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Complexation of β-cyclodextrin with carborane derivatives in aqueous solution

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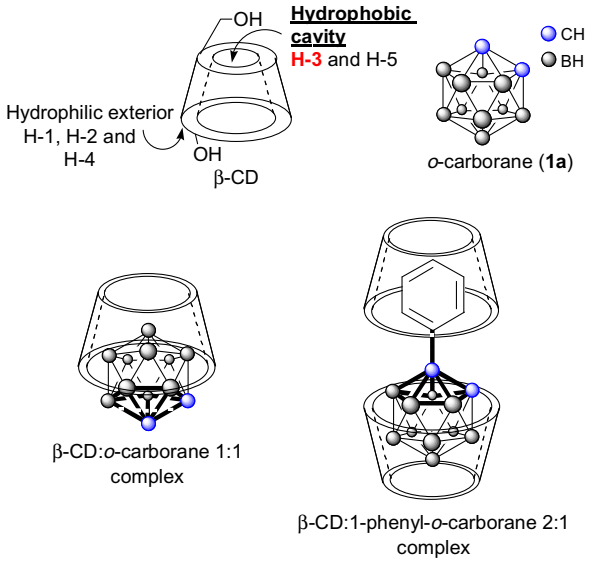
ABSTRACT

β-Cyclodextrin formed the most robust complexes with *o*-carboranols **1b** and **1c** in aqueous solution, and the association constants estimated from NMR titration studies indicated K_a >1 × 10^6 M^{-1} and K_a = 6 × 10^5 M^{-1}, respectively.

Biomedical applications of the unique structural and chemical properties[1] of icosahedral carboranes (dicarba-*closo*-dodecaboranes), for example, in boron neutron capture therapy (BNCT), are of great interest.[2] We have reported the application in medicinal drug design of carborane cages as a hydrophobic component of biologically active molecules,[3] including estrogen receptor (ER) modulators.[4] It was suggested that the carborane cage works as a hydrophobic group for binding to the hydrophobic cavity of ER, and the hydrophobic van der Waals contacts along the spherical carborane cage produce a stronger interaction than that in the case of the native ligand, estradiol. We have investigated the hydrophobicity of carboranes by measuring the partition coefficients, log*P* values, and the hydrophobicity parameter π of carborane groups.[5] However, the hydrophobic interactions of carboranes with the host molecules are not well understood.

Therefore, we examined the spherical shape and hydrophobic surface of carboranes by employing cyclodextrins (CDs). CDs have been widely used as solubilizing agents for lipophilic drugs,[6] as photostabilizers of light-sensitive drugs,[7] and as sustained-release[6] and drug delivery systems[8,9] for various drugs.

Complexation of CDs and carborane was first reported by Harada and Takahashi.[10] 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. However, the stoichiometric ratio, association constant, and complex formation in solution were not well characterized. Recently, Threadgill and co-workers have reported formation of a remarkably robust 2:1 complex of β-CD with 1-phenyl-*o*-carborane.[11] The structure of the complex was determined by NOE and NOESY spectroscopy in solvent, and an excellent fit of the carborane cage with β-CD was confirmed by a molecular modeling study. There are only two reports on the complexation of CDs and carborane, and these deal only with the *o*-carborane isomer. The attractive properties of *m*- and *p*-carboranes[12] mean that complexation of these molecules with β-CD is also of interest.



Here, we present the first report of the robust complexation of β-CD with *o*-, *m*-, and *p*-carborane derivatives in aqueous solution. The stoichiometry and association constant (K_a) of the complexes were estimated on the basis of Job's plots and NMR titration, respectively.[13] Moreover, the association constants of fluorescent sensor-modified β-CD, dansyl-β-CD,[14] with carborane derivatives were estimated from the quenching of the fluorescence derived from the dansyl group. Carborane derivatives examined for complexation ability with β-CD were selected from among simple and common structures that have been used in our medicinal studies.

