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Molecular Recognition by Steroid Cyclophane in Aqueous Solution and Synthetic Bilayer Membrane

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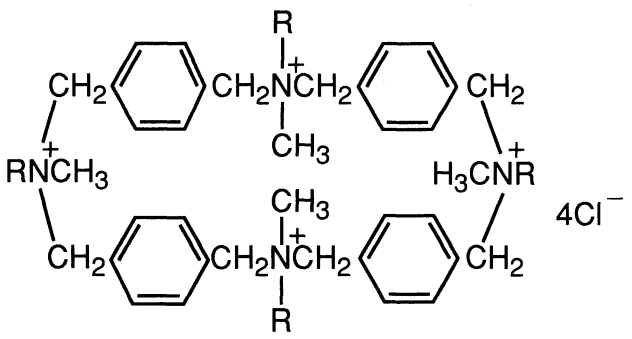
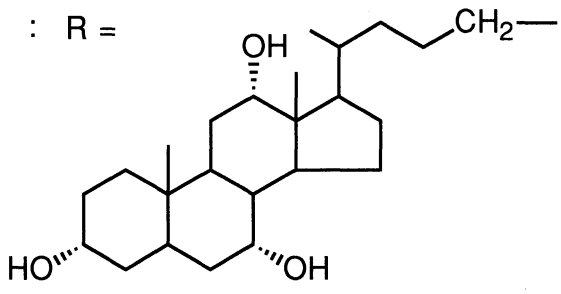
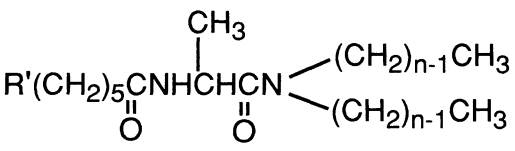
A novel host named "steroid cyclophane," a tetraaza[3.3.3.3]paracyclophane with four steroid moieties, was prepared, and its ability to recognize 8-anilinonaphthalene-1-sulfonate, a typical anionic guest with hydrophobic character, in aqueous media was examined in the presence and absence of synthetic bilayer membranes.

Molecular recognition by artificial hosts is of current interest from the viewpoint of supramolecular chemistry.[1] We have been employing cyclophane derivatives modified with various functional groups as artificial enzymes and receptors[2] and bilayer aggregates formed with synthetic peptide lipids, which have α-amino acid residue(s) interposed between a polar head moiety and a hydrophobic double-chain segment through amide linkages, as structural and functional models of biomembranes.[3] We became interested in the molecular recognition by such a cyclophane derivative which is embedded in a bilayer membrane to form a supramolecular assembly. In this regard, we now prepared a novel water-soluble host, "steroid cyclophane" (**1**),[4]



1 : R =



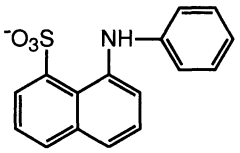


2 : R' = Na^+ $^-O_3S-$, n = 18

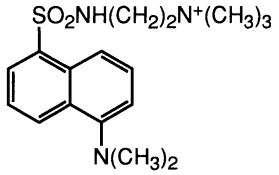
3 : R' = $(CH_3)_3N^+-$ Br^-, n = 14, 16

which is constituted with four steroid moieties and a 2,11,20,29-tetraaza[3.3.3.3]paracyclophane ring. In this communication, we are to report on the molecular recognition ability of **1** in aqueous media in the presence and absence of synthetic bilayer membranes formed with peptide lipids **2** and **3**.

