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Novel Behavior of *O*-Methylated *β*-Cyclodextrins in Inclusion of *meso*-Tetraarylporphyrins

Koji Kano,* Ryuhei Nishiyabu, and Ryoji Doi

Department of Molecular Science and Technology, Doshisha University, Kyotanabe, Kyoto 610-0321, Japan

kkano@mail.doshisha.ac.jp

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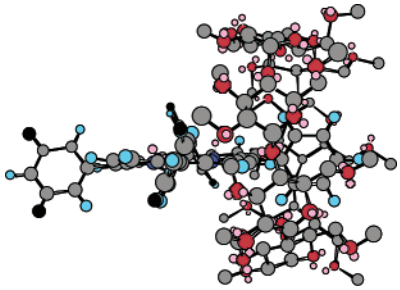

The mechanism for formation of extremely stable 1:2 inclusion complexes of water-soluble *meso*-tetraarylporphyrins with heptakis(2,3,6-tri-*O*-methyl)-*β*-cyclodextrin (TMe-*β*-CD) in aqueous solutions has been studied by means of NMR spectroscopy and isothermal titration calorimetry. To simplify the system, 5,10,15-tris(3,5-dicarboxylatophenyl)-20-phenylporphyrin (**1**) was used as a guest porphyrin, because **1** forms only a 1:1 inclusion complex with cyclodextrin (CD). As host compounds, native *β*-CD and the *O*-methylated-*β*-CDs such as heptakis(2,3-di-*O*-methyl)- (2,3-DMe-*β*-CD), heptakis(2,6-di-*O*-methyl)- (2,6-DMe-*β*-CD), and TMe-*β*-CDs were used. The thermodynamic parameters for complexation such as binding constants (K) and enthalpy ($\Delta H°$) and entoropy changes ($\Delta S°$) were determined by means of isothermal titration calorimetry. The K value for complexation of **1** with CD increases in the order *β*-CD ($K = (1.2 \pm 0.1) \times 10^3\ M^{-1}$) < 2,6-DMe-*β*-CD ($(1.2 \pm 0.1) \times 10^4\ M^{-1}$) ≪ TMe-*β*-CD ($(6.9 \pm 0.4) \times 10^6\ M^{-1}$) < 2,3-DMe-*β*-CD ($(8.5 \pm 0.5) \times 10^6\ M^{-1}$), indicating participation of the secondary OCH_3 groups in extremely strong complexation of **1** with CD. Complex formation of **1** with *β*-CD and 2,6-DMe-*β*-CD is an enthalpically and entropically favorable process, while that with TMe-*β*-CD and 2,3-DMe-*β*-CD is an enthalpically much more favorable but an entropically less favorable process. The thermodynamic parameters suggest that inclusion of **1** into the cavities of TMe-*β*-CD and 2,3-DMe-*β*-CD is promoted by van der Waals interactions, which are stronger than those in the cases of *β*-CD and 2,6-DMe-*β*-CD. ^{13}C NMR spectra show that the conformations of both TMe-*β*-CD and 2,3-DMe-*β*-CD are altered upon inclusion of **1**, while those of *β*-CD and 2,6-DMe-*β*-CD are mostly retained. On the basis of these results, it can be concluded that induced-fit type complexation of **1** with TMe-*β*-CD and 2,3-DMe-*β*-CD causes extremely strong binding of the host to the guest.

Introduction

Cyclodextrins (CDs) are building blocks for constructing supramolecular conjugates.[1] In many cases, however, binding constants (K) for complexation of cyclodextrins (CDs) with guests in aqueous solutions are less than several hundreds M^{-1},[2] while those for enzymes are mostly larger than $10^9\ M^{-1}$. Several attempts have been made to increase the ability of CDs to bind guests. For example, CDs having hydrophobic caps provide more hydrophobic CD cavities as compared with their mother CDs leading to formation of stable inclusion complexes.[3]

(1) (a) Easton, C. J.; Lincoln, S. F. *Modified Cyclodextrins*; Imperial College Press: London, UK, 1999. (b) Breslow, R.; Dong, S. D. *Chem. Rev.* **1998**, *98*, 1997−2011.

(2) Rekharsky, M. V.; Inoue, Y. *Chem. Rev.* **1998**, *98*, 1875−1917.

(3) (a) Tabushi, I.; Shimokawa, K.; Shimizu, N.; Shirakata, H.; Fujita, K. *J. Am. Chem. Soc.* **1976**, *98*, 7855−7856. (b) Ueno, A.; Yoshimura, H.; Saka, R.; Osa, T. *J. Am. Chem. Soc.* **1979**, *101*, 2779−2780. (c) Kuroda, Y.; Yamada, M.; Tabushi, I. *J. Chem. Soc., Perkin Trans. 2* **1989**, 1409−1415. (d) Engeldinger, E.; Armspach, D.; Matt, D. *Chem. Rev.* **2003**, *103*, 4147−4173.

