Circular Dichroism of an Aromatic Guest Induced by a Chiral Steroid Cyclophane in Aqueous Solution and Synthetic Bilayer Membrane

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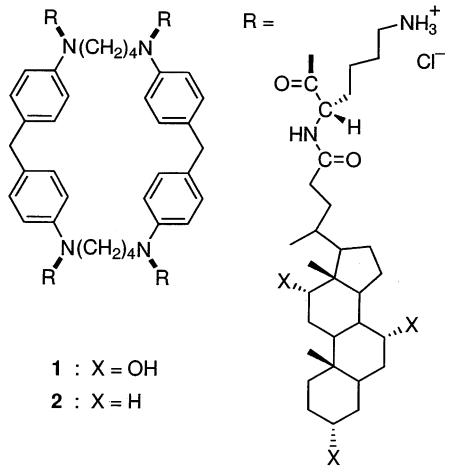
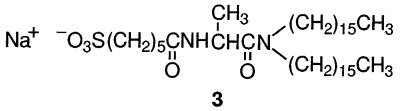
A steroid cyclophane, having L-lysine residues interposed between a tetraaza[6.1.6.1]paracyclophane skeleton and four cholate moieties, furnished a chiral binding site for a hydrophobic aromatic guest in a synthetic bilayer membrane as well as in aqueous solution, as evidenced by induced circular dichroism.

Recently much attention has been focussed on molecular recognition by supramolecular bilayer assemblies with an aim to develop artificial receptors or enzymes.[1,2] As one of molecular elements for such supramolecular systems, we have designed so-called steroid cyclophanes as artificial receptors being capable of recognizing organic molecules in bilayer membranes.[3-5] A steroid cyclophane (**1**) is constituted in combination with three functional components; a 1,6,20,25-tetraaza[6.1.6.1]paracyclophane ring,[6] four bile acid moieties, and four L-lysine residues connecting them. We have clarified that the cationic **1** acts as an efficient cell-surface receptor model for anionic guests, such as 6-(*p*-toluidino)naphthalene-2-sulfonate (TNS), in a bilayer membrane formed with an anionic peptide lipid bearing an L-alanine residue (**3**).[5] Bile acids are well-known natural compounds, and many studies have been carried out for characterization of them and their derivatives as hosts in various physical phases such as solid state,[7] and aqueous[8] and organic media.[9] On these grounds, we are to bring up the novel and potential capacity of bile acid moieties to recognize various organic guests in bilayer assemblies by employing the steroid cyclophanes. In this communication, we report that a steroid cyclophane bearing 3α,7α,12α-trihydroxy-5β-cholan-24-oic acid (cholic acid) moieties (**1**) furnishes a chiral recognition site for a hydrophobic guest in a bilayer membrane composed of **3** as well as in aqueous solution, as clarified by circular dichroism (CD) spectroscopy. An effect of hydroxyl groups placed in the steroid skeleton of **1** on molecular recognition was also examined in comparison with recognition behavior of an analogous steroid cyclophane having no hydroxyl groups (**2**).[10]





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In aqueous acetate buffer (10 mmol dm^{-3}, pH 5.0) at 30.0 °C, the CD spectrum of **1** exhibited two maximum (λ_{max}, nm) at 208 and 236 with molecular ellipticities ([θ], deg cm^2 dmol^{-1}) of −4.9 x 10^4 and +4.8 x 10^4, respectively, reflecting chirality of the L-lysine residues. Upon addition of TNS to this solution, we observed induced CD bands due to TNS incorporated into **1** (Figure 1). A formation constant (K) for the 1:1 host–guest complex of **1** with TNS was evaluated from the induced CD spectral change; 1.3 x 10^6 dm^3 mol^{-1}. The value is in good agreement with that (1.2 x 10^6 dm^3 mol^{-1}) previously obtained by fluorescence spectroscopy.[5] The K value for the complex of **2** with TNS was smaller than the above value with **1**, as evaluated by fluorescence spectroscopy; K = 3.8 x 10^5 dm^3 mol^{-1}. Although the induced CD bands due to TNS bound to **2** were also observed, λ_{max} and $[\theta]_{HG}$[11] values were different from those due to the identical guest bound to **1**: λ_{max} ($[\theta]_{HG}$) values were 276 (+3.8 x 10^4), 310 (+2.1 x 10^4), and 366 (−1.8 x 10^4) for the **1**–TNS complex and 280 (+2.3 x 10^4), 325 (+1.0 x 10^4), and 370 (−0.8 x 10^4) for the **2**–TNS complex. The results imply that the hydroxyl groups placed in the steroid skeleton of **1** give out a significant effect on the guest recognition ability through hydrogen-bonding interactions between them.

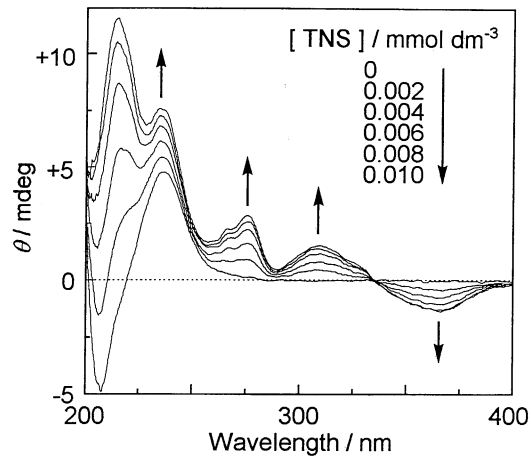

Figure 1. CD spectral change in complexation of TNS with **1** (0.010 mmol dm^{-3}) in an aqueous acetate buffer (10 mmol dm^{-3}, pH 5.0) at 30.0 °C; light path length, 10 mm.