The host (**1**) is soluble in water, and its critical aggregate concentration (cac) was evaluated by means of surface tension measurements based on the Wilhelmy principle; 3.7 x 10^{-5} mol dm^{-3}. We investigated the molecular recognition ability of the steroid cyclophane in an aqueous phosphate buffer (0.01 mol dm^{-3}, pH 8.0) at 30.0 °C in a concentration range below its cac. In order to evaluate the guest recognition behavior of **1** in comparison with that of other cyclophane hosts previously prepared, 8-anilinonaphthalene-1-sulfonate (ANS) was chosen as a hydrophobic fluorescent guest. The fluorescence originated from ANS increased significantly in intensity upon addition of **1**, reflecting the host–guest complexation phenomenon. A formation constant (K) for the 1:1 host–guest complex was evaluated on the basis of the Benesi-Hildebrand relationship[5] in a manner similar to that reported previously;[6] 3.3 x 10^5 dm^3 mol^{-1}. The K value is much greater than the corresponding value for a simple macrocycle, *N,N',N'',N'''*-tetramethyl-2,11,20,29-tetraaza[3.3.3.3]paracyclophane (550 dm^3 mol^{-1} at pH 2),[7] and comparable to those for octopus cyclophanes bearing eight flexible hydrocarbon chains on the same azacyclophane ring.[8] On the other hand, **1** exhibited no capacity of binding the cationic 1-dimethylamino-naphthalene-5-sulfonamidoethyltrimethylammonium species (DASP).



ANS

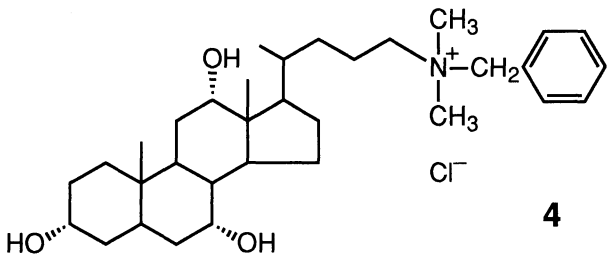


DASP

The microscopic polarity experienced by a guest, ANS, at the guest-binding site provided by **1** was evaluated from a fluorescence maximum (λ_{max}^F) observed for the guest; E_T^N [8,9] 0.41, λ_{max}^F 463 nm. The result, along with additional rationalization by the CPK model study, suggests strongly that the steroid cyclophane is able to incorporate one ANS molecule into its three-dimensionally extended hydrophobic cavity created by the four steroid moieties and the macrocyclic skeleton in a similar manner as performed by an octopus cyclophane having eight flexible hydrophobic branches.[6,8] It is noteworthy, however, that the specific structural rigidity of the steroid moieties of **1** is reflected in the microscopic viscosity at the guest-binding site. A rotational correlation time (θ = 5.7 ns) for the ANS molecule incorporated into **1**, as evaluated from fluorescence polarization and lifetime measurements, is much smaller than that for the identical guest included in the octopus cyclophane (θ = 23.0 ns).[8] This means that the flexible hydrocarbon chains of the octopus cyclophane is capable of grasping the guest more tightly than the rigid steroid moieties of the present host.

We examined molecular recognition behavior of **1** embedded in synthetic bilayer membranes. Anionic lipid **2**[10] afforded bilayer aggregates when dispersed in aqueous media, as confirmed by negative staining electron microscopy. A peak temperature for the gel to liquid-crystalline phase transition (T_m) and the corresponding enthalpy change (ΔH) were evaluated for

2 in the aqueous dispersion state by means of differential scanning calorimetry (DSC); T_m 38.6 °C, ΔH 41.8 kJ mol^{-1}. Upon addition of **1** (2.5 mol%) to the aqueous dispersion of **2**, there was no change in T_m but a 20% increase in half-bandwidth of the endothermic peak, reflecting formation of the hybrid molecular assembly. On the other hand, host **1** was not incorporated effectively into the bilayer domain of **3** (n = 16) as evidenced by DSC measurements. Figure 1 shows fluorescence spectra of ANS in a sonicated solution of **2** with and without the steroid cyclophane. While the anionic ANS alone interacts weakly with the anionic lipid aggregate (E_T^N 0.97, λ_{max}^F 510 nm), the hybrid assembly formed with **1** and **2** strongly incorporates ANS into its hydrophobic domain (E_T^N 0.01, λ_{max}^F 458 nm). The microenvironment around the guest incorporated into the latter aggregate is equivalent to that provided by hexane (E_T^N 0.009). When a steroid derivative (**4**),[11] a monomeric analog of **1** with respect to the steroid fragment, was added in 10 mol% to the



