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Thermodynamical and geometrical characterization of molecular recognition by cage-type and peptide azapara-cyclophanes in aqueous media

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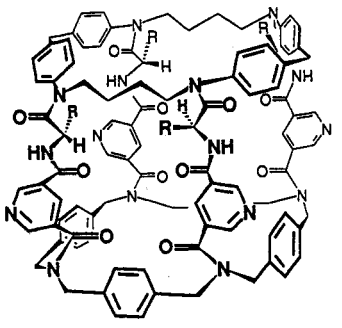
Abstract - A cage-type cyclophane which is constructed with two rigid macrocyclic skeletons, tetraaza[6.1.6.1]paracyclophane and tetraaza[3.3.3.3]paracyclophane, and four chiral bridging components strongly binds anionic and hydrophobic guests, such as 8-anilinonaphthalene-1-sulfonate and 6-*p*-toluidinonaphthalene-2-sulfonate. Thermodynamic parameters were evaluated from temperature-dependent complexation constants for the cage-type host and a peptide cyclophane, which was prepared by introducing four valine residues into a tetraaza[6.1.6.1]-paracyclophane skeleton, with the hydrophobic guests as determined by fluorescence spectroscopy. The complexation of the former and latter hosts with the guests gave positive and negative ΔS values, respectively. The positive ΔS values come primarily from effective desolvation of the guest molecule incorporated into the hydrophobic host cavity and partly from conformational changes around the bridging moieties of the cage-type host upon complexation, as evidenced by fluorescence polarization and circular dichroism measurements. The geometrical arrangements of the guest molecules in the respective inclusion complexes were clarified by detailed ^{1}H NMR analysis.

INTRODUCTION

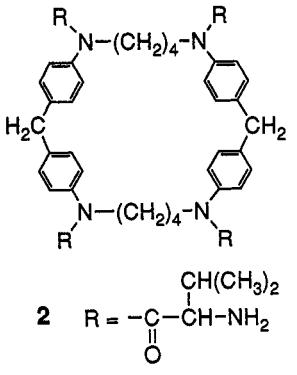
In close functional relation to specific molecular recognition by naturally occurring receptors, various cyclophanes having a sizable internal cavity have been employed as artificial receptors (ref. 1). The molecular recognition ability of such artificial hosts in aqueous media is highly dependent on the hydrophobic character of the host cavity, because other non-covalent host–guest interactions become effective in well desolvated and hydrophobic microenvironments. On these grounds, we have recently designed and prepared cage-type cyclophane **1**, which is constructed with two rigid macrocyclic skeletons, tetraaza[6.1.6.1]paracyclophane and tetraaza[3.3.3.3]paracyclophane, and four bridging components that connect the macrocycles, in order to give out a hydrophobic three-dimensional cavity that is well shielded from the bulk aqueous phase (ref. 2). Each bridging component of the host is composed of an optically active L-valine residue and a pyridine-3,5-dicarbonyl moiety, so that the cage-type host is capable of performing the chirality-based molecular discrimination toward various hydrophobic guests through stereochemical host–guest interactions. Peptide cyclophane **2**, a structural fragment of the cage-type host, was also prepared by introducing four L-valine residues into a rigid tetraaza[6.1.6.1]paracyclophane skeleton (ref. 3). Since such a chemical modification of the macrocyclic skeleton is not enough to create a three-dimensional cavity, the peptide cyclophane is to be expected to provide a cavity of moderate hydrophobic nature as compared to the cage-type host in aqueous media. These macrocyclic hosts holding different hydrophobic cavities are expected to act as useful artificial receptors in different respective modes toward well-known fluorescent guests such as 8-anilinonaphthalene-1-sulfonate (ANS) and 6-*p*-toluidinonaphthalene-2-sulfonate (TNS). Characteristic features of molecular recognition by the artificial receptors toward the guests in aqueous media are clarified in this work from thermodynamic and stereochemical viewpoints (ref. 4).

