Complexation of α-Cyclodextrin with Carborane Derivatives in Aqueous Solution

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Although the complexation of carborane derivatives with β-cyclodextrin (β-CD) is well-known, we present here the first observation of the complexation of carborane derivatives with α-CD in aqueous solution. The stoichiometry and association constant (K_a) of the complexes were estimated from Job's plots and NMR titration studies, respectively. The carborane : α-CD stoichiometry was 1 : 1 in all cases. The complexation ability and selectivity of the carborane derivatives for α-CD are markedly decreased compared with those for β-CD. The interaction between the carborane cage and the hydrophobic cavity of α-CD appears to be weak, probably because the hydrophobic cavity of α-CD is too small to accommodate the carborane cage. The C–H hydrogen and the polar substituents of carborane cage may form hydrogen bonds with secondary alcohol groups at the rim of α-CD. The orientation of the carborane cage influences the inclusion process, and *o*- and *m*-carborane derivatives showed moderately stronger association constants than *p*-carborane derivatives.

Key words carborane; α-cyclodextrin; association constant; NMR titration; host–guest complex

Icosahedral carboranes (dicarba-*closo*-dodecaboranes) have unique structural and chemical properties, such as high boron content, spherical structure, three isomers (*ortho*, *meta* and *para*), a highly hydrophobic surface, and unexpected thermal and chemical stability (Fig. 1).[1,2] Biomedical applications of the carboranes, *e.g.*, in boron neutron capture therapy (BNCT), are of great interest.[3—5] We have reported applications of carboranes in medicinal drug design as a hydrophobic component of biologically active molecules,[6] targeting nuclear receptors, such as estrogen receptor (ER),[7,8] androgen receptor (AR),[9,10] retinoic acid receptor (RAR)[11,12] and retinoid X receptor (RXR).[13] It was suggested that the carborane cage works as a hydrophobic group for binding to the hydrophobic cavity of the ligand-binding domain (LBD) on the nuclear receptors, and that hydrophobic van der Waals contacts along the spherical carborane cage produce a stronger interaction than that in the case of the native ligand. In the development of carborane-containing estrogen ligands, we have investigated the hydrophobicity of carboranes by measuring the partition coefficients, log *P* values, and the hydrophobicity parameter π.[14,15]

Cyclodextrins (CDs) are widely used as host molecules in a number of areas of chemistry where molecular recognition is required, such as material sciences, supramolecular chemistry, molecular-sensing, and artificial enzymes (Fig. 1). Moreover, CDs are of great utility in the field of medicinal chemistry as solubilizing agents for lipophilic drugs,[16—18] as photostabilizers of light-sensitive drugs,[19—21] and as sustained-release[16—18] and drug delivery systems[22—24] for various drugs.

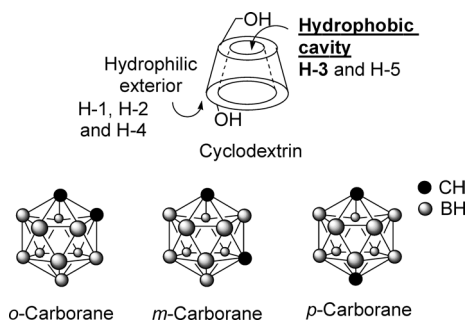

Fig. 1. The Structures of Cyclodextrin and Carboranes

Complexes of α-, β-, and γ-CD with unsubstituted *o*-carborane (**1a**) were isolated, and the stoichiometries of the complexes were determined by elemental analysis and from the ratios of the ^{1}H-NMR peaks.[25] However, the stoichiometric ratio, association constant and complex formation in solution have not been well characterized. Recently, Threadgill and co-workers have reported the formation of a remarkably robust 2 : 1 complex of β-CD with 1-phenyl-*o*-carborane.[26] An excellent fit of the carborane cage with β-CD was confirmed by a molecular modelling study. This work dealt only with the *o*-carborane isomer. The attractive properties of *m*- and *p*-carboranes[27] mean that complexation of these molecules with CDs is also of interest. It is noteworthy that β-CD-bonded chiral stationary phases have been used for enantiomeric resolution in the ten-vertex carborane series,[28,29] implying a strong interaction of ten-vertex carborane with β-CD.