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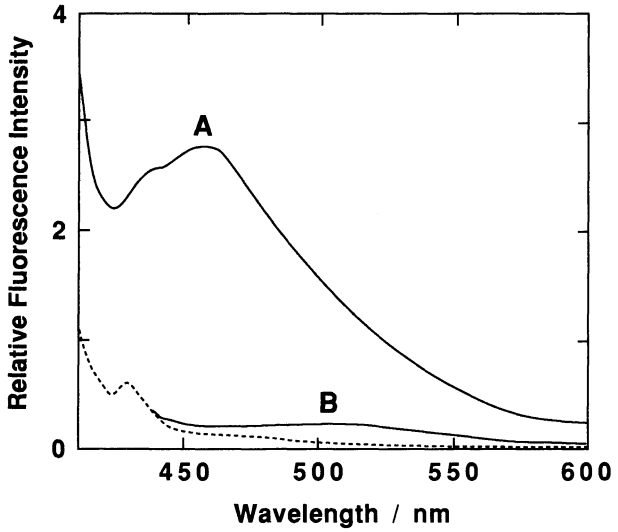

Fig. 1. Fluorescence spectra of ANS (1.0 x 10^{-6} mol dm^{-3}) in a sonicated solution of **2** (4.0 x 10^{-4} mol dm^{-3}); in the presence (A) and absence (B) of **1** (1.0 x 10^{-5} mol dm^{-3}). The dotted line refers to Raman scattering due to water without ANS; excitation wavelength, 375 nm.

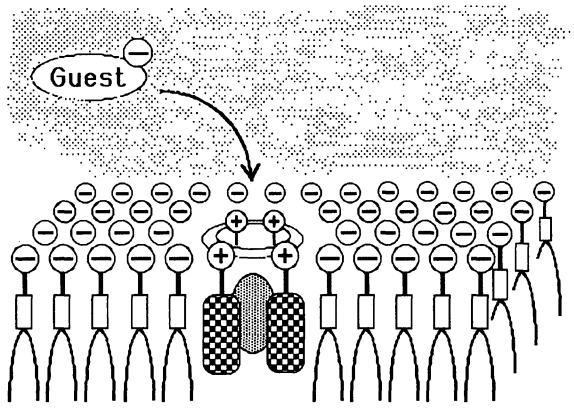

Fig. 2. Schematic illustration of molecular recognition by the steroid cyclophane (**1**) embedded in bilayer membrane formed with anionic peptide lipid **2**.

aqueous dispersion of **2**, the corresponding hybrid assembly was formed as evidenced by a 25% increase in half bandwidth of the DSC peak. However, the ANS binding to the assembly was not significantly enhanced. Although bilayer aggregates formed with cationic lipid **3** are capable of binding ANS, the micropolarity experienced by the guest was found to be much polar (E_T^N 0.72, λ_{max}^F 477 nm for **3** with n = 14)[6] than that in the hybrid assembly formed with **1** and **2**. Thus, it seems that the anionic ANS molecule is selectively included in hydrophobic cavity provided by the cationic steroid cyclophane embedded in the single-walled bilayer membrane formed with the anionic peptide lipid as illustrated in Fig. 2.

In conclusion, it became apparent that the steroid cyclophane behaves as an effective artificial receptor not only in aqueous media but also in the synthetic bilayer membrane. The present host can provide a pronounced hydrophobic microenvironment for molecular recognition by forming the supramolecular assembly with the bilayer membrane. Studies on guest selectivity by the steroid cyclophane are now in progress in our laboratories.

References

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4) Found: C, 70.85; H, 9.26; N, 2.85%. Calcd for $C_{132}H_{208}Cl_4N_4O_{12}\cdot 3H_2O$: C, 70.81; H, 9.63; N, 2.50%.

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10) Found: C, 66.72; H, 11.24; N, 3.49%. Calcd for $C_{45}H_{89}N_2NaO_5S\cdot H_2O$: C, 66.62; H, 11.31; N, 3.45%. Prepared in a manner similar to that stated for analogous peptide lipid bearing different double-chain length: Y. Murakami, J. Kikuchi, T. Takaki, K. Uchimura, and A. Nakano, *J. Am. Chem. Soc.*, **107**, 2161 (1985).

11) Found: C, 71.98; H, 9.73; N, 2.65%. Calcd for $C_{33}H_{54}ClNO_3$: C, 72.30; H, 9.93; N, 2.55%.

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