex (1 : 1) take either negative values, that of the former is more negative. These changes in entropy agreed with the above description that 2-NS is enclosed more tightly in the cavity and the rotary movement of 2-NS is partially restricted. Therefore, it is reasonable that the formation constant of 2-NS–β-CD complex is smaller than that of TNS β-CD complex (1 : 1), because the whole naphthalene ring is more tightly enclosed in the 2-NS–β-CD complex, having no benzene ring; this results in stronger van der Waals force between 2-NS and β-CD, but in a more pronounced decrease in entropy than in the TNS–β-CD complex (1 : 1).

Finally, as mentioned above, the formation constant for 2-NS–β-CD complex obtained by the microcalorimeter was consistent with that from the change in the proton chemical shift. Though the present formation constants were not consistent with that of literature obtained by calorimetry,[13] they were comparable to those reported by Hamai and Watanabe.[15] From the above discussion, 2-NS is included somewhat tightly in the cavity of β-CD and the formation constant of TNS–β-CD complex (1 : 1) which is much larger than that of 2-NS–β-CD complex is only in the neighborhood of $3.0 \times 10^3 \, \mathrm{M}^{-1}$. Thus, the present thermodynamic parameters of 2-NS–β-CD complex containing a smaller formation con-

stant seem reasonable, compared to those reported by calorimetry.

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