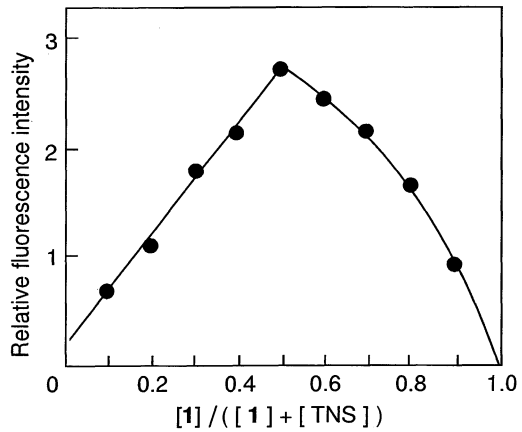

Figure 2. A continuous variation plot for complexation of TNS with **1** embedded in bilayer vesicle formed with **3** in an aqueous acetate buffer (10 mmol dm^{-3}, pH 5.0) at 30.0 °C: total concentrations of TNS and **1**, 0.010 mmol dm^{-3}; a molar ratio of **1** to **3**, 0.025. Excitation and emission wavelengths are 321 and 412 nm, respectively.

We have clarified previously that TNS is effectively bound to the hydrophobic binding site provided by **1** embedded in the bilayer vesicle formed with **3** through hydrophobic and electrostatic interactions.[5] We evaluated in this work both stoichiometry and formation constant for the host–guest complexation in the bilayer membrane by fluorescence spectroscopy under conditions that a composition of the steroid cyclophane relative to the peptide lipid was maintained constant in a molar ratio of 1:40. The continuous variation method applied to the present system (Figure 2) clearly indicates that **1** and TNS forms a 1:1 complex in the bilayer vesicle as well as in aqueous solution. The *K* value was determined on the basis of Benesi-Hildebrand plot;[12] 5.1 x 10^4 dm^3 mol^{-1}. The corresponding *K* value for the complex of TNS with **2** was 1.8 x 10^4 dm^3 mol^{-1}. Although the binding

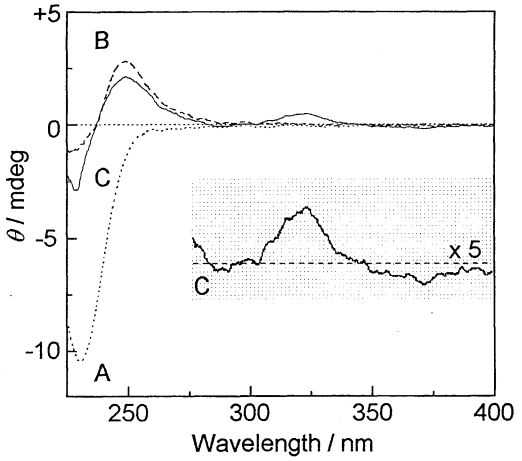

Figure 3. CD spectral change in complexation of TNS with **1** embedded in bilayer vesicle formed with **3** in an aqueous acetate buffer (10 mmol dm^{-3}, pH 5.0) at 30.0 °C: A, **3** (0.40 mmol dm^{-3}) alone; B, **1** (0.010 mmol dm^{-3}) and **3** (0.40 mmol dm^{-3}); C, TNS (0.010 mmol dm^{-3}), **1** (0.010 mmol dm^{-3}), and **3** (0.40 mmol dm^{-3}); light path length, 10 mm.

constants for the steroid cyclophanes embedded in the bilayer membrane were somewhat lower than those in aqueous solution, the superiority of **1** over **2** for molecular recognition of TNS was also retained in the bilayer membrane.

The bilayer vesicle formed with **3** showed a CD band at 231 nm with $[\theta]$ of -2.6×10^3 deg cm^2 $dmol^{-1}$ (Figure 3A). No induced CD was observed upon addition of TNS to this vesicular solution. CD spectra for the hybrid bilayer assembly of **1** with **3** in a molar ratio of 1:40 was nearly identical to a sum of the individual spectra (Figure 3B), strongly suggesting that the steroid cyclophane was incorporated into the membrane without meaningful conformational change.[13] In the present hybrid assembly, we observed induced CD bands due to TNS bound to **1**: λ_{max} ($[\theta]_{HG}$) values were 323 ($+1.9 \times 10^4$) and 371 (-0.7×10^4) (Figure 3C). On the other hand, the induced CD band was not detected in the hybrid assembly composed of **2** and **3**.

In conclusion, we demonstrated here the first example of an artificial cell-surface receptor capable of providing a chiral binding site for an organic guest molecule. The detailed mechanism involved in the molecular recognition is under investigation in our laboratory.

References and Notes

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10 Found: C, 72.35; H, 9.72; N, 6.47%. Calcd for $C_{154}H_{244}Cl_4N_{12}O_8 \cdot H_2O$: C, 72.49; H, 9.72; N, 6.59%.

11 A $[\theta]$ value for a guest upon 100% complexation with a host.

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13 A CD spectrum of the steroid cyclophane is very sensitive to solvent nature, reflecting its conformational change.