However, the host–guest complexations of carboranes with CDs are not well understood. Therefore, we have focused on the interaction between the spherical, hydrophobic surface of carboranes and the hydrophobic pocket of CDs. Recently, we reported the complexation of carborane derivatives with β-CD, including association constant values and stoichiometry in aqueous solution.[30] The interior hydrophobic space of β-CD can accommodate the carborane cage, and the stoichiometry of carborane : β-CD is 1 : 1. The value of the association constant (K_a) was strongly dependent on the carborane isomer and the nature of its substituent(s). The complexation of carborane derivatives other than *o*-carborane with α-CD is yet to be examined and is of interest. Since the hydrophobic space of α-CD is smaller than that of β-CD, 1 : 1 and/or 2 : 1 α-CD : carborane complexes might be formed.

Here, we present the first report of the complexation of α-CD with *o*-, *m*- and *p*-carborane derivatives **1**—**3** in aqueous solution. The stoichiometry and association constant (K_a) of the complexes were estimated from Job's plots and by means of NMR titration, respectively.[31] Carborane derivatives ex-

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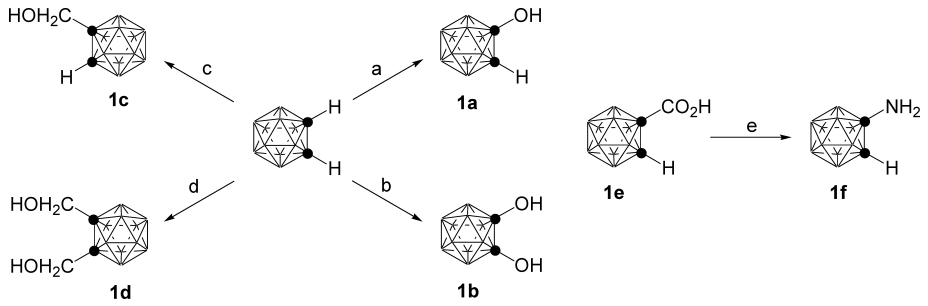

Reagents: a) 1. *n*-BuLi, $(MeO)_3B$, ether; 2. H_2O_2, AcOH; b) 1. *n*-BuLi (2 eq), $(MeO)_3B$ (2 eq), ether; 2. H_2O_2, AcOH; c) *n*-Bu_4NF, $(CHO)_n$, THF (for *o*-carborane) or *n*-BuLi, $(CHO)_n$, ether (for *m*- and *p*-carborane); d) 1. *n*-BuLi (2 eq), $(CHO)_n$, ether; e) 1. DPPA, Et_3N, DMAP, *t*-BuOH, Δ; 2. TFA, CH_2Cl_2.

Chart 1. Synthesis of *o*-Carborane Derivatives **1a**—**d** and **1f**

The *m*- and *p*-carborane derivatives, **2a**—**d**, **2f**, **3a**—**d** and **3f**, were similarly synthesized.

amined for complexation ability with α-CD were selected from among simple structures that have been used in our medicinal studies. The association constants of carborane derivatives for α-CD were compared with those for β-CD.

Synthesis of the carborane derivatives **1a**—**f**, **2a**—**f** and **3a**—**f** is summarized in Chart 1. *C*-Hydroxy-*o*-carborane **1a** and *C*,*C*′-dihydroxy-*o*-carborane **1b** were synthesized by boronation followed by oxidative rearrangement.[32,33] *C*-Hydroxymethyl-*o*-carborane **1c**[34,35] and *C*,*C*′-dihydroxymethyl-*o*-carborane **1d** were prepared by the reaction of *o*-carboranyl anion with paraformaldehyde. *C*-Hydroxycarbonyl-*o*-carborane **1e**, which is commercially available, was transformed into amine **1f** by Curtius rearrangement.[36] The *m*- and *p*-carborane derivatives **2a**—**d**,[32—35] **2f**,[36—39] **3a**—**d**[32—35] and **3f**[36—39] were synthesized in the same manner as the *o*-carborane derivatives.

The stoichiometric ratio of the complexation of α-CD and the carborane derivatives was estimated from Job's plots using the ^{1}H-NMR chemical shift changes of the H-3 proton positioned in the interior of α-CD as an indicator; this signal is very sensitive to the inclusion of guest compounds. Another interior proton, H-5, could not be employed for NMR study because the peak overlapped with those of other protons. Tetramethylsilane (TMS) could not be used as an internal standard, because it was expected to influence the process of complexation of carborane derivatives with α-CD, so trimethylsilyl sodium propionate (TSP) was used as an external standard for the measurements. The total concentration of α-CD and the carborane derivatives was kept constant at 3.0×10^{-3} M. The fitting curves obtained from Job's plots of carboxylic acid derivatives **1e**, **2e** and **3e** with α-CD each showed 1 : 1 stoichiometry in D_2O at 30 °C (Fig. 2). However, the stoichiometry for *C*-hydroxycarboranes **1a**, **2a** and **3a** and *C*,*C*′-dihydroxycarboranes **2b** and **3b** could not be estimated from Job's plots because the H-3 proton signal of α-CD was broadened when α-CD was mixed with these carborane derivatives in D_2O. Other carborane derivatives showed 1 : 1 stoichiometry for the complexation with α-CD.

