The Interaction of 1-Naphthalenesulfonate with β-Cyclodextrin. Studies by Calorimetry and Proton Nuclear Magnetic Resonance Spectroscopy

Juziro NISHIJO,* Yuko USHIRODA, Hiroe OHBORI, Makiko SUGIURA, and Noriko FUJII

Kobe Pharmaceutical University, Motoyama-kitamachi, Higashinada-ku, Kobe 658, Japan.
Received October 16, 1996; accepted January 14, 1997

The interaction of 1-naphthalenesulfonate (1-NS) with β-cyclodextrin was investigated in a 0.1 M phosphate buffer at pH 7.4 by an LKB 2277 microcalorimeter, using a flow-mixed mode at 25 °C. The thermodynamic parameters for inclusion complex formation obtained are as follows: $\Delta G° = -13.2$ kJ/mol ($K = 209\ M^{-1}$), $\Delta H° = -1.87$ kJ/mol, $\Delta S° = 38.1$ J/mol K. The main driving force for inclusion complex formation was considered to be the hydrophobic interaction, whereas the contribution of the van der Waals–London dispersion force was minor. Also, from measurements of the proton nuclear magnetic resonance spectra and the model building with Corey–Pauling–Koltun atomic models, the probable structures of the inclusion complex were determined.

Key words 1-naphthalenesulfonate; β-cyclodextrin; flow-microcalorimeter; thermodynamic parameter; inclusion complex; ^{1}H-NMR

Cyclodextrins (CDs) are increasingly used for enzyme modeling,[1] as catalytic systems, for chromatographic separations,[2] for the microcapsulation of drugs,[3] and in many other applications.[4] Therefore, it is important to clarify 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.

From such standpoints, we have already reported[5] the interaction of 8-anilino-1-naphthalenesulfonate (ANS) with β-CD and it was found that the benzene ring of ANS, not the naphthalene ring, is included in the cavity of β-CD from the secondary hydroxyl group side. In the present study, the interaction of 1-naphthalenesulfonate (1-NS), which has a naphthalene ring and sulfonate group but no benzene ring, with β-CD was investigated in an aqueous solution by using direct calorimetric measurements, measurements of the proton nuclear magnetic resonance (^{1}H-NMR) spectra, and Corey–Pauling–Koltun (CPK) atomic models. Then, it was discussed how the thermodynamic parameters and the inclusion modes are affected, depending on whether or not the guest has a benzene ring.

Experimental

Materials Reagent grade β-CD supplied by Nacalai Tesque, Co. was recrystallized twice from water and dried over P_2O_5 for 5 h at 110 °C in a vacuum before use. 1-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 2 h at 130 °C. The water used was obtained by twice distilling water purified with ion-exchange resin. All other chemicals used were of reagent grade. The compounds Na_2DPO_4 and KD_2PO_4 were obtained by treating Na_2HPO_4 and KH_2PO_4, respectively, with deuterium oxide and vaporizing the solvent; the resulting compounds were used to adjust pD.

The Measurements of the Heat of Reaction An LKB 2277 microcalorimeter (thermal activity monitor) was used in the flow-mixed mode at 25 °C. The solutions of β-CD and 1-NS were 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 Pharmaicia LKB model REC 2 recorder potentiometer. All data were corrected for dilution heat effect.

^{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. Two-dimensional nuclear Overhauser enhancement spectroscopy (NOESY) experiments were performed using the phase-sensitive method (hyper-complex method) with a mixing time of 1000 ms. Two-dimensional rotating frame 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.

Results

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. 1,

$$\text{1-NS} + \beta\text{-CD} \rightleftarrows \text{1-NS}-\beta\text{-CD}$$

$$K = \frac{X}{(NS_0 - X)(CD_0 - X)} \tag{1}$$

where NS_0, CD_0 and X represent the total concentration of 1-NS, total concentration of β-CD, and the concentration of 1-NS–β-CD complex, respectively. Here, the concentration is a molar concentration.

