Binding Studies of Adamantanecarboxylic Acid and a Naphthyl-Bound β-Cyclodextrin by Variable Temperature ^{1}H NMR

Shelli R. McAlpine and Miguel A. Garcia-Garibay*

Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90024

Received July 22, 1996

Introduction

Chromophores attached to cyclodextrins may help monitor the state of binding between cyclodextrin and external guests, and recent efforts have been directed to the development of compounds capable of acting as molecular sensors.[1−9] It is expected that sensitivity to external guests should depend on the interaction between the guest and the probe and on whether they may share the hydrophobic cavity of the host or they must compete for it. We recently showed that a short-tethered naphthyl group attached at the 3-hydroxy position of β-cyclodextrin (compound **1**, Scheme 1) displays a temperature-dependent inside−outside isomerism that is capable of modifying its binding and sensing capabilities.[10] In this paper, we report a variable temperature ^{1}H NMR study with **1** and 1-adamantanecarboxylic acid (AdCA)[11−14] that supports a binding scheme (Scheme 1) similar to that recently proposed from fluorescence measurements with 2-(*p*-toluidino)-6-naphthalenesulfonate (TNS).[10]

Results and Discussion

Compound **1** was prepared and purified as described previously.[10,15−17] Samples for ^{1}H NMR measurements were prepared by addition of equimolar AdCA to 10 mM solutions of **1** at 25 °C. Control measurements were carried out with pure **1**, (2-naphthyl)methyl methyl ether, pure AdCA, and AdCA included in β-cyclodextrin. All measurements were carried out in D_2O in a Bruker ARX at 500 MHz with presaturation of residual water in a temperature-controlled probe. The temperature-dependence of the spectra was measured between 25 and 80 °C and after equilibration at ambient temperature (annealing). While interpretation of spectral changes corresponding to the cyclodextrin hydrogens between 3.0 and 5.0 ppm is difficult because of insufficient resolution, changes in the aromatic (7.20−8.20 ppm) and aliphatic (1.00−2.20 ppm) regions reflect changes in the environment of the naphthyl group and the AdCA guest. These are shown in Figures 1 and 2, respectively.

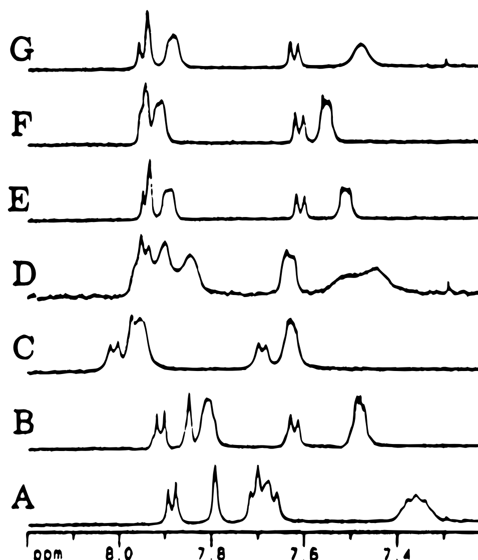
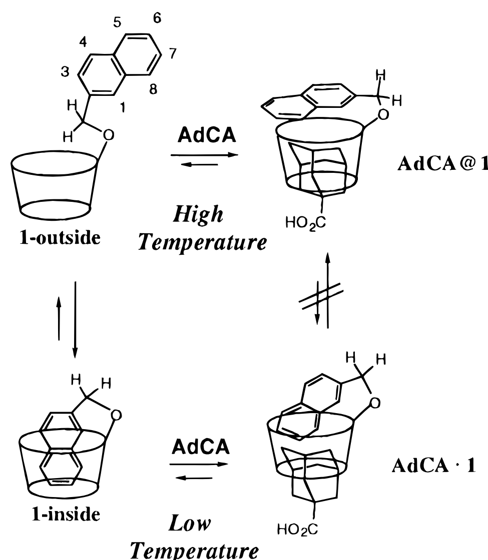


Figure 1. ^{1}H NMR (500 MHz, D_2O) spectra of the aromatic region of 4 mM **1** at 25 °C (A), 50 °C (B), and 80 °C (C), after addition of equimolar AdCA at 25 °C (D), 50 °C (E), and 80 °C (F), and after cooling down and equilibrating at 25 °C (G).