MICROENVIRONMENTAL PROPERTIES OF CYCLOPHANE CAVITIES

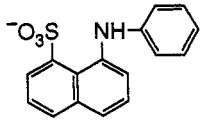
Both cage-type cyclophane **1** and peptide cyclophane **2** are soluble in acidic aqueous media and behave as



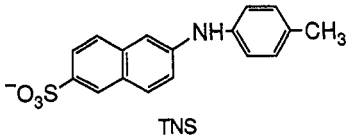
1 R = $-CH(CH_3)_2$



2 R = $-C(=O)-CH(CH_3)_2-CH-NH_2$



ANS



TNS

polycationic hosts. The guest-binding behavior of the hosts toward fluorescent probes, ANS and TNS whose emission is extremely sensitive to change in microenvironmental polarity experienced by the molecules, was examined by fluorescence spectroscopy in aqueous acetate buffer [0.01 mol dm^{-3}, pH 4.1, μ 0.10 (KCl)] at 20, 30, 40, and 50 °C. The fluorescence intensity originated from each guest increased along with a concomitant blue shift of the fluorescence maximum upon addition of the host. The binding constants (K) of **1** and **2** toward ANS and TNS were evaluated on the basis of Benesi-Hildebrand relationship for 1:1 host–guest interactions (ref. 5), and are summarized in Table 1. Good linear correlations based on double-reciprocal plots of the extent of change in fluorescence intensity upon addition of the host against the total concentration of the host were obtained at various temperatures. As is obvious from the data in Table 1, the K values for the cage-type host with the guests are much greater than the corresponding values for the peptide cyclophane. The free energies (ΔG), enthalpies (ΔH) and entropies (ΔS) for formation of host–guest complexes were evaluated from the temperature-dependent K values as listed in Table 2.

TABLE 1. Binding constants (K/dm^3 mol^{-1}) for host-guest complexes of cyclophanes with hydrophobic guests in aqueous acetate buffer (0.01 mol dm^{-3}, pH 4.1, μ 0.10 with KCl)[a]

Guest	Host	Temperature/K			
		293	303	313	323
ANS	**1**	3.3×10^4	2.8×10^4	2.1×10^4	1.8×10^4
TNS	**1**	6.9×10^4	5.8×10^4	4.2×10^4	3.4×10^4
ANS	**2**	2.2×10^3	1.6×10^3	1.0×10^3	7.5×10^2
TNS	**2**	2.6×10^4	1.4×10^4	1.0×10^4	6.4×10^3

[a] Concentrations in mol dm^{-3}: guests, 1.0×10^{-6}; cyclophanes, 5.0×10^{-6} – 5.0×10^{-5} mol dm^{-3}.

Large favorable ΔH values were obtained for all the complexation processes of **1** and **2** with ANS and TNS. On the other hand, the complexation of cage-type host **1** with the guests gave positive ΔS values, while negative values were assigned to that of peptide cyclophane **2**. The positive ΔS values must come primarily from effective desolvation of the guest molecules incorporated into the hydrophobic host cavity and partly from conformational changes around the guest-binding sites of **1** upon complexation with the guest. Different modes of desolvation and conformational change in the course of complexation of

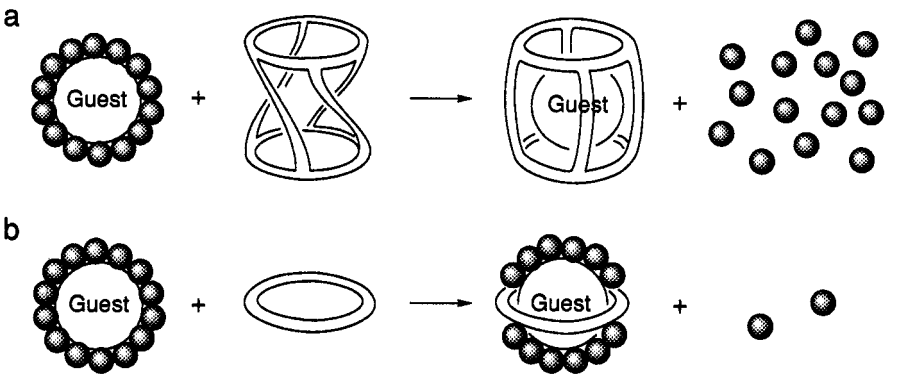


Fig. 1. Schematic illustration of inclusion behavior of the cage-type (**a**, top) and peptide (**b**, bottom) cyclophanes; the shaded circle denotes a water molecule

TABLE 2. Thermodynamic parameters for host–gust complexes of cyclophane hosts with hydrophobic guests in aqueous acetate buffer (0.01 mol dm^{-3}, pH 4.1, μ 0.10 with KCl) at 313 K