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Utilization of Coulomb interactions is an effective method to stabilize inclusion complexes of charged guests with oppositely charged CD derivatives.[4] CD dimers where two CD moieties are bridged with linkers tend to form extremely stable sandwich-type inclusion complexes with $K > 10^{11}$ M^{-1}.[5] We[6] as well as Tonellato et al.[7] found an extremely strong ability of heptakis(2,3,6-tri-*O*-methyl)-β-CD (TMe-β-CD) to include phenyl substituents at the meso-positions of water-soluble tetraarylporphyrins affording trans-type 1:2 inclusion complexes. The binding constants ($K_1 \cdot K_2$) were estimated to be 1.6×10^{16} and 2.9×10^{13} M^{-2} for 5,10,15,20-tetrakis(4-carboxylatophenyl)porphyrin and 5,10,15,20-tetrakis(4-sulfonatophenyl)-porphyrin (TPPS), respectively.[7,8] It was impossible to determine individual K_1 and K_2 for complexation of TPPS with TMe-β-CD in aqueous solution because K values were too large. Then we determined the K_1 and K_2 values for the TPPS complex in 75% (v/v) ethylene glycol−25% (v/v) H_2O to be $(2.0 \pm 1.3) \times 10^4$ and $(5.8 \pm 1.5) \times 10^4$ M^{-1}, respectively.[9] The K values are still large even in the aqueous organic solvent. It should be very important to clarify the reason(s) for such a novel stability of the porphyrin−TMe-β-CD complexes to obtain basic information for designing new supramolecular systems with CDs. The present study deals with this subject. We noticed intramolecular hydrogen bonding at the secondary OH group sides of CDs as a factor that controls inclusion phenomena of the CDs. It has been well-known that the intramolecular hydrogen bonding between the OH groups at the 2-positions of the glucopyranoses and those at the 3-positions of the adjacent glucopyranoses stabilizes the symmetric structures of the cavities of the native CDs.[10] Meanwhile, no intramolecular hydrogen bonds were formed with TMe-β-CD leading to a flexible cavity of this CD.[11] We assumed the difference in the flexibility of the CD cavities as the origin of the difference in the stability of the porphyrin−CD complexes. As host CDs, we used heptakis(2,3-di-*O*-methyl)-β-CD (2,3-DMe-β-CD) and heptakis(2,6-di-*O*-methyl)-β-CD (2,6-DMe-β-CD) besides β-CD and TMe-β-CD (Chart 1). As a guest molecule, we chose 5,10,15-tris(3,5-dicarboxylatophenyl)-20-phenylporphyrin (**1**) because this water-soluble porphyrin forms only 1:1 complexes with CDs. NMR spectroscopy and isothermal titration calorimetry revealed the importance of induced-fit type inclusion in the formation of very stable complexes of TMe-β-CD and 2,3-DMe-β-CD.

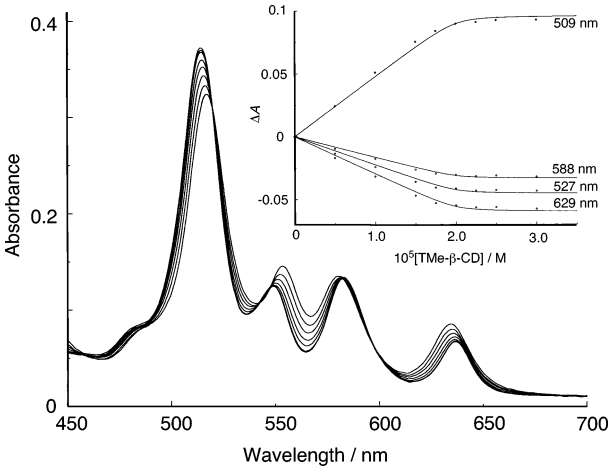
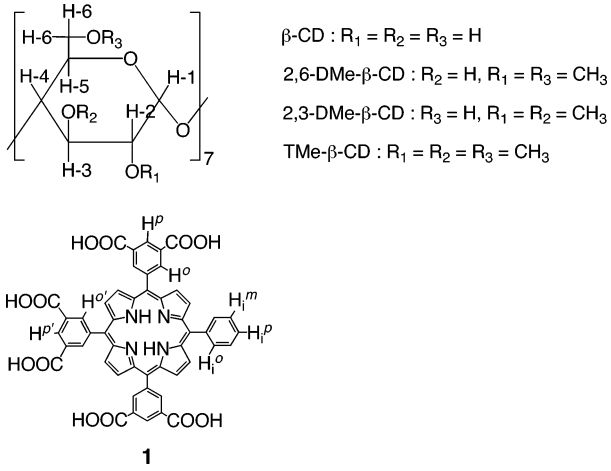


FIGURE 1. Absorption spectral changes of **1** (2.0×10^{-5} M) in 0.1 M phosphate buffer at pH 7.0 upon addition of TMe-β-CD [(0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.5, 5.0, 7.0) $\times 10^{-5}$ M] at 25 °C. The inset represents the changes in absorbances (ΔA) of **1** at various wavelengths upon addition of TMe-β-CD.

CHART 1. Structures of Hosts and a Guest



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Results and Discussion

Structures of Inclusion Complexes. Figure 1 shows the absorption spectral changes of **1** in phosphate buffer

(4) (a) Tabushi, I.; Shimizu, N.; Sugimoto, T.; Shiozuka, M.; Yamamura, K. *J. Am. Chem. Soc*. **1977**, *99*, 7100−7102. (b) Matsui, Y.; Okimoto, A. *Bull. Chem. Soc. Jpn*. **1978**, *51*, 1219−1223. (c) Tabushi, I.; Kuroda, Y.; Mizutani, T. *J. Am. Chem. Soc*. **1986**, *108*, 4514−4518. (d) Matsui, Y.; Ogawa, K.; Mikami, S.; Yoshimoto, M.; Mochida, K. *Bull. Chem. Soc. Jpn*. **1987**, *60*, 1219−1223. (e) Eliseev, A. V.; Schneider, H.-J. *J. Am. Chem. Soc*. **1994**, *116*, 6081−6088. (f) Kano, K.; Kitae, T.; Takashima, H.; Shimofuri, Y. *Chem. Lett*. **1997**, 899−900. (g) Kano, K.; Kitae, T.; Shimofuri, Y.; Tanaka, N.; Mineta, Y. *Chem. Eur. J*. **2000**, 2705−2713.