Scheme 1 summarizes the structure and synthesis of *o*-carborane derivatives with *C*-hydroxy- (**1b**),[15] *C*,*C*′-dihydroxy (**1c**),[15]

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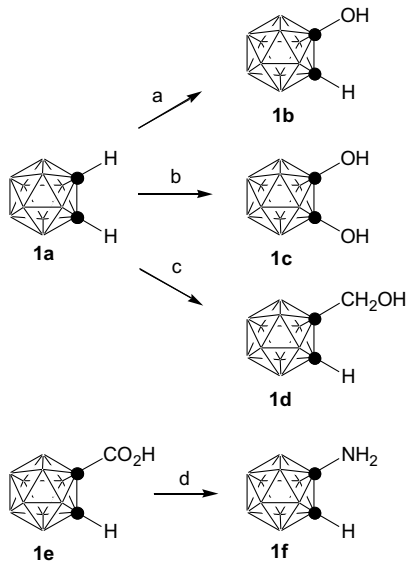

Scheme 1. Synthesis of *o*-carborane derivatives **1b–d** and **1f**. The *m*- and *p*-carborane derivatives, **2b–d**, **2f**, **3b–d**, and **3f**, were similarly synthesized. Reagents: (a) (1) *n*-BuLi, $(MeO)_3B$, ether; (2) H_2O_2, AcOH; (b) (1) *n*-BuLi (2 equiv), $(MeO)_3B$ (2 equiv), ether; (2) 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) DPPA, Et_3N, DMAP, *t*-BuOH, Δ; (2) TFA, CH_2Cl_2.

C-hydroxymethyl (**1d**),[16] *C*-hydroxycarbonyl (**1e**), which is commercially available, and *C*-amino (**1f**)[17] groups. Compounds **1b** and **1c** were synthesized by boronation followed by oxidative rearrangement. Compound **1d** was prepared by the reaction of *o*-carboranyl anion with paraformaldehyde. Carboxylic acid **1e** was transformed into amine **1f** by Curtius rearrangement. The *m*- and *p*-carborane derivatives **2b–d**,[15,18] **2f**,[17,19] **3b–d**,[15,18] and **3f**[17,20,21] were synthesized in the same manner as *o*-carborane derivatives. The fluorescent sensor-modified β-CD, (6-(*N*-dansyl-glycylamino)-6-deoxy-β-cyclodextrin), was synthesized by the reaction of 6-amino-6-deoxy-β-CD[22] with *N*-dansylglycine.[14]

The stoichiometric ratio of the complexation of β-CD and 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 β-CD as an indicator; this signal is very sensitive to the inclusion of guest compounds. Another interior proton, H-5, could not be applied for NMR study because the peak overlapped with those of other protons. Since an internal standard, tetramethylsilane (TMS), was expected to influence the process of complexation of carborane derivatives with β-CD, trimethylsilyl sodium propionate (TPS) was used as an external standard for the measurements. Each of the fitting curves obtained from Job's plots of **1b**, **2b**, and **3b** with β-CD showed 1:1 stoichiometry in D_2O at 30 °C (Fig. 1). Compounds **1c–f**, **2c–f**, and **3c–f** also formed 1:1 complexes with β-CD. However, it was impossible to examine the complexation of non-substituted carboranes with β-CD in aqueous solution, because these carboranes are not soluble in water. Furthermore, there was no change in the ^{1}H NMR chemical shift of the H-3 proton or other protons in either component upon complexation in DMSO-d_6 solution or in a mixed solution of 1:1 DMSO-d_6/D_2O, in which the carboranes **1a**, **2a**, and **3a** are soluble. The carborane derivatives **1b–f**, **2b–f**, and **3b–f** also did not show any change of ^{1}H NMR chemical shifts in DMSO-d_6 solution or in a mixed solution of 1:1 DMSO-d_6/D_2O.[23]