The ^{1}H-NMR titration study was carried out at 30 °C in D_2O with a 400 MHz NMR spectrometer. TSP was used as an external standard for the measurements. The association constant (K_a) were estimated from plots of Δ(ppm) at H-3 *vs.* carborane concentration employing nonlinear least-squares method, according to Eq. 1.

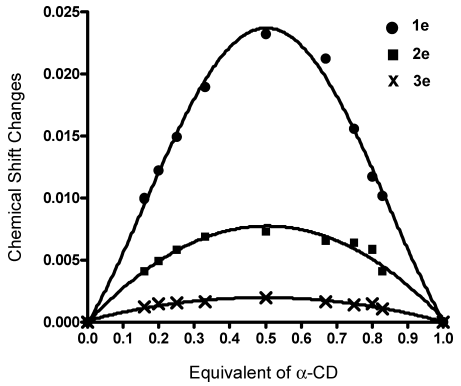

Fig. 2. Job's Plots for Carborane Carboxylic Acids **1e**, **2e** and **3e** with α-CD in D_2O at 30 °C

The total concentration of α-CD and carboxylic acid was kept constant at 3.0×10^{-3} M. The chemical shift changes were measured at the interior H-3 proton of α-CD and calculated by means of the formula [Δ (ppm)]×[α-CD]/([α-CD]+[carboxylic acid]). Equivalents of α-CD were estimated as the ratio of [α-CD]/([α-CD]+[carboxylic acid]).

$$\Delta\delta_{obs} = \frac{\Delta\delta}{2K_a[H]}\left[1 + K_a[H] + K_a[G] - \{(1 + K_a[H] + K_a[G])^2 - 4K_a^2[H][G]\}^{0.5}\right] \quad (1)$$

The nonlinear least-squares method was used for the estimation of association constant (K_a), based on the above equation. K_a: association constant; [*H*]: concentration of host compound; [*G*]: concentration of guest compound; $\Delta\delta_{obs}$: δ (chemical shift value after addition of guest compound)$-\delta_0$ (chemical shift value at the concentration of [*G*]=0); $\Delta\delta$: chemical shift value when a host : guest 1 : 1 complex is formed.

The titration curve of carboxylic acid derivatives **1e**, **2e** and **3e** with α-CD are shown in Fig. 3. There is no plateau area in any of the titration curves, indicating that strong complexation may not occur, because the carborane derivatives showed a low solubility in D_2O and the NMR measurement with high concentration was impossible under the condition.[31] Table 1 summarizes the association constants estimated from NMR titration studies of carborane derivatives with α-CD and β-CD in aqueous solution.

The titration curve arising from the complexation of amino-*p*-carborane **3f** with α-CD was complex and the association constant could not be estimated, in spite of the 1 : 1 stoichiometry in the Job's plot. The association constants of

Table 1. Association Constant K_a (M^{-1}) Values of Carborane Derivatives **1**—**3** with α-CD and β-CD in D_2O at 30 °C

Substituent	*o*-Carborane (**1**)		*m*-Carborane (**2**)		*p*-Carborane (**3**)	
	α-CD	β-CD	α-CD	β-CD	α-CD	β-CD
OH (**a**)	—[a]	$>10^6$	—[a]	41500	—[a]	9600
$(OH)_2$ (**b**)	970	6.0×10^5	—[a]	25300	—[a]	9800
CH_2OH (**c**)	320	8300	830	2400	30	54000
$(CH_2OH)_2$ (**d**)	620	n.t.[b]	1500	n.t.[b]	80	n.t.[b]
CO_2H (**e**)	500	37000	370	5600	220	4400
NH_2 (**f**)	2300	2500	1500	3900	—[c]	3800

a) NMR titration study could not be carried out because the H-3 proton signal of α-CD was broadened. *b*) Not tested. *c*) The titration curve was too complex to allow estimation of the association constant.