(5) (a) Tabushi, I.; Kuroda, Y.; Shimokawa, K. *J. Am. Chem. Soc*. **1979**, *101*, 1614−1615. (b) Fujita, K.; Ejima, S.; Imoto, T. *J. Chem. Soc., Chem. Commun*. **1984**, 1277−1278. (c) Fujita, K.; Ejima, S.; Imoto, T. *Chem. Lett*. **1985**, 11−12. (d) Breslow, R.; Greenspoon, N.; Guo, T.; Zarzycki, R. *J. Am. Chem. Soc*. **1989**, *111*, 8296−8297. (e) Coates, J. H.; Easton, C. J.; van Eyk, S. J.; Lincoln, S. F.; May, B. L.; Whalland, C. B.; Williams, M. L. *J. Chem. Soc., Perkin Trans. 1* **1990**, 2619−2620. (f) Breslow, R.; Chung, S. *J. Am. Chem. Soc*. **1990**, *112*, 9659−9660. (g) Breslow, R.; Halfon, S.; Zhang, B. *Tetrahedron* **1995**, *51*, 377−388.

(6) Kano, K.; Tanaka, N.; Minamizono, H.; Kawakita, Y. *Chem. Lett*. **1996**, 925−926.

(7) Carofiglio, T.; Fornasier, R.; Lucchini, V.; Rosso, C.; Tonellato, U. *Tetrahedron Lett*. **1996**, *37*, 8019−8022.

(8) Hamai, S.; Koshiyama, T. *J. Photochem. Photobiol. A*: *Chem*. **1999**, *127*, 135−141.

(9) Kano, K.; Nishiyabu, R.; Asada, T.; Kuroda, Y. *J. Am. Chem. Soc*. **2002**, *124*, 9937−9944.

(10) (a) Stezowski, J. J.; Jogun, K. H.; Eckle, E.; Bartels, K. *Nature* **1978**, *274*, 617−619. (b) Saenger, W. *Angew. Chem*. **1980**, *92*, 343−361. (c) Hamilton, J. A.; Sabesan, M. N.; Steinrauf, L. *Carbohydr. Res*. **1981**, *89*, 33−53. (d) Chacko, K. K.; Saenger, W. *J. Am. Chem. Soc*. **1981**, *103*, 1708−1715. (e) Tokuoka, R.; Fujiwara, T.; Tomita, K. *Acta Crystallogr., Sect. B* **1981**, *37*, 1158−1160 (f) Lindner, K.; Saenger, W. *Carbohydr. Res*. **1982**, *99*, 103−115. (g) Harata, K. *Bull. Chem. Soc. Jpn*. **1982**, *55*, 2315−2320. (h) Harata, K. *Chem. Lett*. **1984**, 641−644. (i) Steiner, T.; Mason, S. A.; Saenger, W. *J. Am. Chem. Soc*. **1990**, *112*, 6184−6190.

(11) (a) Harata, K.; Uekama, K.; Otagiri, M.; Hirayama, F. *Bull. Chem. Soc. Jpn*. **1983**, *56*, 1732−1736. (b) Harata, K.; Uekama, K.; Otagiri, M.; Hirayama, F. *J. Inclusion Phenom*. **1984**, *1*, 279−293. (c) Harata, K.; Uekama, K.; Imai, T.; Hirayama, F.; Otagiri, M. *J. Inclusion Phenom*. **1988**, *6*, 443−460. (d) Harata, K. *J. Chem. Soc., Chem. Commun*. **1988**, 928−629. (e) Harata, K.; Hirayama, F.; Arima, H.; Uekama, K.; Miyaji, T. *J. Chem. Soc., Perkin Trans. 2* **1992**, 1159−1166. (f) R. Caira, M.; Griffith, V. J.; Nassimbeni, L. R.; van Oudtshoorn, B. *J. Chem. Soc., Perkin Trans. 2* **1994**, 2071−2072.

