Inclusion Mode of 8-Anilino-1-naphthalenesulfonate (ANS) in the ANS-β-Cyclodextrin Complex

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Synopsis. The inclusion mode of 8-anilino-1-naphthalenesulfonate (ANS) in the ANS-β-cyclodextrin complex with a molar ratio of 1:1 in deuterium oxide was investigated by measurements of the proton nuclear magnetic resonance spectra and studies with Corey Pauling Koltun models. It was found that in the preferential inclusion mode of ANS, the whole benzene ring of ANS is enclosed at a considerable depth in the cavity of β-cyclodextrin from the side of the secondary hydroxyl group, and a great portion of the naphthalene ring is outside the cavity.

8-Anilino-1-naphthalenesulfonate (ANS) is known to be a fluorescent probe for exploring hydrophobic regions.[1] The fluorescence of this agent is quenched in water, but in hydrophobic environments it is augmented substantially with shifts of emission toward shorter wavelengths.[2] Also, it has been reported that fluorescence emitted by ANS increases markedly when cyclodextrins such as β- or γ-cyclodextrin related to biological substances, were added to an aqueous solution.[3,4] The fluorescence intensity is thought to originate from the presence of ANS in the cavity of the cyclodextrin molecule.

It has already been reported that ANS forms an inclusion complex with β-cyclodextrin with a molar ratio of 1:1.[4] In the present study, the authors investigated the inclusion mode of ANS in the ANS-β-cyclodextrin complex by using Corey Pauling Koltun (CPK) models and by considering proton nuclear magnetic resonance (^{1}H NMR) data.

Experimental

Materials. Reagent-grade β-cyclodextrin supplied by Nakarai Chemicals, Ltd. was recrystallized twice from water and dried over P_2O_5 for 5 h at 110 °C in a vacuum before use. ANS ammonium salt supplied by Nakarai Chemicals, Ltd. was recrystallized twice from water before use.

^{1}H NMR Spectra. All ^{1}H NMR spectra were measured in deuterium oxide at 30 °C, using an XL-500 NMR (499.84 MHz) spectrometer. Two-dimensional rotating frame nuclear overhause effect spectroscopy (ROESY) experiments were performed in the phase-sensitivity mode, using the 2D hyper complex method (State-Haberkorn method). Spectra were acquired with 256 t_1 increments and each t_1 increment consisted of 1 k data points from 16-32 transients. The HOD signal was suppressed by presaturation for 1 s and a spinlock mixing pulse of 250 ms was used. Data sets were multiplied by a Gaussian function and zero-filled to 1 K data points in f_1 prior to Fourier transformation. Tetramethylsilane (TMS) was used as an external reference.

Results and Discussion

^{1}H NMR spectra and CPK models were used to estimate the inclusion mode of ANS in the ANS-β-cyclodextrin complex. A ^{1}H NMR spectrum of 1.5×10^{-2} M ANS was recorded in deuterium oxide at 30 °C, using TMS as an external reference. The assignments of the proton signals of ANS were undertaken on the basis of homonuclear decoupling and comparison with ^{1}H NMR spectra of analogous compounds.[5,6] The assignments were also confirmed with ROESY experiments. The results were as follow: 7-H (8.097 ppm, dd), 5-H (7.859 ppm, dd), 4-H (7.459 ppm, dd), 2-H (7.424 ppm, dd), 3-H (7.316 ppm, t), 6-H (7.297 ppm, t), 3′-H and 5′-H (7.084 ppm, nearly t), 2′-H and 6′-H (6.905 ppm, nearly d), 4′-H (6.704 ppm, nearly t). The proton signals due to ANS shifted after adding β-cyclodextrin. The changes in chemical shifts induced by adding 1.5×10^{-2} M β-cyclodextrin (1 M=1 mol dm^{-3}) are shown in Table 1. The 7-H, 6-H, and 3-H signals of the naphthalene ring shifted downfield slightly. The other proton signals shifted upfield. The upfield shift of the 2-H signal due to the naphthalene ring is prominent, followed by the 4-H shift; the upfield shift of the 5-H signal is slight. On the other hand, the upfield shifts of the signals due to 3′-H, 5′-H, and 4′-H of the benzene ring are prominent, though those of 2′-H and 6′-H are only slight.