Also, if ΔHm, the heat produced when 1-NS and β-CD are mixed, and V, the volume of the solution, are used, then $\Delta H°$, the change in enthalpy for the complex formation, is represented in Eq. 2.

$$\Delta H° = \frac{\Delta Hm}{XV} \tag{2}$$

$$\Delta Hm = \Delta H° V \left\{ \left(NS_0 + CD_0 + \frac{1}{K} \right) - \sqrt{\left(NS_0 + CD_0 + \frac{1}{K} \right)^2 - 4NS_0CD_0} \right\} \Big/ 2 \tag{3}$$

From Eqs. 1 and 2, the heat of mixture, ΔHm, is represented in Eq. 3. If $R = CD_0/NS_0$ and $A = NS_0 \cdot K$, Eq. 4 is valid.[6]

$$\Delta Hm = \frac{\Delta H° V NS_0 \{ (A + AR + 1) - \sqrt{(A + AR + 1)^2 - 4A^2R} \}}{2A} \tag{4}$$

Figure 1 shows the plots of ΔHm, the heat produced, to

* To whom correspondence should be addressed.

the R, ratio of concentration of β-CD to that of 1-NS, when 1-NS and β-CD are mixed, the concentration of 1-NS being constant (2.50×10^{-3} M) and the concentration of β-CD being changed (2.50×10^{-4} M—2.50×10^{-3} M). Formation constant K and the enthalpy change for complex formation ΔH° can be estimated from Eq. 4 using the nonlinear squares program MULTI.[7] The values obtained at 25 °C are shown in Table 1. Also, Fig. 1 shows the binding curve for β-CD with 1-NS, together with the theoretical curves obtained using the calculated parameters. The change in entropy (ΔS°) accompanying the complexation was determined in the usual way.[8] The results obtained are shown in Table 1. Also, the thermodynamic parameters reported already,[9] which were determined by calorimetric titration in buffered aqueous solution at pH 7.20 (0.1 M sodium phosphate), are added in Table 1. It was found that the present formation constant is smaller than that of the literature.

^{1}H-NMR spectra and a CPK model were used to estimate the structure of the inclusion complex between 1-NS and β-CD. In Fig. 2a, a ^{1}H-NMR spectrum of 1.0×10^{-3} M 1-NS in 0.1 M phosphate buffered deuterium oxide solution at 25 °C is shown. The assignments of the proton signals of 1-NS were undertaken on the basis of ^{1}H–^{1}H chemical shift correlation spectroscopy (COSY) and NOESY measurements. In the presence of β-CD, proton signals due to 1-NS shifted slightly. When β-CD (3.0×10^{-3} M) was added to the 1-NS (1.0×10^{-3} M), the upfield shift of 5-H and the downfield shift of 8-H of 1-NS were a little prominent, whereas the shift of protons of the rest was only slight, as shown in Fig. 2b. The magnitudes of shifts of proton signals due to 1-NS in the presence of β-CD at various concentrations are shown in Fig. 3a.

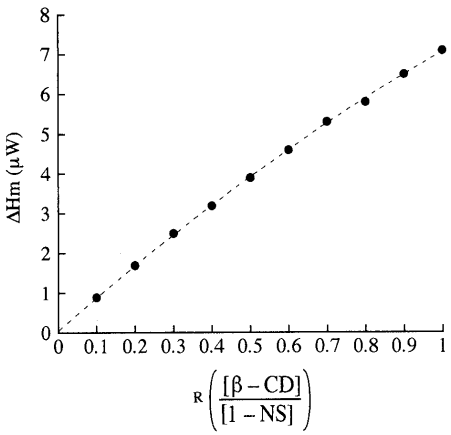

Fig. 1. Dependence of Heat Flow, Corresponding to Complex Formation, on the Molar Concentration Ratio of β-CD to 1-NS (R) at 25 °C

●, observed heat flow, which is the mean of 3 repeated runs; ---, theoretical curve, which was obtained from Eq. 4 using the calculated parameters.

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

	K (M^{-1})	ΔG° (kJ/mol)	ΔH° (kJ/mol)	ΔS° (J/mol K)
1-NS	209±15	−13.2	−1.87±0.12	38.1±3.8
Literature[8]	2510	−19.1	−6.23	44.5