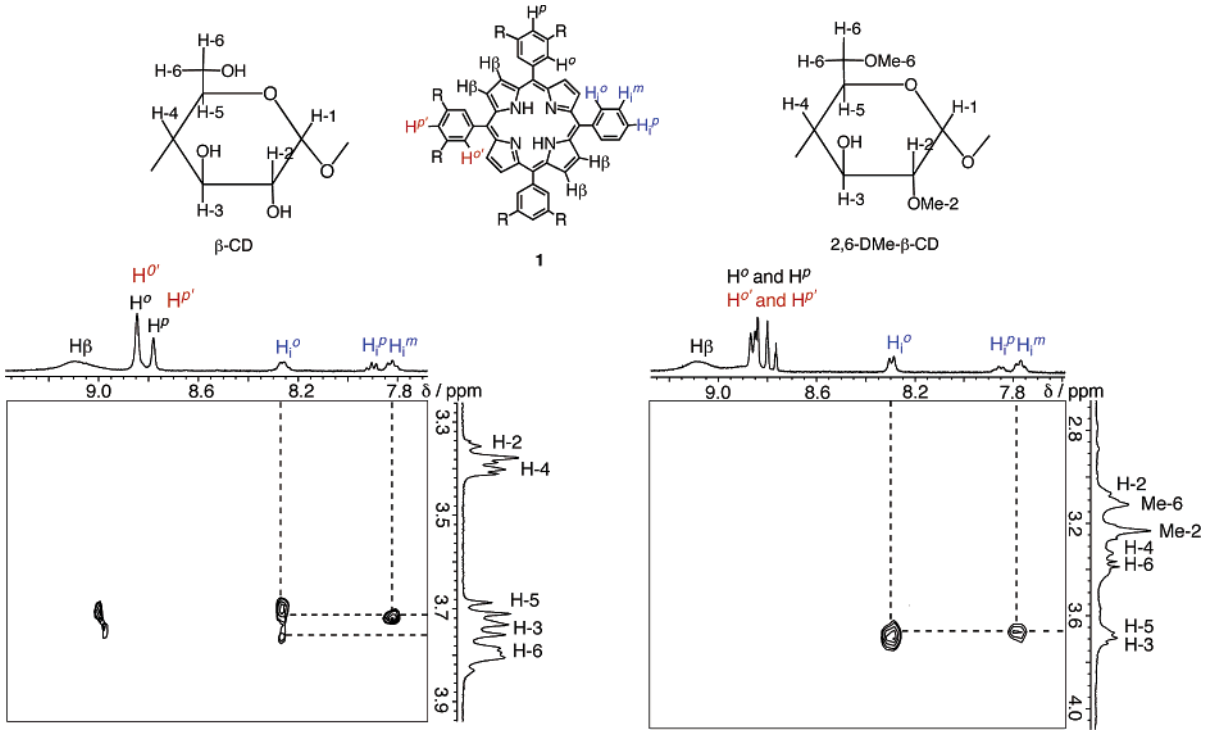

FIGURE 2. ROESY spectra of the **1**−*β*-CD and **1**−2,6-DMe-*β*-CD systems in 0.1 M phosphate buffer at pD 7.0 and 25 °C: [**1**] = 1×10^{-3} M; [*β*-CD] = 3×10^{-3} M, [**1**] = [2,6-DMe-*β*-CD] = 2×10^{-3} M. The mixing time of the ROESY measurements was 250 ms.

(0.1 M, pH 7.0) upon addition of TMe-*β*-CD. Such spectral changes showing several isosbestic points suggest the occurrence of a simple complexation process. As indicated in an inset of Figure 1, the spectral changes were sharply saturated after the addition of 1 equiv of TMe-*β*-CD, suggesting the formation of a stable 1:1 complex of **1** and TMe-*β*-CD. From a curve fitting using a nonlinear least-squires method,[9] the binding constant (*K*) was roughly estimated to be 1.1×10^7 M^{-1}. Essentially the same spectral changes were observed with the **1**−2,3-DMe-*β*-CD system leading to 1.2×10^7 M^{-1} as *K* (Supporting Information). Meanwhile, the spectral changes of **1** were more insensitive toward addition of *β*-CD or 2,6-DMe-*β*-CD as compared with the cases of TMe-*β*-CD and 2,3-DMe-*β*-CD and no spectral saturation was observed upon addition of these CDs more than equivalent. The absorption spectral changes afforded the *K* values of 1.7×10^3 and 2.0×10^4 M^{-1} for the complexes of *β*-CD and 2,6-DMe-*β*-CD, respectively.

The 2D NMR technique was applied to determine the structures of the 1:1 complexes of **1** and the CDs. The ROESY spectra of the **1**−*β*-CD and **1**−2,6-DMe-*β*-CD systems are shown in Figure 2. In the **1**−*β*-CD system, strong cross-peaks between the H_i^o and H_i^m protons of **1** and the protons at the 5-positions (H-5) of *β*-CD and a cross-peak between H_i^o and H-3 of *β*-CD were observed. No cross-peak was detected between H_i^m and H-3. Such results clearly indicate that the phenyl moiety of **1** is incorporated into the cavity from the secondary OH group side of *β*-CD. The patterns in the correlation peaks for the **1**−2,6-DMe-*β*-CD system were essentially the same as those for the **1**−*β*-CD system.

The ROESY spectra of the **1**−2,3-DMe-*β*-CD and **1**−TMe-*β*-CD systems in D_2O are shown in Figure 3. The most remarkable difference in the ROESY spectra between the *β*-CD and 2,6-DMe-*β*-CD systems and the 2,3-DMe-*β*-CD and TMe-*β*-CD ones is the cross-peaks between the H_i^m protons of **1** and the H-6 protons of the CDs which were observed for the complexes of 2,3-DMe-*β*-CD and TMe-*β*-CD while no such cross-peaks were found for the *β*-CD and 2,6-DMe-*β*-CD complexes. Another point is the cross-peaks between the H^o protons of the dicarboxylatophenyl groups, which are not included into the CD cavities, and the secondary OCH_3 protons of 2,3-DMe-*β*-CD and TMe-*β*-CD. Such a correlation was not observed in the case of the 2,6-DMe-*β*-CD complex. In addition, the protons due to the OCH_3 groups at the 3-positions of 2,3-DMe-*β*-CD and TMe-*β*-CD strongly correlate with the H^p protons of the carboxylatophenyl groups, which are located outside the CD cavities. These results clearly indicate the deeper penetration of the phenyl group of **1** into the cavities of 2,3-DMe-*β*-CD and TMe-*β*-CD as compared with the cases of *β*-CD and 2,6-DMe-*β*-CD. A schematic representation explaining the result of the ROESY spectrum of the **1**−TMe-*β*-CD complex is shown in Figure 4.

Binding Constants and Thermodynamics. The thermodynamic parameters are essentially important for discussing the mechanism of host−guest complexation. Then we applied isothermal titration calorimetry as the most reliable method for determining *K* as well as enthalpy ($\Delta H°$) and entropy changes ($\Delta S°$) for complexation.[2] The calorimetric titration curves for complexation of **1** with 2,6-DMe-*β*-CD and 2,3-DMe-*β*-CD are shown in Figure 5. The patterns of these titration curves reveal that the **1**−2,3-DMe-*β*-CD complex showing a sigmoid titration curve is much more stable than the **1**−2,6-DMe-*β*-CD complex. The titration curves for the *β*-CD and