Then, ^{1}H NMR spectra of β-cyclodextrin were measured in the presence and absence of ANS. It was found that all the proton signals due to β-cyclodextrin shifted upfield in the presence of ANS. The results are shown in Table 1. The upfield shift of the signal due to 5-H inside the cavity of β-cyclodextrin was the most prominent, followed by those due to 3-H and 6-H inside

Table 1. The Induced Chemical Shifts[a] of ANS in the Presence of β-Cyclodextrin and β-Cyclodextrin in the Presence of ANS

ANS (Δ ppm)									β-Cyclodextrin (Δ ppm)					
7-H	6-H	5-H	4-H	3-H	2-H	2′-H,6′-H	4′-H	3′-H,5′-H	1-H	2-H	3-H	4-H	5-H	6-H
+0.012	+0.007	−0.009	−0.020	+0.010	−0.035	−0.003	−0.029	−0.043	−0.034	−0.045	−0.048	−0.029	−0.097	−0.046

a) Signs + and − indicate downfield and upfield shifts, respectively.

the cavity. The upfield shifts of the signals due to protons outside the cavity was slight. It is believed that these upfield shifts of the β-cyclodextrin signals result mainly from the magnetic anisotropy of the benzene or naphthalene ring of the ANS molecule.

From the investigation of CPK models, three kinds of possible inclusion modes of ANS in the ANS-β-cyclodextrin complex can be considered, as shown in Fig. 1. The fact that the extent of the upfield shift of the 5-H signal of β-cyclodextrin is the greatest suggests that the inclusion mode in Fig. 1b cannot be the preferential one. The inclusion mode in Fig. 1a would result in a prominent upfield shift of the 5-H signal. But it is difficult to evaluate the extent of the upfield shift of the β-cyclodextrin 3-H because the effects of $-SO_3^-$ and >NH groups in the neighborhood on the shift of the 3-H signal are not clear. Finally, the inclusion mode shown in Fig. 1c can result in an upfield shifts of the 5-H signal that is prominent and of the 3-H signal that is slight. Consequently, it was difficult to decide the inclusion mode by the induced chemical shifts, in this case. Therefore, the ROESY spectrum was measured and is shown in Fig. 2. In a ROESY spectrum, a cross-peak connecting two proton resonances indicate that those proton nuclei are in close proximity in the ground state of the molecule. The cross-peak in the ROESY spectrum of Fig. 2 connecting the 5-H resonance of β-cyclodextrin to the 2′-H, 6′-H, 3′-H, and 5′-H resonances of ANS indicate clearly that the whole benzene ring of ANS is enclosed at a considerable depth in the cavity of β-cyclodextrin from the side of the secondary hydroxyl group. Though a small cross-peak connecting the 5-H resonance of β-cyclodextrin to the 4′-H resonance of ANS is observed, the cross-peak is consistent with the inclusion mode of ANS mentioned above. Also, if the inclusion mode of ANS in ANS-β-cyclodextrin is the one shown in Fig. 1c, the cross-peak connecting the 3-H resonance of β-cyclodextrin to the 3′-H and 5′-H resonance of ANS must be observed. As this cross-peak is observed, though the intensity is very small, and a cross-

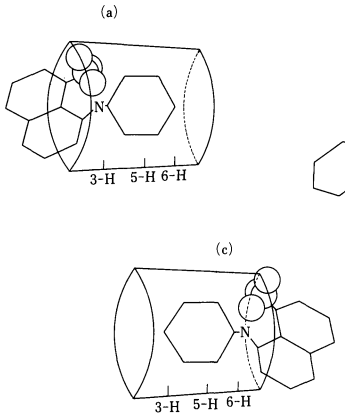

Fig. 1. Possible inclusion modes of ANS based on CPK space-filling models.