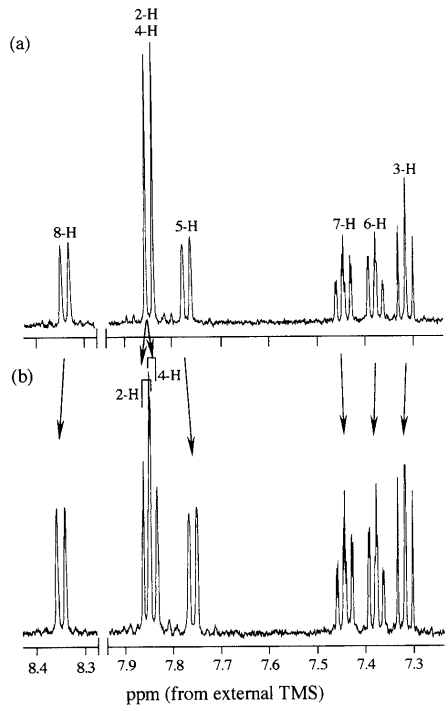

Fig. 2. ^{1}H-NMR Spectra of 1-NS in the Presence of β-CD in 0.1 M Phosphate Buffered Deuterium Oxide Solution (pD7.4) at 25 °C

(a) 1-NS alone (1.0×10^{-3} M), (b) 1-NS (1.0×10^{-3} M) + β-CD (3.0×10^{-3} M).

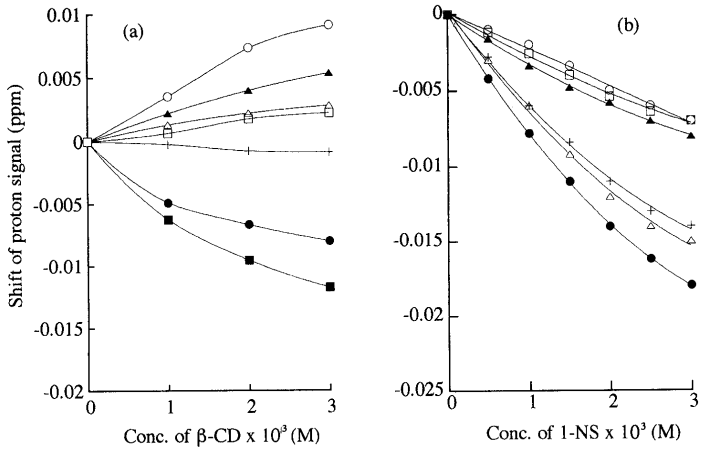

Fig. 3. Induced ^{1}H-NMR Chemical Shifts of (a) 1-NS (1.0×10^{-3} M) in the Presence of β-CD and (b) β-CD (1.0×10^{-3} M) in the Presence of 1-NS

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

A ^{1}H-NMR spectrum of 1.0×10^{-3} M β-CD solution is shown in Fig. 4a. The spectrum is different from that (Fig. 4b) in the presence of 3.0×10^{-3} M 1-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 1-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. Also, 6-H lying on the inner surface of the primary hydroxyl group side shifted upfield to the same extent as the 3-H. 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 prominent and their magnitudes were similar. The magnitudes of the upfield shifts of the signals due to β-CD in the presence of 1-NS at various concentrations are shown in Fig. 3b.

As the possible structures for the inclusion complex, judging from the investigation using the CPK model and taking into account that the $-SO_3^-$ group is hydrophilic, three kinds of structures described in Fig. 5 are considered, a) the naphthalene ring is included shallowly at the head of 4-H and 5-H of 1-NS in the cavity of β-CD from the secondary hydroxyl group side, sulfonate group being outside the cavity, b) the naphthalene ring is enclosed at the head of 6-H of 1-NS, not so deeply, and the long axis of the naphthalene ring inclines slightly to the molecular axis of β-CD in order that the sulfonate group might avoid being included in the cavity. About half the benzene ring- (if the naphthalene ring consists of two benzene rings) binding sulfonate group and sulfonate group are left outside the cavity, and c) the naphthalene ring is enclosed shallowly at the head of the 6-H of 1-NS, from the primary hydroxyl group side, and the long axis of the naphthalene ring inclines to the molecular axis of β-CD more than in Fig. 5b since the cavity of the primary hydroxyl group side is more narrow than that of the secondary hydroxyl group side. Almost all of the benzene ring-binding sulfonate group and sulfonate group are left outside the cavity.