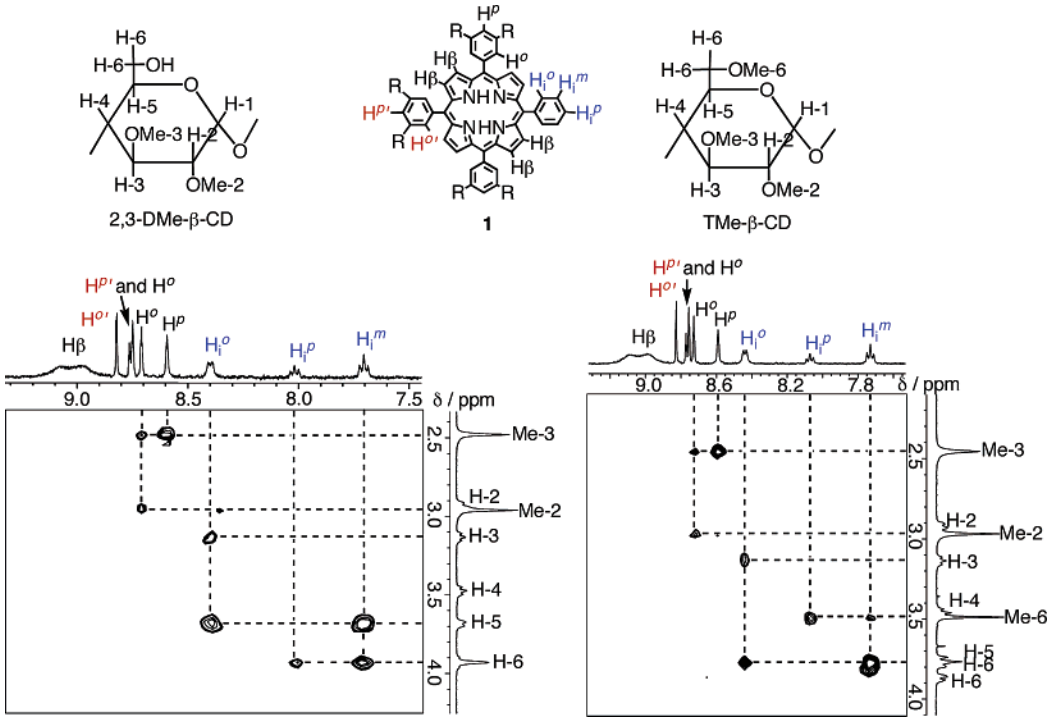

FIGURE 3. ROESY spectra of the **1**−2,3-DMe-β-CD and **1**−TMe-β-CD systems in 0.1 M phosphate buffer at pD 7.0 and 25 °C: [**1**] = [CDs] = 1×10^{-3} M. The mixing time of the ROESY measurements was 250 ms.

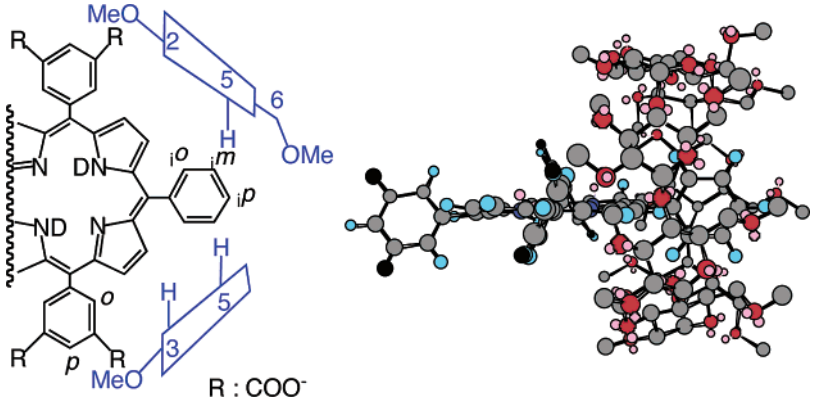

FIGURE 4. Schematic representation of the **1**−TMe-β-CD complex explaining the result of the ROESY spectrum. In the molecular model (right-hand side), all hydrogen atoms of TMe-β-CD are omitted for clarity and the CO_2^- groups of **1** are indicated by solid black circles.

TMe-β-CD systems were quite similar to those for the 2,6-DMe-β-CD and 2,3-DMe-β-CD ones, respectively (Supporting Information). From the analysis of the titration curves, the thermodynamic parameters were determined and the results are summarized in Table 1. On the basis of the thermodynamic data, the β-CD derivatives can be classified into two groups: namely the β-CD and 2,6-DMe-β-CD group in which the intramolecular hydrogen-bonding belt is formed at the secondary OH group side and the 2,3-DMe-β-CD and TMe-β-CD group where no hydrogen-bonding belt is formed. The former group shows fairly large K values while the later group forms extremely stable inclusion complexes with **1**. The K value for the **1**−2,3-DMe-β-CD complex is 700 times larger than that for the **1**−2,6-DMe-β-CD complex. The thermodynamic characteristics of the former group are negative and small enthalpy changes ($\Delta H°$) and positive and small entropy changes ($\Delta S°$). In the later group, however, the extremely strong complexation is promoted by the negative and quite large $\Delta H°$ accompanied by the somewhat unfavorable $\Delta S°$. The difference in the stability of the complexes of **1** between these two groups is ascribed to the difference in the $\Delta H°$ values, suggesting that van der Waals interactions between the host and the guest in the later group are much stronger than those in the former group.

The marked effect of *O*-methylation of CD on complex formation with **1** was also observed in the cases of γ-CD and TMe-γ-CD. The K value for TMe-γ-CD was much larger than that for γ-CD (Table 1). In complexation of **1** with γ-CD and TMe-γ-CD, however, the contribution of $\Delta H°$ and $\Delta S°$ to the complex stability is opposite to the cases of the β-CD derivatives. Namely, complexation of native γ-CD is enthalpically more favorable but entropically less favorable, while that of TMe-γ-CD is enthalpically less favorable and entropically more favorable.