Scheme 1



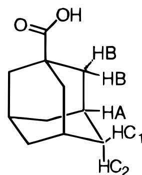
The ^{1}H NMR spectrum of (2-naphthyl)methyl methyl ether in D_2O is nearly temperature independent and can be described in terms of α- (H1, H4, H5, and H8) and β-naphthyl (H3, H6, and H7) hydrogens. In contrast, the spectrum of pure **1** has a high temperature-dependence as illustrated by measurements at 25, 50, and 80 °C (Figure 1A−C), which are in excellent agreement with those previously interpreted in terms of the inside−outside conformational equilibrium of **1**.[10] The complex pattern and upfield shifts at 25 °C (Figure 1A) are assigned to the inside isomer while temperatures of 50

(1) Ueno, A.; Suzuki, I.; Osa, T. *J. Chem. Soc., Chem. Commun.* **1988**, 1373−1374.
(2) Ueno, A.; Suzuki, I.; Osa, T. *J. Am. Chem. Soc.* **1989**, *111,* 6391−6397.
(3) Nakamura, A.; Saitoh, K.; Toda, F. *Chem. Lett.* **1989**, 2209−2212.
(4) Fijita, K.; Tahara, T.; Koga, T.; Imoto, T. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 300−305.
(5) Ueno, A.; Moriwaki, F.; Osa, T.; Hamada, F.; Murai, K. *Bull. Chem. Soc. Jpn.* **1986**, *59,* 465−470.
(6) Ueno, A.; Moriwaki, F.; Tomita, Y.; Osa, T. *Chem. Lett.* **1985**, 493−496.
(7) Wang, Y.; Ikeda, T.; Ueno, A.; Toda, F. *Chem. Lett.* **1992**, 863−866.
(8) Hamada, Y. K.; R, I. *J. Inclusion Phenom. Mol. Recognit. Chem.* **1993**, *15,* 273−279.
(9) Ueno, A.; Minato, S.; Suzuki, I.; Fukushima, M.; Okhubo, M.; Osa, T.; Hamada, F.; Murai, K. *Chem. Lett.* **1990**, 605−608.
(10) McAlpine, S. R.; Garcia-Garibay, M. A. *J. Am. Chem. Soc.* **1996**, *118,* 2750−2751.
(11) Hamilton, J. A. *Carbohydr. Res.* **1985**, *142,* 21−37.
(12) Jaime, C.; Redondo, J.; Sanchez-Ferrando, F.; Virgili, A. *J. Mol. Struct.* **1991**, *248,* 317−329.
(13) Palepu, R.; Reinsboroough, V. C. *Aust. J. Chem.* **1990**, *43,* 2119−2123.
(14) Hamilton, J. A.; Sabesan, M. N. *Acta Crystallogr.* **1982**, *38,* 3063−3069.
(15) Smith, S. H.; Forrest, S. M.; Williams, D. C.; F., C. M.; Acquavella, M. F.; Abelt, C. J. *Carbohydr. Res.* **1992**, *230,* 289−97.
(16) Abelt, C. J.; Pleier, J. *J. Org. Chem.* **1988**, *53,* 2159−2162.
(17) Abelt, C. J.; Lokey, J. S.; Smith, S. H. *Carbohydr. Res.* **1989**, *192,* 119−130.

S0022-3263(96)01379-5 CCC: $12.00 © 1996 American Chemical Society

and 80 °C (1B and 1C) increase the population of the outside isomer. The latter possesses a downfield-shifted spectrum characteristic of a water-exposed[18] and symmetric naphthyl group.[10]

Changes in the naphthyl region of **1** caused by the presence of AdCA are shown in spectra 1D–G. The spectrum of pure **1** changes upon initial mixing at 25 °C (1D), after the samples are heated to 50 and 80 °C (1E and 1F) and after cooling back to 25 °C (1G). The naphthyl region in 1D shows significant broadening and downfield shifting as compared to the spectrum of pure **1**. While downfield shifts suggest a moderate increase in water exposure,[18] spectral broadening is consistent with slower equilibria, which may be caused by coinclusion of the two hydrophobic groups (i.e., AdCA·**1**). In fact, heating the sample to 50 and 80 °C simplifies the spectrum by increasing resolution while shifting toward the simpler downfield pattern of the α- and β-hydrogens characteristic of **1**-outside (Scheme 1).[10] Interestingly, while temperature-dependent changes in the ^{1}H NMR spectrum of **1** (Figure 1A–C) are reversible in the absence of external guests, comparison of spectra 1G and 1D shows that annealing the complex of AdCA and **1** results in a spectrum that is different from that initially observed at 25 °C. We postulate that spectra 1D and 1G reflect structures that differ in the extent of inclusion of the naphthyl and adamantyl groups. We suggest that thermal activation allows for formation of the most stable complex (i.e., AdCA@**1**), which becomes the predominant species after slow cooling. The annealed spectrum (1G) is sharper and downfield shifted, suggesting an increase in water exposure of the naphthyl group, which may act as a cap when the hydrophobic cavity is occupied with AdCA.