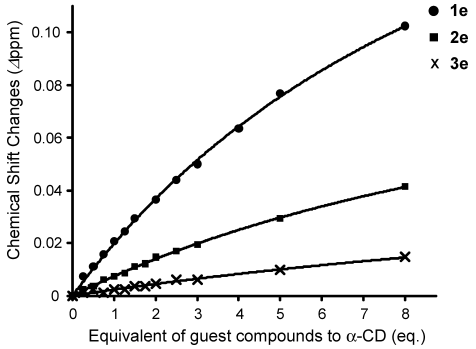

Fig. 3. NMR Titration of Carborane Carboxylic Acids, **1e**, **2e** and **3e**, with α-CD Based on the Chemical Shift Changes (Δ ppm) of the Interior C-3 Proton of α-CD

The titration study was performed in D_2O at 30 °C. [α-CD]=2×10^{-4} M. A squared multiple of correlation coefficient (R^2) of the compounds for the titration curves is 0.99, 0.99 and 0.98, respectively.

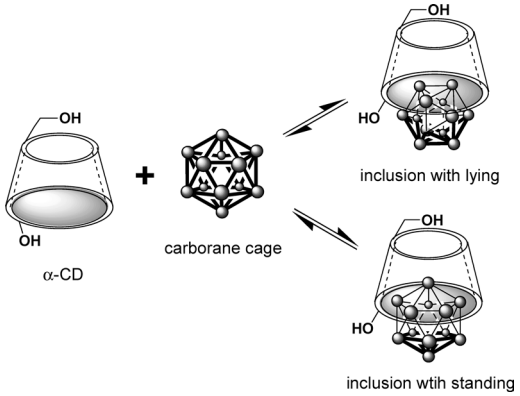

Fig. 4. Proposed Inclusion Modes Based on the Orientation of the Carborane Cage

carborane derivatives for α-CD were greatly decreased as compared with those for β-CD. This is considered to reflect a mismatch between the size of the carborane cage and that of the α-CD interior: most of the carborane cage would be excluded from α-CD. Singularly low associations of *p*-carborane derivatives with α-CD are considered to arise from the orientation of the carborane cage in the inclusion process. There are alternative inclusion modes, the lying type and standing type, based on the carborane cage orientation (Fig. 4). Since the hydrophobic α-CD interior is incompatible with inclusion of acidic carborane C–H hydrogen and polar substituents, *o*- and *m*-carborane derivatives would favor a lying orientation in the inclusion process. On the other hand, standing type orientation would be favored by *m*- and *p*-carborane derivatives, because the C–H hydrogen and *C*-substituents of carborane can be inserted into the center of the α-CD interior. The C–H hydrogen and *C*-substituents of *o*- and *m*-carborane derivatives would be able to form weak hydrogen bonds with secondary alcohol groups at the rim of α-CD,[40] and this may be the reason why *o*- and *m*-carborane derivatives showed stronger association constants with α-CD than *p*-carborane derivatives.

In conclusion, we found that the complexation of carborane derivatives with α-CD in aqueous solution is much weaker than that with β-CD. The major factor accounting for the low binding affinity is considered to be impaired hydrophobic interaction owing to dimensional misfit between the carborane cage and the α-CD core. The *o*- and *m*-carborane derivatives showed somewhat greater binding affinity than *p*-carborane derivatives, and this might reflect more effective hydrogen bond formation between the substituents of *o*- and *m*-carborane and the hydroxyl groups at the rim of α-CD.

Experimental

General Melting points were determined with a Yanaco micro melting point apparatus and were not corrected. ^{1}H and ^{13}C-NMR spectra were recorded with JEOL JNM-EX-270 spectrometer. Chemical shifts for ^{1}H-NMR spectra were referenced to tetramethylsilane (0.0 ppm) as an internal standard. Chemical shift for ^{13}C-NMR spectra were referenced to residual ^{13}C present in deuterated solvents. The splitting patterns are designated as follows: s (singlet), d (doublet), t (triplet) and m (multiplet). Mass spectra were recorded on a JEOL JMS-DX-303 spectrometer. Job's plot and NMR titration studies were carried out with JEOL JNM-LA-400 spectrometer. Chemical shifts for ^{1}H-NMR spectra in D_2O were referenced to trimethylsilyl sodium propionate (TSP; 0.0 ppm) as an external standard. The reagents and solvents were purchased from Aldrich Chemical Co., Kanto Chemicals, Tokyo Chemical Industries, or Wako Chemicals, Inc., and all isomers of dicarba-*closo*-dodecaboranes and *C*-hydroxycarbonyl carboranes **1e**, **2e** and **3e** were purchased from KATCHEM. Unless otherwise noted, they were used as received. Compounds **1a**—**c**, **1f**, **2a**—**c**, **2f**, **3a**—**c**, and **3f** were synthesized according to the literature procedures.