Guest	Host	ΔG/kJ mol^{-1}	ΔH/kJ mol^{-1}	$T\Delta S$/kJ mol^{-1}
ANS	**1**	−25.9	−17.1	8.8
TNS	**1**	−27.7	−19.5	8.3
ANS	**2**	−18.0	−29.1	−11.0
TNS	**2**	−24.0	−35.6	−11.7

hosts **1** and **2** with the guest are schematically shown in Fig. 1. The desolvation of the guest molecule upon complexation with cage-type host **1**, which is well known as caused by the classical hydrophobic effect (ref. 6), was evidenced by various fluorescence parameters. The microenvironmental polarity experienced by the incorporated guest molecule was evaluated from the fluorescence maximum in a manner similar to that reported previously (ref. 7). Host **1** provides relatively apolar microenvironments for the hydrophobic guests, ANS and TNS (Table 3). The E_T^N value for ANS in host **1** (E_T^N = 0.23; identical with the value for ethyl acetate) is smaller than that for ANS upon complexation with cyclophane **2** (E_T^N = 0.73; 0.654 and 0.762 for ethanol and methanol, respectively) (ref. 8). Thus, the three-dimensional cavity provided intramolecularly by the two macrocyclic rings and the four bridging components of host **1** is considered to be significantly apolar and well shielded from the bulk aqueous phase. Furthermore, relatively large fluorescence polarization values (P) were obtained for ANS and TNS incorporated into the cage-type cyclophane (Table 3). This also indicates that a highly desolvated microenvironment is apparently provided by host **1** so that the tight host–guest interaction, which brings about the marked motional repression of the entrapped guest, becomes effective. In addition, these values are comparable to those for guests incorporated into an octopus cyclophane, having L-aspartate residues as connector units interposed between a rigid 2,11,20,29-tetraaza-[3.3.3.3]paracyclophane skeleton and four double-chain hydrocarbon segments (0.33 and 0.32 for ANS and TNS, respectively) (ref. 7).

TABLE 3. Microenvironmental polarity parameters (E_T^N) and steady-state fluorescence polarization values (P) for guests incorporated into cyclophanes in aqueous acetate buffer (0.01 mol dm^{-3}, pH 4.1, μ 0.10 with KCl) at 30 °C

Guest	Host	E_T^N(λ_{ex}/nm; λ_{em}/nm)[a]	P
ANS	**1**	0.23 (375; 462)	0.40
TNS	**1**	0.58 (322; 422)	0.29
ANS	**2**	0.73 (375; 479)	0.10
TNS	**2**	0.84 (322; 461)	0.09

[a] Excitation and emission maxima are given in parentheses, in this sequence.

The conformational change of cage-type cyclophane **1**, caused by the induced-fit binding of the guest molecule, was detected by means of circular dichroism (CD) and FT-IR measurements. Host **1** (5.0 x 10^{-5} mol dm^{-3}) alone shows a CD band at 244 nm with molecular ellipticity ([θ], deg cm^2 $dmol^{-1}$) of 4.7 x 10^4 in aqueous acetate buffer [0.01 mol dm^{-3}, pH 4.1, μ 0.10 (KCl)] at 30 °C (Fig. 2). Upon addition of an equimolar amount of ANS, the CD band intensity at the identical wavelength was weakened; [θ], 3.0 x 10^4 deg cm^2 $dmol^{-1}$ (Fig. 2). In addition, C=O stretching bands in D_2O/DMSO-d_6 (9:1 v/v) for the amide group of host **1** [1%(w/v)] appeared at 1640 and 1648 cm^{-1} in the absence and presence of ANS, respectively, suggesting that **1** forms stronger intramolecular hydrogen bonds in the absence of the guest. The results are consistent with the following states of affairs. The four pyridyl moieties bound to the chiral L-valine residues in the bridging components of **1** approach close to each other and are subjected to conformational fixation owing to the formation of intramolecular hydrogen bonds in the absence of the guest. Moreover, the decrease in CD band intensity and the shift of C=O stretching band of host **1**, both being induced by complexation with the guest, are attributable to conformational and configurational changes around the bridging moieties of **1** so as to form thermodynamically stable complexes. On the other hand, the complexation of cyclophane **2** with ANS and TNS gave unfavorable ΔS values, in a manner similar to those exercised by various monocyclic cyclophanes and cyclodextrins with small apolar organic molecules in aqueous media (ref. 9, 10). It is noteworthy that the desolvation effect exerted by the hydrophobic cavity of **2** on an incorporated guest molecules is insufficient, as the cavity provided by cyclophane **2** seems to be relatively shallow for incorporation. In addition, the conformational flexibility of the macrocyclic framework and the multiple hydrophobic branches of host **2** is reduced upon complexation with the guest.