It is considered that upfield shifts of proton signals lying on the inner surface of β-CD result mainly from the magnetic anisotropy of the naphthalene ring of the 1-NS molecule. Therefore, the result that the magnitude of the upfield shifts of the signals due to the protons on the inner surface of β-CD are in the order of 5-H > 3-H $\geq$ 6-H suggests that the structure shown in Fig. 5a is not preferential, because the upfield shift of the signal due to the 3-H of β-CD may be most prominent in Fig. 5a. In the structure of the inclusion complex of Fig. 5b, it can be explained why the upfield shift of the 5-H of β-CD is more prominent than that of the 6-H of β-CD, but it can't be explained why the upfield shift of the 5-H of β-CD is more prominent than that of the 3-H of β-CD or why the upfield shift of the 3-H is the same as that of the 6-H of β-CD. Also, in the inclusion mode of Fig. 5c, it is expected that the upfield shift of the 6-H of β-CD is much more prominent than that of the 3-H of β-CD, and therefore, it can't be preferential. Thus, it was impossible to reasonably explain the induced chemical shifts of β-CD in the presence of 1-NS by one of three inclusion modes shown in Fig. 5. However, if the inclusion complex has

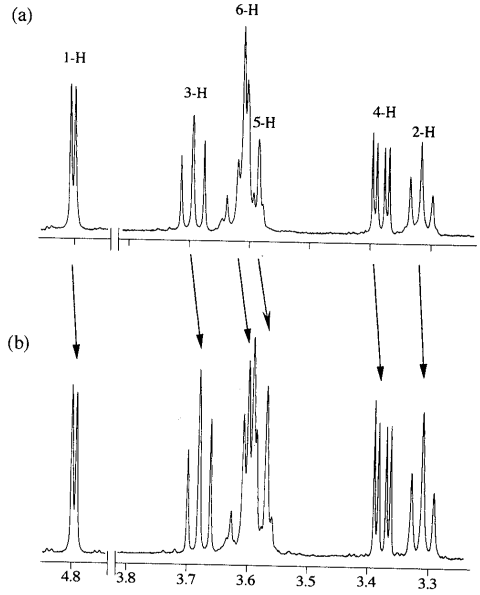

Fig. 4. ^{1}H-NMR Spectra of β-CD (1.0×10^{-3} M) in the Presence of 1-NS in 0.1 M Phosphate Buffered Deuterium Oxide Solution (pD 7.4) at 25 °C

(a) β-CD alone (1.0×10^{-3} M), (b) β-CD (1.0×10^{-3} M) + 1-NS (3.0×10^{-3} M).

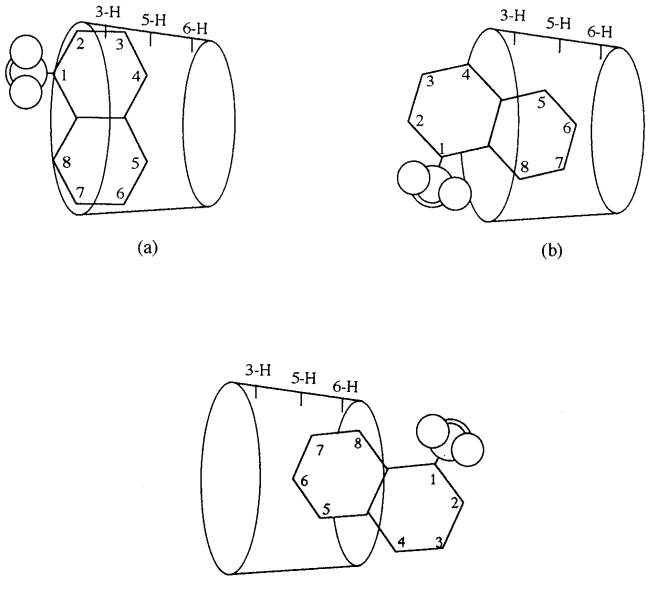

Fig. 5. Possible Structures of the Inclusion Complex of 1-NS with β-CD in Aqueous Solution Based on CPK Space-Filling Models

the two inclusion modes shown in Figs. 5b and 5c, the upfield shift of the 5-H signal of β-CD may be most prominent, and those of 6-H and 3-H may be similar with one other. Therefore, from the induced chemical shifts by complexation it was assumed that the complex structure described in Figs. 5b and 5c are preferential.