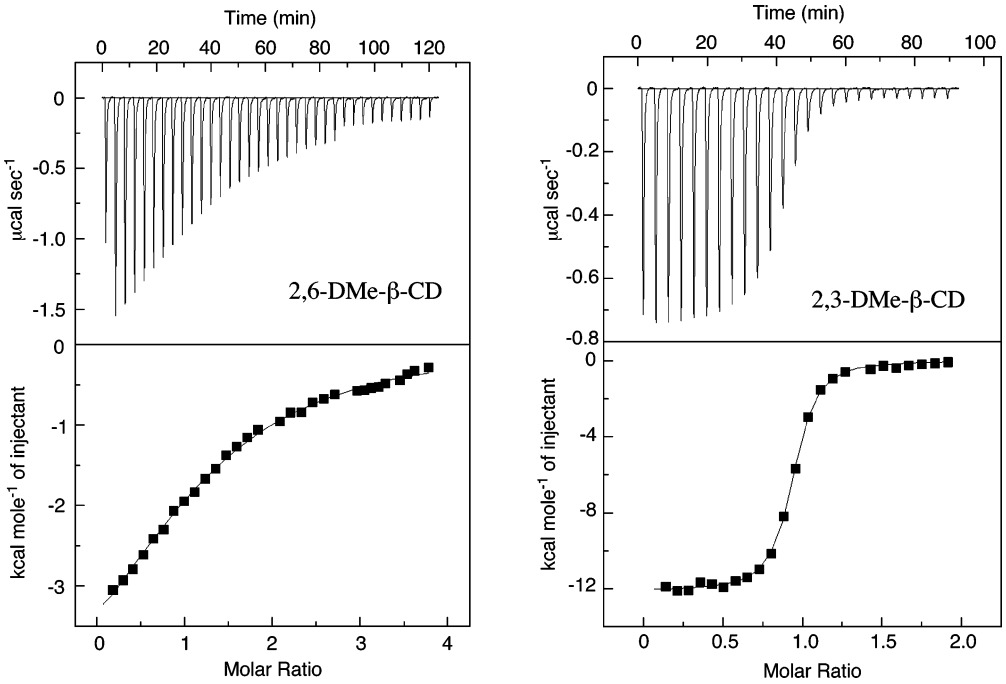

FIGURE 5. Calorimetric titrations of **1** (1×10^{-4} M for 2,6-DMe-β-CD and 2×10^{-5} M for 2,3-DMe-β-CD) with 25 aliquots (10 μL each) of 2,6-DMe-β-CD (2×10^{-3} M, left) and 2,3-DMe-β-CD (2×10^{-4} M, right) in 0.1 M phosphate buffer solutions at pH 7.0 and 25 °C. The top panels show the raw data, denoting the amount of generated heat after each injection of the CD. The bottom panels show the plots of the amount of heat generated per injection as a function of the molar ratio of **1** to CD.

TABLE 1. Thermodynamic Parameters for Complexation of 1 with Various CDs in 0.1 M Phosphate Buffer at pH 7.0 and 25 °C

host	K/M^{-1}	$\Delta H°$/kJ mol^{-1}	$T\Delta S°$/kJ mol^{-1}
α-CD	33 ± 4	-53 ± 6	-44 ± 6
TMe-α-CD	nd[a]	nd[a]	nd[a]
β-CD	$(1.2 \pm 0.1) \times 10^3$	-13 ± 0.5	4.3 ± 0.6
2,6-DMe-β-CD	$(1.2 \pm 0.1) \times 10^4$	-23 ± 1	0.5 ± 1.2
2,3-DMe-β-CD	$(8.5 \pm 0.5) \times 10^6$	-51 ± 0.5	-11 ± 0.5
TMe-β-CD	$(6.9 \pm 0.4) \times 10^6$	-59 ± 0.5	-20 ± 0.5
γ-CD	$(6.4 \pm 0.3) \times 10^2$	-48 ± 1	-32 ± 1
TMe-γ-CD	$(2.9 \pm 0.1) \times 10^4$	-24 ± 0.5	1.7 ± 0.5

[a] Not determined.

Relative instability of the γ-CD complex is ascribed to the negative and large $\Delta S°$. Although no evidence has been obtained, intermolecular hydrogen bonding between the secondary OH groups of γ-CD and the CO_2^- groups of the carboxylatophenyl groups at the 5- and 15-positions of **1** might be a candidate that explains the large and negative $\Delta H°$ and $\Delta S°$ in the γ-CD system. In general, intermolecular hydrogen bonding hardly occurs in aqueous solution because of strong hydration to the hydrogen-bonding sites. The hydrogen bond, however, might be formed if there is a proximity effect between a hydrogen-bond donor and an acceptor due to complex formation.[12] The cavities of α-CD and TMe-α-CD are so small that inclusion of the phenyl group of **1** hardly occurs with these host CDs.

Complexation-Induced Conformational Changes of CDs. It has been known that the intramolecular hydrogen bonds between the OH groups at the 2-positions of the glucopyranoses and those at the 3-positions of the adjacent glucopyranoses stabilize the cyclic structures of native CDs.[10] In the case of 2,6-DMe-β-CD, the intramolecular hydrogen bonds between the ethereal oxygen atoms at the 2-positions and the OH groups at the 3-positions, OH···OCH_3, also provide a rigid cavity of this CD.[13] Meanwhile, no hydrogen bonds are formed in the case of TMe-β-CD leading to a flexible structure of this per-*O*-alkylated CD.[11] Although no X-ray structure has been reported with 2,3-DMe-β-CD, the flexible nature is also expected because no hydrogen bonding occurs at the secondary OCH_3 group side. The flexible nature of TMe-β-CD causes induced-fit type inclusion of guests.[11] Conformational change of a CD upon complexation can be followed by means of ^{13}C NMR spectroscopy. If the conformational change occurs upon complexation, the NMR signals due to the ^{13}C atoms at the 1- (C-1) and 4-positions (C-4) of the CD shift to lower magnetic fields more remarkably than other ^{13}C NMR signals.[14] In this study, the shift in the chemical shift upon complexation ($\Delta\delta$) is defined as

$$\Delta\delta = \delta_c - \delta_f \quad (1)$$

where δ_c and δ_f are the observed chemical shifts of the ^{13}C NMR signals of the CD in the presence and the absence of a guest. To cancel the effect of the extent of complexation, saturated complexation induced shifts

(12) (a) Miyake, K.; Yasuda, S.; Harada, A.; Sumaoka, J.; Komiyama, M.; Shigekawa, H. *J. Am. Chem. Soc*. **2003**, *125*, 5080−5085. (b) Kano, K.; Kobayashi, S. *Bull. Chem. Soc. Jpn*. **2003**, *76*, 2027−2034.