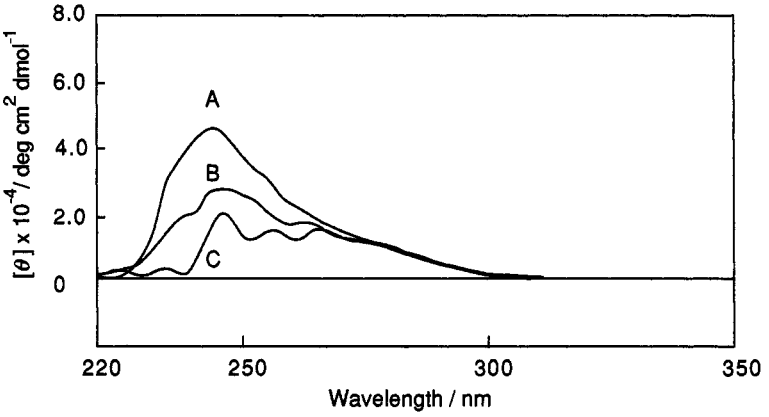


Fig. 2. CD spectra of **1** (5.0 x 10^{-5} mol dm^{-3}) in aqueous acetate buffer (0.01 mol dm^{-3}, pH 4.1, μ 0.10 with KCl) at 30 °C: without any guest (A); in the presence of the guests (5.0 x 10^{-5} mol dm^{-3}), ANS and TNS (B and C, respectively)

GEOMETRICAL ARRANGEMENTS OF HOST AND GUEST MOLECULES IN COMPLEXES

The inclusion interactions of hosts **1** and **2** with ANS and TNS were investigated by means of ^1H NMR spectroscopy in aqueous media at 303 K. Upon addition of peptide cyclophane **2** to D_2O/CD_3OD (4:1 v/v) containing individual guests, all the ^1H NMR signals due to the guests were subjected to marked up-field shifts, except for H-3' and methyl protons of TNS, reflecting formation of the host–guest complexes. The binding constants (K) for 1:1 host–guest complexes and the complexation-induced shifts (CIS) (ref. 11), the shifts of NMR signals for the guest upon 100% complexation, were evaluated by means of the computer-aided least squares curve fitting method applied to NMR titration data. The K values for complexation of host **2** with ANS and TNS are 1.1 x 10^3 and 1.2 x 10^4 mol^{-1} dm^3, respectively. The evaluated CIS values are shown in Fig. 3. The CIS values for TNS upon complexation with cyclophane **2** prove that the naphthalene moiety of the guest is fully incorporated into the internal macrocyclic cavity of **2**, while the toluidino moiety is located outside the cavity, in a manner similar to that performed by **2** toward steroid hormones (ref. 2). On the other hand, it becomes apparent that both benzene and naphthalene moieties of ANS, though the latter moiety being partially, are incorporated into the internal cavity of **2**. Complementary information on the inclusion mode is accessible by spin-lock rotating frame NOE experiments (Roesy) (ref. 12). The intermolecular cross peaks between H-2' protons of TNS and aromatic ortho-protons of **2** were observed in the Roesy spectrum, indicating the geometrical arrangement of the host and guest molecules in the corresponding complex, that was postulated on the

basis of CIS values, is quite reasonable. The intermolecular NOE peaks observed between H-4 proton of ANS and aromatic meta-protons of **2** also provide definite evidence for the postulated complex structure based on CIS values. Low energy conformations of host–guest complexes in the gas phase were examined by molecular mechanics (BIOGRAF, Dreiding-I and Dreiding-II) calculations (ref. 13) on an IRIS-4D/220GTX workstation. The result reveals that such a difference in geometrical arrangement between the guest molecules when they are incorporated into the cavity of **2** comes from the different molecular shapes of the guests; TNS is more slender than ANS, so that the former guest is capable of penetrating the macrocyclic skeleton of **2** while the latter is not. In both host–guest complexes, the sulfonate moiety of each guest prefers, for reasons of favorable solvation, to be placed in the outside region of the cavity (Fig. 4).