To confirm the structure of the inclusion complex, ROESY spectra were measured. As a result of examing the experimental conditions, a ROESY spectrum was measured in the solution containing 1-NS (5.0×10^{-2} M) and β-CD (5.0×10^{-2} M) with a spin-lock mixing pulse of 250 ms (Fig. 6). Unambiguous cross peaks were observed between the 3-H of β-CD and the 3-H, 6-H, and 7-H of

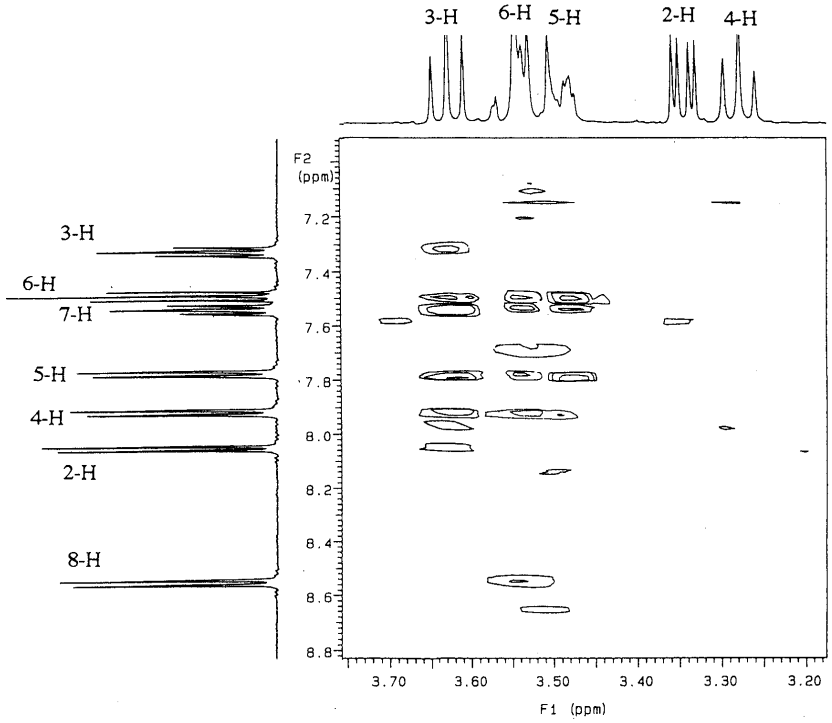

Fig. 6. ROESY Spectrum of Solution Containing 1-NS (5.0×10^{-2} M) and β-CD (5.0×10^{-2} M) in the 0.1 M Phosphate Buffered Deuterium Oxide Solution (pD 7.4) at 25 °C

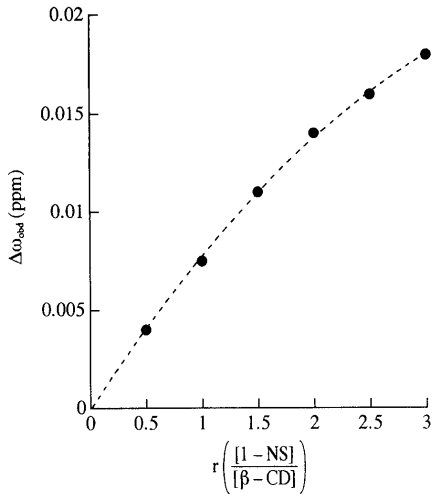

Fig. 7. Plots of $\Delta\omega_{obd}$ Values of the 5-H Signal due to β-CD *versus* the Molar Concentration Ratio of 1-NS to β-CD at 25 °C

●, observed value, which is the mean of repeated runs; ---, theoretical curve, which was obtained from Eq. 5 using the calculated parameters.

1-NS. Therefore, it is certain that the inclusion complex shown in Fig. 5a exists. Also, cross peaks were observed between the 5-H, 6-H, and 7-H of 1-NS and the 5-H of β-CD. Further, cross peaks were observed between the 5-H and 4-H of 1-NS and the 3-H of β-CD. These cross peaks support the structure of an inclusion complex of Fig. 5b. Also, cross peaks observed between the 8-H, 7-H, 5-H, and 4-H of 1-NS and the 6-H of β-CD support the structure shown in Fig. 5c. It is presumed that the two structures shown in Figs. 5b and 5c are preferential according to the induced chemical shifts. But, considering that the ROESY spectra gives direct information, it is reasonable to conclude that the structure shown in Fig. 5a exists too, in addition to the structures shown in Figs. 5b and 5c.

Harata and Uedaira.[10] suggested that 1-substituted naphthalene can be included in two modes in the β-CD cavity by the circular dichroism spectra and by calculation of the rotational strength. The structures of the two species were similar to those in Figs. 5a, 5b and 5c.