(13) (a) Czugler, M.; Eckle, E.; Stezowski, J. J. *J. Chem. Soc., Chem. Commun*. **1981**, 1291−1292. (b) Harata, K.; Hirayama, F.; Uekama, K.; Tsoucaris, G. *Chem. Lett*. **1988**, 1585−1588. (c) Harata, K. *J. Chem. Soc., Chem. Commun*. **1993**, 546−547. (d) Steiner, T.; Saenger, W. *Carbohydr. Res*. **1995**, *275*, 73−82.

(14) (a) Inoue, Y. *Annu. Rep. NMR Spectrosc*. **1993**, *27*, 60−101. (b) Botsi, A.; Yannakopoulou, K.; Perly, B.; Hadjoudis, E. *J. Org. Chem*. **1995**, *60*, 4017−4023.

TABLE 2. ^{13}C NMR Chemical Shifts (ppm) of the CDs in 0.1 M Phosphate Buffer at pD 7.0 and 25 °C

CD	C-1	C-2	C-3	C-4	C-5	C-6	2-CH_3	3-CH_3	6-CH_3
β-CD	104.68	74.89	75.89	83.95	74.64	63.11	-		
2,6-DMe-β-CD	101.88	84.10	74.82	84.16	72.84	73.40	62.03		61.23
2,3-DMe-β-CD	99.93	82.83	83.78	79.76	74.45	63.37	60.93	62.56	
TMe-β-CD	99.67	82.71	83.61	79.65	73.03	73.46	60.74	62.38	61.04

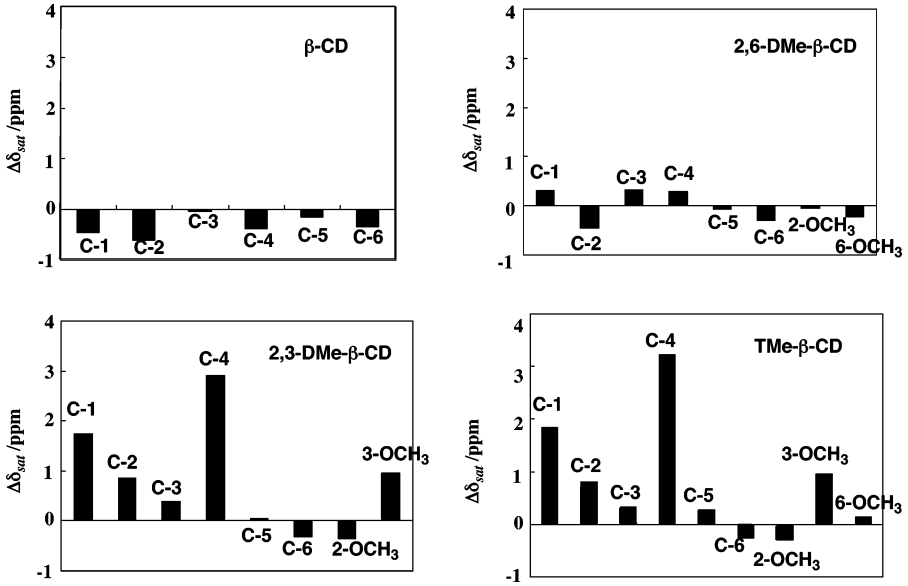

FIGURE 6. Saturated complexation-induced shifts ($\Delta\delta_{sat}$) of the ^{13}C NMR signals due to the CDs complexed with **1** in 0.1 M phosphate buffer at pD 7.0 and 25 °C.

($\Delta\delta_{sat}$) obtained from the following equation should be applied:[15]

$$\Delta\delta_{sat} = (\delta_c - \delta_f)/\chi \quad (2)$$

where χ is the mole fraction of the complex under certain conditions which can be calculated from the K value. The $\Delta\delta_{sat}$ value means the shift in a chemical shift when all guest molecules are complexed with the CD. Equation 2 was applied for the **1**−β-CD and **1**−2,6-DMe-β-CD systems. Meanwhile, since the ^{13}C NMR signals of 2,3-DMe-β-CD and TMe-β-CD complexed with **1** appeared at magnetic fields different from those of the free CDs, $\Delta\delta_{sat}$ for these systems could be obtained directly from eq 1. The ^{13}C NMR chemical shifts of each CD were assigned by C−H COSY and COLOC spectra in D_2O and are shown in Table 2. The $\Delta\delta_{sat}$ values for each CD complexed with **1** are shown in Figure 6. The marked downfield shifts of the ^{13}C NMR signals for the carbons at the 1- and 4-positions of 2,3-DMe-β-CD and TMe-β-CD were clearly observed upon complexation with **1**, while the shifts of ^{13}C NMR signals of β-CD and 2,6-DMe-β-CD were quite small. The remarkable difference between two groups of the CD derivatives is interpreted in terms of that the induced-fit type complexation occurs in the cases of 2,3-DMe-β-CD and TMe-β-CD leading to optimize the van der Waals contacts between the host and the guest, although the cyclic structures of the β-CD and 2,6-DMe-β-CD cavities are hardly changed upon complexation.

Conclusion

The aim of the present study is clarification of the reasons for forming extraordinarily stable inclusion complexes of water-soluble tetraarylporphyrins and TMe-β-CD. The K value for the **1**−TMe-β-CD (6.9×10^6 M^{-1}) is 5800-times larger than that for the **1**−β-CD complex (1.2×10^3 M^{-1}). Such a big difference is caused by the induced-fit type complexation of TMe-β-CD with **1** where TMe-β-CD alters its structure to optimize the van der Waals contacts between the host and the guest, although the structure of β-CD is scarcely altered upon complexation because of the intramolecular hydrogen-bonding belt at the secondary OH group side of β-CD. Induced-fit type complexation of per-*O*-methylated CDs has been discussed in the past two decades.[16] However, there has been no example of complex formation of TMe-β-CD, which shows the K value higher than 10^6 M^{-1} except for the porphyrin−TMe-β-CD systems. The present study revealed the role of the hydrophobic, planar, and large porphyrin framework that participates in maximization of van der Waals contacts with TMe-β-CD or 2,3-DMe-β-CD.