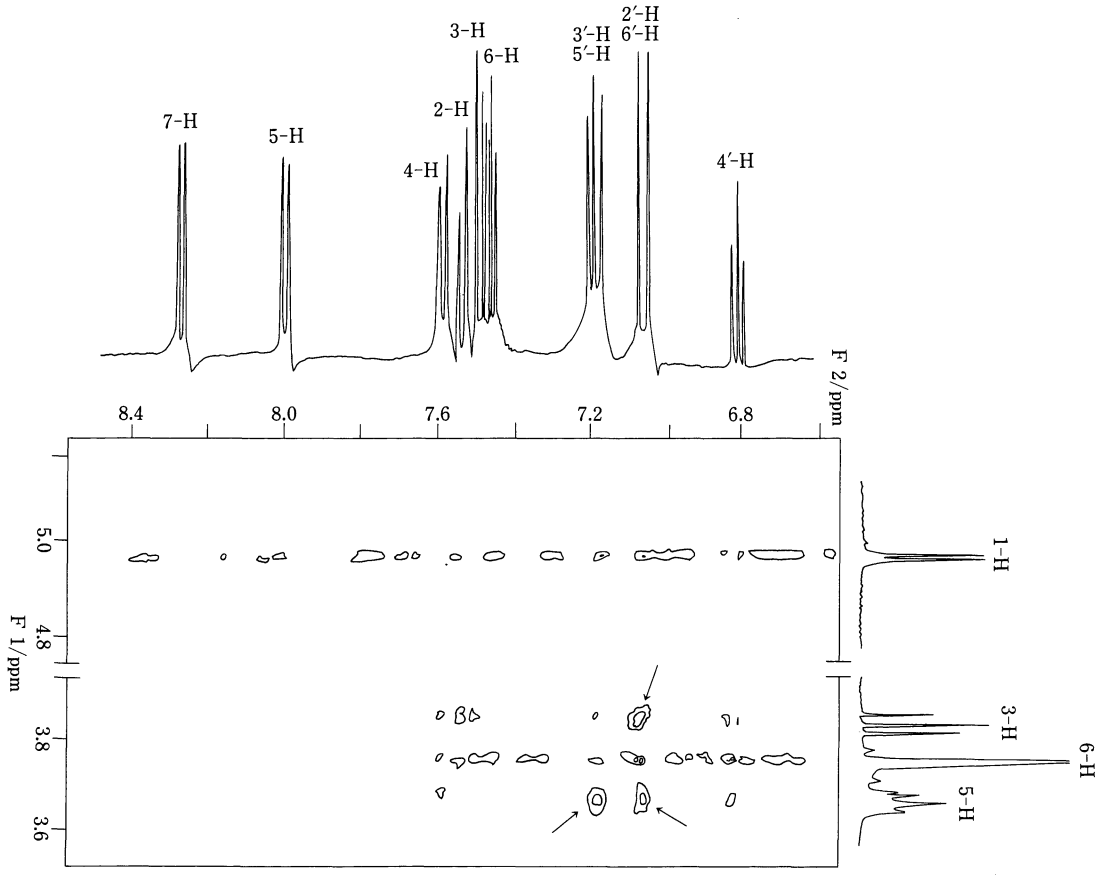

Fig. 2. 2D ROESY spectrum of a solution containing ANS (2.0×10^{-2} M) and β-cyclodextrin (2.0×10^{-2} M) in deuterium oxide. Cross-peaks with strong intensities are indicated by arrows.

peak connecting 3-H of β-cyclodextrin to 4′-H of ANS seems to appear, the inclusion mode of ANS shown in Fig. 1c may exist slightly. On the other hand, cross-peaks connecting the 3-H resonance of β-cyclodextrin to the 3-H, 2-H, and 4-H resonances of ANS and the 5-H resonance of β-cyclodextrin to the 4-H resonance of ANS are found in Fig. 2, though the intensities of the signals are small. If the inclusion mode of ANS in the ANS-β-cyclodextrin complex is the one shown in Fig. 1b, these cross-peaks may be observed. Further, in addition to these cross-peaks, those connecting the 3-H resonance of β-cyclodextrin to the 5-H and 6-H resonances of ANS and the 5-H resonance of β-cyclodextrin to the 5-H resonance of ANS should be observed, if the inclusion mode of ANS is the one shown in Fig. 1b. However, such cross-peaks are not observed in Fig. 2. Also, taking into consideration the fact that the 5-H signal of β-cyclodextrin shows the most prominent upfield shift in the presence of ANS (Table 1), it is reasonable to consider that the cross-peaks having small intensities are noise or that the inclusion mode of ANS shown in Fig. 1b may exist only slightly. Consequently, it was determined that the inclusion mode of ANS in the ANS-β-cyclodextrin complex shown in Fig. 1a is the most preferential one in deuterium oxide. This structure is different from that of the inclusion complex of ANS with β-cyclodextrin previously reported.[7]

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