***C*,*C′*-Bis-hydroxymethyl-*o*-carborane (1d)** To a solution of *o*-carborane (150 mg, 1.04 mmol) in 5 ml of dry ether was added dropwise a solution of 2.56 M *n*-BuLi in hexane (0.9 ml, 2.28 mmol) at 0 °C under an Ar atmosphere. The mixture was stirred for 5 min at the same temperature. Solid paraformaldehyde (51 mg, 1.62 mmol) was added in one portion, and the whole was stirred for 30 min at room temperature. The reaction was quenched with 10% HCl aqueous solution and the mixture was extracted with AcOEt. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated. The residue was purified by means of column chromatography on silica gel with *n*-hexane : AcOEt 10 : 1 to give **1d** (193 mg, 91%) as a colorless solid. Colorless cubes (*n*-hexane-ether). mp 208—210 °C. ^{1}H-NMR (270 MHz, DMSO) δ (ppm): 0.8—3.50 (10H, br m), 4.00 (4H, d, J=6.8 Hz), 6.12 (2H, t, J=6.8 Hz). ^{13}C-NMR (67.8 MHz, DMSO) δ (ppm): 62.0, 81.5. MS (EI) *m*/*z*: 204 (M^+), 186 (100%); HR-MS

(EI) *m*/*z*: 204.2153 (Calcd for $C_4H_{16}B_{10}O_2$: 204.2156).

***C*,*C′*-Bis-hydroxymethyl-*m*-carborane (2d)** Compound **2d** was prepared by a similar method to that described for the synthesis of **1d**; 89% yield. Colorless cubes (*n*-hexane–ether). mp 155—156 °C. ^{1}H-NMR (270 MHz, DMSO) δ (ppm): 0.8—3.5 (10H, br m), 3.60 (4H, s), 5.81 (2H, br s). ^{13}C-NMR (67.8 MHz, DMSO) δ (ppm): 63.7, 78.7. MS (EI) *m*/*z*: 204 (M^+), 186 (100%). HR-MS (EI) *m*/*z*: 204.2153 (Calcd for $C_4H_{16}B_{10}O_2$: 204.2156).

***C*,*C′*-Bis-hydroxymethyl-*p*-carborane (3d)** Compound **3d** was prepared by a similar method to that described for the synthesis of **1d**; 94% yield. Colorless cubes (*n*-hexane–ether). mp 159—160 °C. ^{1}H-NMR (270 MHz, DMSO) δ (ppm): 0.8—3.5 (10H, br m), 3.32 (4H, s), 5.49 (2H, s). ^{13}C-NMR (67.8 MHz, DMSO) δ (ppm): 64.4, 82.1; MS (EI) *m*/*z*: 204 (M^+), 186 (100%). HR-MS (EI) *m*/*z*: 204.2153 (Calcd for $C_4H_{16}B_{10}O_2$: 204.2156).

Studies of Stoichiometry of Complexation between α-CD and Carborane Derivatives 2—6 (Job's Plot Analysis) Job's plot analysis was performed using ^{1}H-NMR spectroscopy in D_2O at 30 °C. The total concentration of α-CD and carboranol was kept constant at 3.0×10^{-3} M. The chemical shift changes were measured at the interior H-3 proton of α-CD and calculated by means of the formula $[\Delta(\text{ppm})]\times[\alpha\text{-CD}]$. Equivalents of α-CD were estimated as the ratio of $[\alpha\text{-CD}]/([\alpha\text{-CD}]+[\text{carboranols}])$. All Job's plots were found to exhibit maxima at 0.5, indicating that α-CD forms a 1 : 1 complex with each of the carborane derivatives **1**—**3**.

NMR Titration Studies of Carborane Derivatives with α-CD All NMR titrations were carried out by adding aliquots of a solution of carborane derivative to a solution of α-CD (2.0×10^{-4} M) in D_2O at 30 °C. After each addition, the NMR spectrum was recorded and referenced to external TSP (0.0 ppm). Association constants (K_a) were estimated from plots of Δ(ppm) *vs.* [carborane] employing a nonlinear least-squares method based on Eq. 1. GraphPad Prism 4 software was used for the calculation of association constants.

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