Interaction of a tetraarylporphyrins and CD in aqueous solution was reported first by Manka and Lawrence.[17]

(15) Kano, K.; Hasegawa, H. *J. Am. Chem. Soc*. **2001**, *123*, 10616−10627.

(16) (a) Harata, K.; Uekama, K.; Otagiri, M.; Hirayama, F. *J. Inclusion Phenom.* **1984**, *2*, 583−594. (b) Harata, K.; Uekama, K.; Otagiri, M.; Hirayama, F. *Bull. Chem. Soc. Jpn*. **1987**, *60*, 497−502. (c) Harata, K.; Tsuda, K.; Uekama, K.; Otagiri, M.; Hirayama, F. *J. Inclusion Phenom.* **1988**, *6*, 135−142. (d) Kano, K.; Ishimura, T.; Negi, S. *J. Inclusion Phenom. Mol. Recognit. Chem*. **1995**, *22*, 285−298. (e) Rontoyianni, A.; Mavridis, I. *J. Inclusion Phenom. Mol. Recognit. Chem*. **1998**, *32*, 415−428. (f) Cardinael, P.; Peulon, V.; Perez, G.; Coquerel, G.; Toupet, L. *J. Inclusion Phenom. Macrocyclic Chem*. **2001**, *39*, 159−167.

(17) (a) Manka, J. S.; Lawrence, D. S. *J. Am. Chem. Soc*. **1990**, *112*, 2440−2442. (b) Dick, D. L.; Rao, T. V. S.; Sukumaran, D.; Lawrence, D. S. *J. Am. Chem. Soc*. **1992**, *114*, 2664−2669.

They used a cationic tetraarylporphyrin and 2,6-DMe-β-CD as the guest and the host, respectively, and proposed the proper structure of the complex. Later, Ribó et al. reported the structure of the TPPS-β-CD complex.[18] We found that TPPS forms the 1:2 inclusion complex with TMe-β-CD whose stability is much higher than that of the TPPS-β-CD and TPPS-2,6-DMe-β-CD complexes.[6,9] The present study revealed the reasons why the TMe-β-CD complex is much more stable than the β-CD and 2,6-DMe-β-CD ones as mentioned above. The pK_a value of TPPS in aqueous solution is 5.4, which is reduced to 0.4 upon complexation with TMe-β-CD.[6] This means that two TMe-β-CD molecules include the sulfonatophenyl groups deeply affording a very hydrophobic environment around the porphyrin center. Such novel inclusion behavior of TMe-β-CD can be utilized to prepare a met-myoglobin model, which shows selective anion binding to Fe(III)-TPPS in aqueous solution,[19] and a myoglobin model, which binds dioxygen in aqueous solution.[20] Hemoproteins such as myoglobin (hemoglobin) and cytochrome *c* bind iron porphyrin near the surfaces of the proteins. Such structures might be important to contact with dioxygen or to transport an electron to another protein as well as to exclude water molecules from the porphyrin centers. Metalloporphyrin−TMe-β-CD systems are expected to be the simplest hemoprotein models which work in aqueous solution. The present study might inform us about basic aspect that serves the purpose of designing a new metalloprotein model.

Experimental Section

Chemicals. 1 was prepared previously in our laboratory.[21] All *O*-methylated CDs except for TMe-β-CD were prepared according to the procedures described in a literature.[22] Crude DMe-β-CDs were purified repeatedly by silica gel column chromatography with chloroform−methanol until no peaks of impurities were detected by means of FAB MS spectroscopy. β-CD, TMe-β-CD (Nacalai), and other materials were purchased and used without further purification.

Measurements. UV−vis spectra were recorded on a Shimadzu UV-2100 spectrophotometer having a thermostated cell holder. ^{1}H and ^{13}C NMR spectra were taken on a JEOL JNM-A400 spectrometer (400 MHz) in D_2O (CEA, 99.9%) with sodium 3-trimethyl[2,2,3,3-$^2H_4$]propionate (TSP, Aldrich) as an external standard. All signals were identified by measuring H−H and C−H COSY, ROESY, and COLOC spectra. Microcalorimetric measurements were carried out with a MicroCal Isothermal Titration Calorimeter VP-ITC. The cell of the calorimeter was filled with a phosphate buffer solution (0.1 M, pH 7.0) of **1** that was added by 25 aliquots (10 μL each) to a CD solution made by the same buffer at 298.15 K. The data were subsequently analyzed with use of the ORIGIN software program for a 1:1 complex formation.

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Supporting Information Available: Absorption spectral changes of **1** upon addition of the CDs, ^{1}H NMR spectra of β-CD, 2,6-DMe-β-CD, 2,3-DMe-β-CD, and TMe-β-CD in D_2O containing various amounts of **1**, and calorimetric titrations for the **1**-β-CD and **1**-TMe-β-CD systems. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) Ribó, J. M.; Farrera, J.-A.; Valero, H. L.; Virgili, A. *Tetrahedron* **1995**, *51*, 3705−3712.
(19) Kano, K.; Kitagishi, H.; Tamura, S.; Yamada, A. *J. Am. Chem. Soc*. **2004**, *126*, 15202−15210.
(20) Kano, K.; Kitagishi, H.; Kodera, M.; Hirota, S. *Angew. Chem., Int. Ed*. **2005**, *44*, 435−438.
(21) Kano, K.; Nishiyabu, R.; Yamazaki, T.; Yamazaki, I. *J. Am. Chem. Soc*. **2003**, *125*, 10625−10634.
(22) Takeo, K.; Mitoh, H.; Uemura, K. *Carbohydr. Res*. **1989**, *187*, 203−221.