Binding studies of β-CD with carborane derivatives **1b–f**, **2b–f**, and **3b–f** by using ^{1}H NMR techniques revealed amazingly selective binding and very high affinities of β-CD for the *o*-carboranols, **1b** and **1c**. The NMR titration curves of β-CD with **1b** and **1c** in D_2O at 30 °C are shown in Figure 2. Table 1 summarizes the association constants estimated from NMR titration studies of carborane derivatives with β-CD in D_2O at 30 °C. The association constant of β-CD for **1b** in D_2O is close to the limit of calculation with NMR titration methods (K_a >1 × 10^6 M^{-1}), while the value for **1c** was 6.0 × 10^5 M^{-1}. *m*-Carboranols **2b** and **2c** also showed high binding affinities for β-CD, K_a = 41,500 M^{-1} and 25,300 M^{-1}, respectively. The *p*-carboranols **3b** and **3c** had unexpectedly moderate association constants. Interestingly, *C*-hydroxymethyl-*p*-carborane **3d** bound strongly to β-CD with K_a = 54,000 M^{-1}, although the association constants of the *o*- and *m*-isomers, **1d** and **2d**, were only 8300 and 2400 M^{-1}, respectively. Compound **3d** had exceptionally high affinity for β-CD among the *p*-carborane derivatives. There may be an intermolecular hydrogen bond in addition to a hydrophobic interaction between the carborane cage and β-CD. However, it was difficult to observe putative hydrogen bonding of the hydroxy-

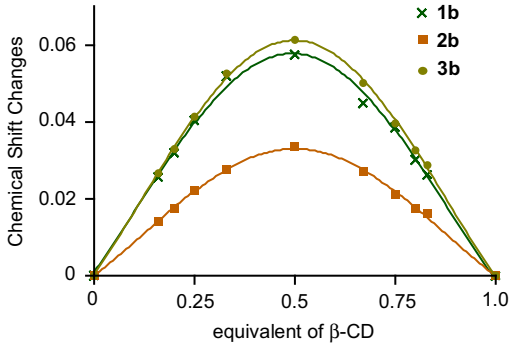

Figure 1. Job's plots for carboranol **1b, 2b**, and **3b** with β-CD in D_2O at 30 °C. The total concentration of β-CD and carboranol was kept constant at 3.0 × 10^{-3} M. The chemical shift changes were measured at the interior H-3 proton of β-CD and calculated by means of the formula [Δ(ppm)] × [β-CD (equiv)]. Equivalents of β-CD were estimated as the ratio of [β-CD]/([β-CD] + [carboranols]).

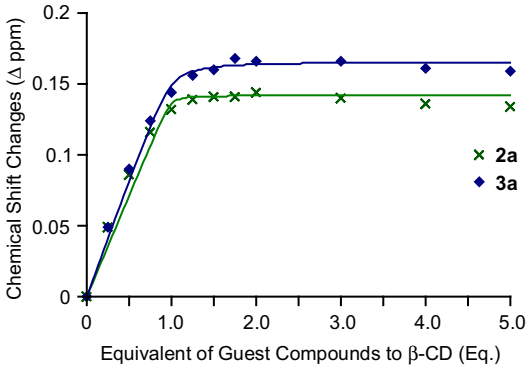

Figure 2. NMR titration of carboranols, **2a** and **3a**, with β-CD based on the chemical shift changes (Δppm) of the interior C-3 proton of β-CD. The titration study was performed in D_2O at 30 °C. [β-CD] = 2.0 × 10^{-4} M.

Table 1
Association constants (K_a) of carborane derivatives **1b–f**, **2b–f**, and **3b–f** with β-CD in D_2O at 30 °C

Substituent	*o*-Carborane	*m*-Carborane	*p*-Carborane
OH	>10^6 (**1b**)	41,500 (**2b**)	9600 (**3b**)
$(OH)_2$	6.0 × 10^5 (**1c**)	25,300 (**2c**)	9800 (**3c**)
CH_2OH	8300 (**1d**)	2400 (**2d**)	54,000 (**3d**)
CO_2H	37,000 (**1e**)	5700 (**2e**)	4400 (**3e**)
NH_2	2500 (**1f**)	3900 (**2f**)	3800 (**3f**)

Compound numbers are shown in parentheses.

methyl group of **3d** with β-CD from the ^{1}H NMR spectra, because of peak overlapping. Carboranyl carboxylic acids **1e**, **2e**, and **3e** bound moderately with β-CD and showed low association constants for carboranyl amines, **1f** (2500 M^{-1}), **2f** (3900 M^{-1}), and **3f** (3800 M^{-1}), with little selectivity.