Discussion

Next, the structures shown in Fig. 5 will be discussed from the standpoint of the induced chemical shifts of 1-NS in the presence of β-CD. The signal due to the 5-H of 1-NS shifted upfield most prominently in the proton signals of 1-NS, followed by the 4-H of 1-NS in the presence of β-CD. It has been known that 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 by the C–C bond anisotropy effect, and so on.[11-13] The 5-H of 1-NS exists in the cavity without close contact with the atoms on the inner surface of the host molecule in either structure shown in Fig. 5. This might induce considerable upfield shifts of the signals due to 5-H. Also, the 4-H of 1-NS exists in the cavity without close contact in the structures in Figs. 5a and 5b, but with weak contact in the structure shown in Fig. 5c. Therefore, the upfield shift of the signal due to the 4-H of 1-NS would not be so prominent as that of the 5-H of 1-NS. On the other hand, the signal due to the 8-H of 1-NS shifted downfield prominently in the presence of β-CD, followed by the 2-H of 1-NS. Since the 8-H tends to come into contact with atoms on the inner surface of β-CD in the structures described in Fig. 5c, the downfield shift of the 8-H signal might result from the contact, namely, by a steric compression effect.[14] Also, the signal of 2-H of 1-NS shifted downfield, though the downfield shift was smaller than that of 8-H. The 2-H of 1-NS do not come into close contact in either structure shown in Fig. 5. In the structures of Figs. 5b and 5c, a $-SO_3^-$ group approaches the

cavity of β-CD, and it is therefore assumed that the $-SO_3^-$ group would form a hydrogen bond with the hydroxyl group of β-CD to result in a decrease in the electron density of 2-H, inducing the downfield shift of 2-H. The other proton signals (3-H, 6-H, 7-H) of 1-NS shifted downfield, although only slightly. The results are explained on the basis of the assumption that steric compression effects are superior to any other effect, because these protons tend to come into contact with atoms on the inner surface of β-CD in either structure shown in Fig. 5.

In the case of complexation in aqueous solution, various driving forces have been reported to play a big part in the interaction.[15] However, according to Tabushi *et al.*,[16] the van der Waals–London dispersion forces and hydrophobic interaction are the major driving forces for the inclusion complexation between cyclodextrin and a guest molecule. In the present study, it was found that the complexation was accompanied by a negative change in enthalpy and by a positive change in entropy. These thermodynamic parameters suggest that the van der Waals–London dispersion force and hydrophobic interaction are the main contributors to inclusion complex formation.

The authors have already reported the thermodynamic parameters for the inclusion complex of ANS, the structure of which has an anilino group at position 8 of 1-NS,[12] with β-CD, as determined by fluorescence spectrophotometry. The thermodynamic parameters were determined as follows: $K=70.9\,\mathrm{M}^{-1}$ at 25 °C, $\Delta H^\circ=-7.95$ kJ/mol, $\Delta S^\circ=8.8$ J/mol K. The two primary driving forces, van der Waals–London dispersion force and a hydrophobic interaction, were responsible for the complex formation of ANS with β-CD. In the ANS–β-CD complex, as reported already, the benzene ring of ANS, not the naphthalene ring, is rather loosely packed in the cavity. Therefore, it seemed reasonable that the change in enthalpy (ΔH°) would take a small negative value and the change in entropy (ΔS°) a small positive one in the ANS–β-CD complex. In the present study, as possible structures of the inclusion complex between 1-NS and β-CD, the three structures shown in Fig. 5 were estimated. When these structures were examined again using CPK models, it was confirmed that the naphthalene ring moiety of 1-NS is included more shallowly than the benzene ring of ANS, because the sulfonate group of 1-NS prevents the naphthalene ring from penetrating deeply into the cavity in the structures of Figs. 5b and 5c, and the long axis of the naphthalene ring is inclined to the axis of the cavity in such a way that the sulfonate group goes away from the margin of the cavity. Also, in the structure of Fig. 5a, the naphthalene ring is included shallowly because of the contact of the 3-H and 6-H of 1-NS with atoms on the inner surface of the cavity. Thus, it could be estimated that 1-NS is included more shallowly in the cavity than ANS, and is included more loosely in the complex of 1-NS with β-CD as well as ANS.

Consequently, it seems reasonable that the change in enthalpy (ΔH°) for the complex formation of 1-NS–β-CD is much less negative than that for the complex formation of ANS–β-CD, and the changes in entropy (ΔS°) are more positive. Therefore, the fact that the formation constant of the complex of 1-NS is $209\,\mathrm{M}^{-1}$, being larger than that of ANS, is mainly attributable to a more positive entropy change for the complex formation of 1-NS than that for the complex formation of ANS.