Support for the above interpretation was searched for by analysis of the aliphatic region of the spectra. The temperature-dependence of AdCA (Figure 2A) and its inclusion complex with β-cyclodextrin (Figures 2B–D) was investigated in order to help us interpret changes in the aliphatic region of the spectra assigned to AdCA·**1** and AdCA@**1** (Scheme 1 and Figure 2E–H). The spectrum of free AdCA in D_2O (Figure 2A) has an insignificant temperature-dependence between 25 and 80 °C. There are four groups of signals corresponding to HA (1.91 ppm), HB (1.78 ppm), and the diastereotopic signals of HC_1 and HC_2, which are centered at 1.62 ppm and separated by only 0.05 ppm (see structure for labeling). Inclusion of AdCA within β-cyclodextrin (Figure 2B) causes unequal upfield shifts for the four groups of signals. The most affected are those assigned to HB and HC_1, and the least affected are those assigned to HA and HC_2. An X-ray structure of the β-cyclodextrin complex shows that inclusion of AdCA occurs via the primary hydroxyl side.[14] If a similar structure is formed in solution, signals assigned to HC_1 and HB may be affected by their close proximity to the oxygens of the glycosidic 1,4-linkages. Increasing the temperature to 50 and 80 °C (Figure 2C,D) leaves signals of HA and HB virtually unchanged while signals corresponding to HC_1 and HC_2 converge toward one another.

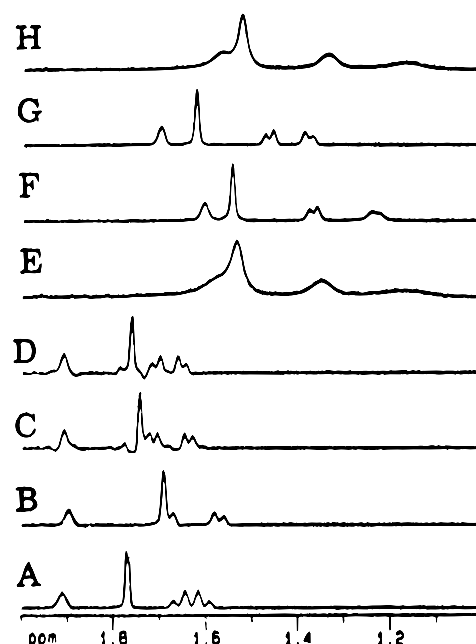


Figure 2. ^{1}H NMR (500 MHz, D_2O) spectra of the aliphatic region of AdCA (A), the inclusion compound of AdCA in β-cyclodextrin at 25 °C (B), 50 °C (C), and 80 °C (D), and samples containing AdCA and **1** at 25 °C (E), 50 °C (F), and 80 °C (G) and after cooling down and equilibrating at 25 °C (H).