These results show that β-CD favors carborane cages in the order of *ortho* > *meta* > *para*, except for *C*-hydroxymethyl-*p*-carborane **6c**. The hydrophobic interactions between β-CD and carborane cages depend largely on the orientation of the carborane cage in the inclusion process, and there are two types of inclusion modes, the lying type and the standing type, in the orientation of the carborane cage (Fig. 3).

Hydrophobic interaction between β-CD and the carborane cages is the major driving force in the inclusion process, but hydrogen bonding between primary or secondary hydroxyl groups of β-CD and substituents of the carboranes, as well as the orientation of the carborane cage, and the directions of substituents may also be important factors in the inclusion process. Moreover, the hydrogen bonds of carborane C–H hydrogen with hydroxyl groups of β-CD are particularly important factors in forming a robust complex.[11,24]

Plausible inclusion modes of carboranols **1b**, **2b**, and **3b** with β-CD are shown in Figure 4, based on the association constants given in Table 1. In general, the hydroxyl groups of β-CD can form hydrogen bonds with polar substituents of a guest molecule, and the hydrophobic cavity favors a hydrophobic structure over a hydrophilic one. The *C*-hydroxyl group and C–H hydrogen in carboranols are regarded as polar substituents and the carborane cage consisting of ten B–H parts is regarded as a hydrophobic structure. Therefore, we suggest that the lying type inclusion mode would be favorable for **1b**, and that **2b** and **3b** might prefer the standing inclusion mode in view of hydrogen bond formations and repulsion of carborane C–H hydrogen with the hydrophobic interior side of β-CD. In the proposed inclusion modes of **1b** and **2b** with β-CD, hydrogen bonding of carborane C–H hydrogen with the secondary hydroxyl group of β-CD is possible, as reported by Threadgill and co-workers.[11] It is also important to note that the association constant is strongly influenced by the pK_a value of the *C*-hydroxyl group: the pK_a values reported for the *ortho* (**1b**), *meta* (**2b**), and *para* (**3b**) compounds are 5.33, 8.39, and 9.03, respectively.[25] In the case of *p*-carboranol **3b**, no hydrogen bond formation between compound **3b** and β-CD may be formed. In other words, the inclusion process of **3b** and β-CD is totally dependent on hydrophobic interaction. To determine the inclusion mode in detail, we will undertake DFT calculation studies.

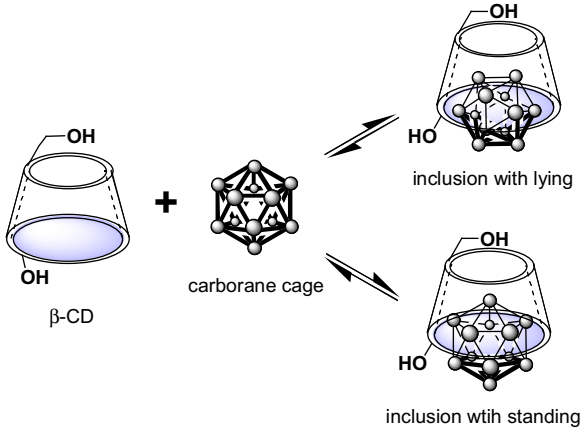

Figure 3. Alternative orientations of the carborane cage in the inclusion process.

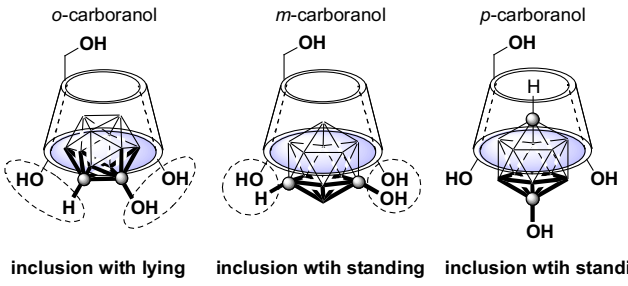

Figure 4. Plausible inclusion modes of carboranols **1b**, **2b** and **3b** with β-CD. The dashed lines indicate intermolecular hydrogen bonding formations.