As mentioned above, the present formation constant for the 1-NS–β-CD complex was smaller than that of literature. Thus, another determination of the formation constant was carried out from the change in the chemical shifts of 5-H due to β-CD shown in Fig. 3b, using Eq. 5.[17]

$$\Delta\omega_{obd}=\frac{\Delta\omega_0\left(1+r+\frac{1}{a}-\sqrt{\left(1+r+\frac{1}{a}\right)^2-4r}\right)}{2} \tag{5}$$

where $r=NS_0/CD_0$, $a=K\cdot CD_0$. Also, $\Delta\omega_{obd}$ is the change in the chemical shift observed, and $\Delta\omega_0$ is the complexation shift.[8] Figure 7 shows the plots of the $\Delta\omega_{obd}$ of 5-H due to β-CD, to the r, ratio of concentration of 1-NS, to that of β-CD. Formation constant K and complexation shift $\Delta\omega_0$ can be estimated from Eq. 5 using the nonlinear squares program MULTI,[7] and K was $220\pm5\,\mathrm{M}^{-1}$ ($\Delta\omega_0=0.048\pm0.003$ ppm). In this way, the formation constant obtained from the microcalorimeter was consistent with that from the change in the proton chemical shift. Though the present formation constant K was not consistent with that of literature, the reason would perhaps be attributable to differences in experimental conditions. Regrettably, detailed experimental conditions such as the concentrations of 1-NS and β-CD used in the experiment were not described in the literature.[9] From the above discussion, as 1-NS is included rather loosely and shallowly, the present thermodynamic parameters containing a smaller formation constant seems reasonable.

References

1) Breslow R., *Science*, **218**, 532—537 (1982).
2) Armstrong D. W., Ward T. J., Armstrong R. D., Beesly T. E., *Science*, **232**, 1132—1135 (1986).
3) Uekama K., Otagiri M., *CRC Crit. Rev. Ther. Drug Carrie Syst.*, **3**, 1—40 (1987).
4) Szejtli J., "Cyclodextrin Technology," Kluwer Academic Dordrecht, The Netherlands, 1988, pp. 186—293
5) Nishijo J., Yasuda M., Nagai M., Sugiura M., *Bull. Chem. Soc. Jpn.*, **65**, 2869—2871 (1992).
6) Lamberts L., Zeegers-Huyskens T., *J. Chim. Phys.*, **60**, 435—441 (1963).
7) Yamaoka K., Nakagawa T., *J. Parmacobio-Dyn.*, **6**, 595—606 (1983).
8) *a*) Nishijo J., Yonetani I., Tagahara K., Suzuta Y., Iwamoto E., *Chem. Pharm. Bull.*, **34**, 4451—4456 (1986); *b*) Nishijo J., Yonetani I., Iwamoto E., Tagahara K., Sugiura M., *J. Pharm. Sci.*, **79**, 14—18 (1990).
9) Inoue Y., Hakushi T., Lin Y., Tong L.-H., Shen B.-J., Jin D.-S., *J. Am. Chem. Soc.*, **115**, 475—481 (1993).
10) Harata K., Uedaira H., *Bull. Chem. Soc. Jpn.*, **48**, 375—378 (1975).
11) Nakajima T., Sunagawa M., Hirihashi T., Fujioka K., *Chem. Pharm. Bull.*, **32**, 384—400 (1984).
12) Nishijo J., Nagai M., *J. Pharm. Sci.*, **80**, 58—62 (1991).
13) Nishijo J., Nagai M., Yasuda M., *Carbohydr. Res.*, **245**, 43—56 (1993).
14) Wood D.J., Hruska F.E., Saenger W., *J. Am. Chem. Soc.*, **99**, 1735—1740 (1977).
15) Komiyama M., Bender M.L., *J. Am. Chem. Soc.*, **100**, 2259—2260 (1978).
16) Tabushi I., Kiyosuku Y., Sugimoto T., Yamamura K., *J. Am. Chem. Soc.*, **100**, 916—919 (1978).
17) Wiese M., Cordes H.-P., Chi H., Seydel J. K., Backensfeld T., Miiller B. W., *J. Pharm. Sci.*, **80**, 153—156 (1991).