Because of limited solubility of host **1** in D_2O/CD_3OD (4:1 v/v) for reasonable NMR measurements, the measurements were carried out in D_2O/DMSO-d_6 (7:3 v/v). ^1H NMR signals of the identical guests showed slight up-field shifts upon complexation with an equimolar amount of host **1**. The corresponding CIS values were evaluated on the basis of the binding constants determined in a medium identical with that used for NMR measurements by fluorescence spectroscopy (K; 4.3 x 10^3 and 1.8 x 10^3 dm^3 mol^{-1} for ANS and TNS, respectively). The evaluated CIS values for ANS and TNS upon complexation with host **1** are much smaller than those for the identical guests upon complexation with cyclophane **2**. Consequently, the guest molecules are undoubtedly placed in the three-dimensional cavity provided intramolecularly by the two macrocyclic skeletons and the four bridging components of host **1**.

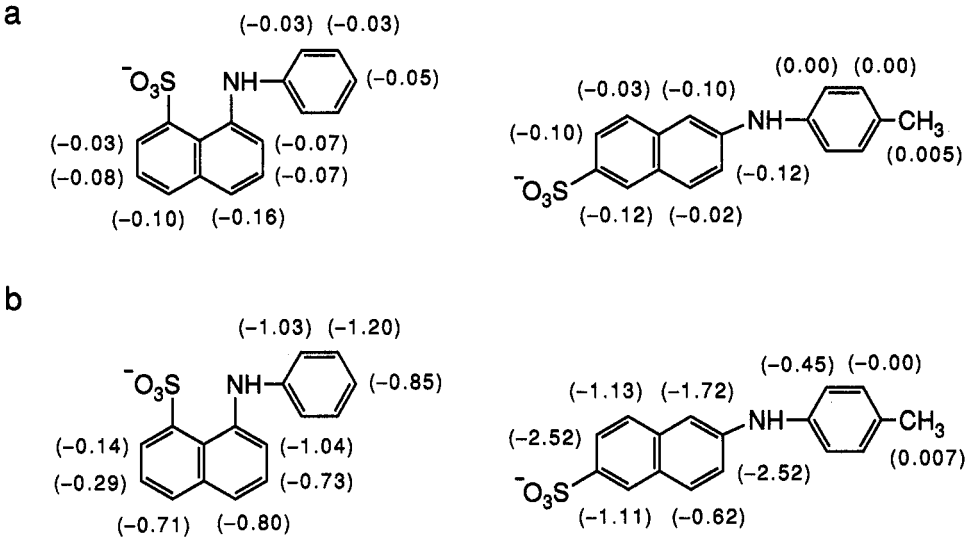


Fig. 3. CIS values (ppm) for ANS and TNS upon complexation with cage-type cyclophane **1** (**a**, top) and peptide cyclophane **2** (**b**, bottom).

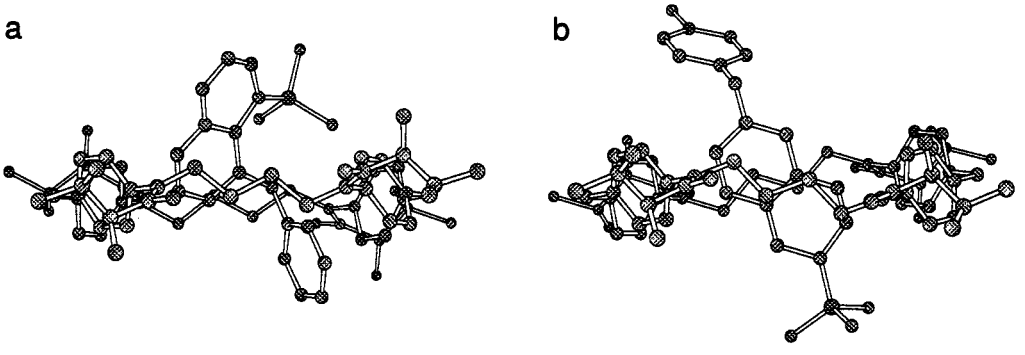


Fig. 4. Computer optimized conformations for complexes of peptide cyclophane **2** with ANS (**a**, left) and TNS (**b**, right); all protons are removed for simplicity to show geometrical arrangements of host and guest molecules.

CONCLUSION

The present cage-type cyclophane (**1**) furnishes a three-dimensional internal cavity that is well shielded from the bulk aqueous phase when an appropriate guest molecule is included. In addition, a guest molecule incorporated into the cage-type host is effectively desolvated and its molecular motion is remarkably repressed relative to that captured by the peptide cyclophane (**2**). We believe that our concept on molecular design provides a useful guidepost for preparation of multifunctional receptor models that are capable of performing effective molecular discrimination in aqueous media and bilayer membranes (ref. 3).

Acknowledgment
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