Binding of AdCA with compound **1** shows a remarkable diamagnetic shielding consistent with a close proximity of the naphthyl and adamantyl groups (Figure 2E–H). The aliphatic regions in Figure 2E–H are complementary of the aromatic regions shown in Figure 1D–G and spectral changes also observed upon mixing at 25 °C (Figure 2E), with increasing temperatures to 50 and 80 °C (Figure 2F,G), and after cooling to 25 °C (Figure 2H). The upfield shift of AdCA protons in Figure 2E suggests a close proximity with the naphthyl group inside the cavity. Those shifts are consistent with an apical approach to the face of the naphthyl group where HC_1, HC_2, and HA are most upfield shifted. That a close interaction between the naphthyl group and AdCA is mediated by the hydrophobic cavity of **1** was shown by analysis of aqueous solutions of AdCA and (2-naphthyl)methyl methyl ether where the above shifts did not occur, even after addition of β-cyclodextrin (see Supporting Information). The broadening of spectrum 2E at 25 °C is consistent with dynamic effects due to restricted motion and slow association–dissociation equilibria. The spectrum shows HA and HB shifted closer together with HA moving from 1.92 ppm to 1.60 ppm and HB moving from 1.75 to 1.55 ppm. These shifts are consistent with structures such as those shown in Scheme 1 with AdCA binding from the primary hydroxyl side and with the HC_2 protons pushing the naphthyl group partially outside of the cyclodextrin cavity (i.e., AdCA·**1**). Signals corresponding to HC_2 should come in closest contact with the naphthyl group, and they do shift the most (ca. 0.55 ppm). Signals corresponding to HB are on average farthest from the naphthyl group and shift by only 0.2 ppm, while signals corresponding to HA and HC_1 shift together by an intermediate value of 0.33 ppm. Heating the sample to

(18) Schneider, H.-J.; Blatter, T.; Simova, S. *J. Am. Chem. Soc.* **1991**, *113,* 1996–2000.

50 and 80 °C (Figure 3F,G) results in spectral changes consistent with partial displacement of the AdCA and naphthyl groups outside the cavity. Finally, in agreement with observations in the naphthyl region (Figure 1), the spectrum obtained after the complex is allowed to cool down to 25 °C (spectrum H) has sharper signals than those obtained upon initial mixing at the same temperature (Figure 2E).

Conclusions

The ^{1}H NMR spectra of Figures 1 and 2 reflect the structures and interactions between the covalently modified cyclodextrin and the external hydrophobic guest. Spectral shifts suggest that complexation of AdCA occurs via the primary hydroxyl side (6-OH). This is in agreement with known X-ray structural data of the inclusion compound of AdCA and β-cyclodextrin and with the fact that the (2-naphthyl)methyl ether is expected to hinder the secondary hydroxyl side where it is attached to one of the 3-OH positions of **1**. Changes in the aromatic and aliphatic regions are consistent with structures involving a partial coinclusion of the naphthyl and adamantyl groups at low temperatures and with structures involving capping of included AdCA by the naphthyl substituent. Further studies involving different fluorescent labels and photophysical measurements are currently in progress.

Experimental Section

General. All solvents used were of the highest purity commercially available and were used as received. β-Cyclodextrin was generously donated by American Maize Products and was used as received. Adamantanecarboxylic acid was purchased from Aldrich and used as received. 3−*O*-(2-Methylnaphthyl)-β-cyclodextrin **(1)** was prepared as described in the literature.[10,15] (2-Naphthyl)methyl methyl ether was prepared from 2-naphthaldeyde (Aldrich) by standard procedures. Inclusion complexes for ^{1}H NMR studies were prepared by dissolving known amounts of guest and host in D_2O. Separations and purifications were carried out in a Waters 600E HPLC with a photodiode array detector and a C18 m Bondapak column of dimensions 3.9 mm × 300 mm.

Variable Temperature NMR. ^{1}H NMR spectra were obtained in a Bruker 500 MHz ARX NMR instrument with a 5 mm inverse broad band probe. Measurements were taken after spinning the sample in the probe for at least 10 min at each selected temperature. The instrument was only shimmed at 298 K, but the probe was tuned at every temperature before spectra were taken. Thirty-two scans with a 30 deg excitation pulse were accumulated at each temperature. Chemical shifts were measured relative to external acetic anhydride (δ = 3.08 ppm). Residual protons in D_2O shifted from 4.70 ppm at 298 K to 4.40 ppm at 323 K and to 4.10 ppm at 353 K. The host to guest ratio was confirmed by integration of the anomeric hydrogens relative to the signals of the guest.

Acknowledgment. We acknowledge support by the National Science Foundation (CHE-9320619) and the NIH for a Chemistry and Biochemistry Interface Training Grant to S.R.M. (NIH-GM08496). We also thank Dr. J. M. Strouse for help with NMR measurements.

Supporting Information Available: ^{1}H NMR spectra of AdCA and (2-naphthyl)methy methyl ether and of AdCA, (2-naphthyl)methy methyl ether, and β-cyclodextrin, and complete spectra corresponding to Figures 1 and 2 (4 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO9613794