Next, the complexation of modified β-CD with carboranols and carborane carboxylic acids was assessed by using fluorescence sensor-modified β-CD covalently linked with a dansyl group as an indicator.[14] The fluorescence is quenched when the guest molecule drives the dansyl group from β-CD. Figure 5 shows the changes of fluorescence intensity upon addition of the guest compound **1b** in H_2O at 25 °C and the result of fluorescence titration using the fluorescence intensity at λ_{max} (540 nm). As **1b** was added to the dansyl-β-CD solution, the fluorescence intensity of the dansyl group at 540 nm decreased, depending on the concentration of **1b**. However, about 500 equiv amounts of **1b** had to be added before the titration curve reached a plateau. The titration curves of compounds **1c**, **1e**, **2b**, **2c**, **2e**, **3b**, **3c**, and **3e** were similar to that of **1b**.

The association constants of dansyl-β-CD with the tested compounds, estimated from the fluorescence titration data, are summarized in Table 2. Unfortunately, dansyl-linked β-CD showed no selectivity for various carborane derivatives, and the K_a values fell within the range of 2000–5000 M^{-1} regardless of substituent and carborane isomer. The exact reasons for the low association constants and selectivity among them are not clear, but it seems that the exchange process between the dansyl group and carborane cage has a great impact on the complexation of β-CD and carborane derivatives.

In conclusion, we found that β-CD forms robust complexes with *o*-carboranols **1b** and **1c** in aqueous solution, and that not every carborane forms a robust complex with β-CD. Compound **3d** had an exceptionally strong association constant among *p*-carborane derivatives for β-CD. The inclusion process is typically dominated by hydrogen bonding and cage orientation, as well as by hydrophobic interactions. It will be interesting to examine the binding abilities of carboranes with other CDs, α- and γ-CDs, in aqueous

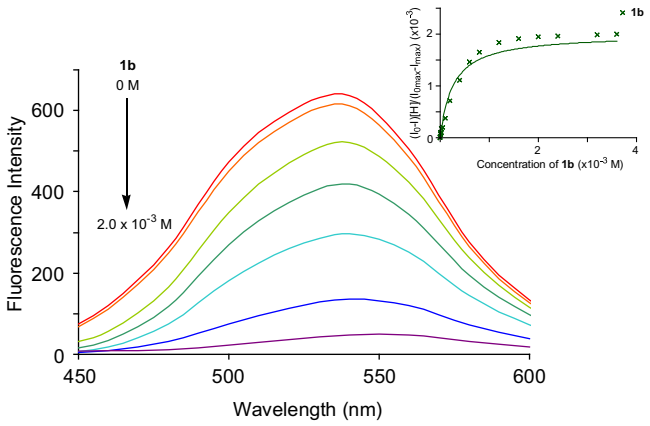

Figure 5. Fluorescence intensity changes upon complexation of dansyl-β-CD (2.0×10^{-6} M) with **1b** (0, 0.01, 0.1, 0.2, 0.4, 0.8 and 2.0×10^{-3} M) in H_2O at 25 °C. The inset shows the fluorescence titration curve. The excitation wavelength was 370 nm, and emission intensity was measured at 540 nm (λ_{max}).

Table 2
Association constants (K_a) of dansyl-β-CD with carborane derivatives in H_2O at 25 °C

Substituent	*o*-Carborane	*m*-Carborane	*p*-Carborane
OH	3900 (**1b**)	4900 (**2b**)	3400 (**3b**)
$(OH)_2$	3000 (**1c**)	2000 (**2c**)	2000 (**3c**)
CO_2H	4300 (**1e**)	3600 (**2e**)	2900 (**3e**)

Compound numbers are shown in parentheses.

solution in order to understand in detail the mechanism of the complexation. CD-carborane complexes have potential value not only in the development of novel carborane-containing drugs, but also in the field of materials science.

Acknowledgments

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