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Cyclophane-based tetra(resorcinarene) as a host for both histone and hydrophobic molecular guests

Osamu Hayashida[a,b,*] and Masaki Uchiyama[c]

[a]*PRESTO, JST, 4-1-8 Honcho Kawaguchi, Saitama 332-0012, Japan*
[b]*Institute for Materials Chemistry and Engineering, Kyushu University, Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan*
[c]*Department of Chemistry and Biochemistry, Kyushu University, Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan*

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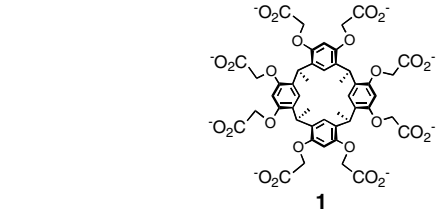
Abstract—A cyclophane-based resorcinarene tetramer, which is constructed with a tetraaza[6.1.6.1]paracyclophane and four resorcinarenes bearing hepta(carboxylic acid) residues that connect the macrocycle through amide linkages, was prepared. The binding constant of the cyclophane-based resorcinarene with immobilized histone was determined to be $1.3 \times 10^7\ M^{-1}$ by surface plasmon resonance measurements, which was 31-fold larger than that of the resorcinarene monomer bearing octacarboxylic acids. Moreover, the cyclophane-based tetra(resorcinarene) acted as a host toward hydrophobic molecular guests such as 6-*p*-toluidinonaphthalene-2-sulfonate.

Histones are small basic proteins that make up a substantial portion of eukaryotic nucleoproteins. Recently, histones that adopt posttranslational modifications such as methylation, acetylation, and phosphorylation, have been suggested to play an important role in diverse biological processes such as gene regulation and chromosome condensation.[1,2] Although there are many approaches for isolating histones by immunoprecipitation methods using antibodies, there have been surprisingly few studies on the development of artificial compounds that interact with histones and recognize bioorganic and chemical modifications of histones. Naturally occurring histone has a high content of the amino acid lysine, and shows an isoelectric point of 10.8. Therefore, artificial compounds such as resorcinarene derivative having eight anionic polar side chains **1**[3] are expected to be candidates for receptors capable of binding histone through electrostatic interactions. Accordingly, in order to enhance the favorable interactions between these artificial compounds and histones, we designed a novel polytopic resorcinarene having 28 carboxylate residues **4** on the basis of a molecular design that allows the assembly of four anionic resorcinarenes on an azaparacyclophane skeleton. So-called cluster effects by tetramerization of resorcinarene moieties are expected to enhance the cooperative binding. In addition, the cyclophane cavity has intrinsic potential to act as a host for guest inclusion, and the resulting cyclophane-based tetra(resorcinarene) is expected to be



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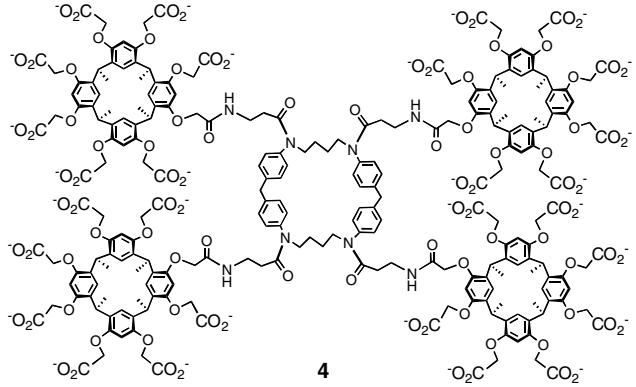

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Keywords: Cyclophane; Resorcinarene; Histone; Guest-binding; Multivalency.
* Corresponding author. Tel.: +81 92 642 4281; fax: +81 92 642 2715; e-mail: ohaya@ms.ifoc.kyushu-u.ac.jp

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utilized in molecular guest delivery systems toward histone. We describe herein the synthesis of cyclophane-based tetra(resorcinarene) **4**, which is constructed with a tetraaza[6.1.6.1]paracyclophane[4] and four resorcinarenes bearing heptacarboxylic acid residues that connect the macrocycle through amide linkages. In addition, the binding behavior of **4** with histone was examined by surface plasmon resonance (SPR) measurements, while its guest-binding abilities were evaluated in aqueous media by fluorescence spectroscopy.

Cyclophane-based resorcinarene **4** was prepared by following the reaction sequence given in Scheme 1. A resorcinarene derivative bearing a carboxylic acid residue **3** was obtained by partial hydrolysis from octa-ester[5] **2** in a 14% yield. Precursor **6** was synthesized by condensation of **3** with a tetraamine derivative of cyclophane **5**[6] in the presence of benzotriazol-1-yloxy-tris(dimethylamino)-phosphonium hexa-fluorophosphate (BOP) in dry *N*,*N*-dimethylformamide. Cyclophane-based resorcinarene **4** was obtained by alkali hydrolysis of the ester groups of **6**. All the new compounds were fully characterized by means of spectroscopy (^{1}H and ^{13}C NMR, and TOF-MS) and elemental analysis.[7]

Based on our investigation of the CPK molecular model, the molecular size of **4** in the extended conformation is 3.8–5.0 nm in the XY plane, while resorcinarene monomer **1** has a size of ca. 1.5 nm (Fig. 1). Cyclophane-based resorcinarene **4** provides a hydrophobic cavity suitable for encapsulating small, complementary organic molecules as a guest, as well as four heptaanionic resorcinarene derivatives with reasonably separated distances, which can be expected to confer the advantage of enhanced solubility in aqueous neutral media at biological pH. From a practical standpoint, cyclophane-based resorcinarene bearing 28 carboxylic acid residues **4** had good solubility of >0.4 g mL^{-1} in aqueous 2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid (HEPES) buffer (0.01 M, pH 7.4, with 0.15 M NaCl).

Cyclophane-based tetra(resorcinarene) **4** (10 μM) was agglutinated by histone (270 μg mL^{-1}), which was readily monitored by the visible turbidity of the solution due to a polyion complexation of these components to give an insoluble material. On the other hand, upon addition of histone to HEPES buffer containing **1**, the turbidity

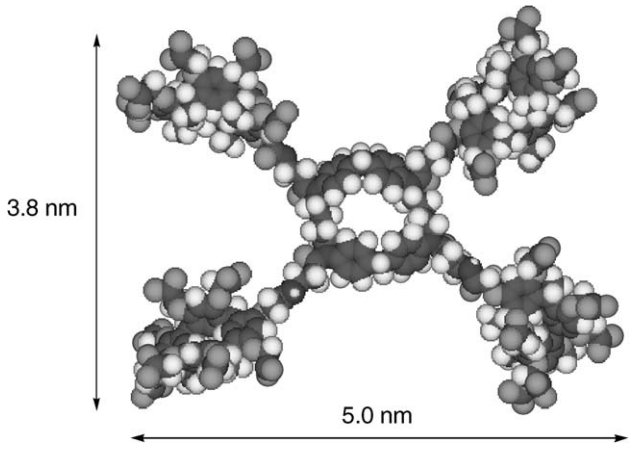
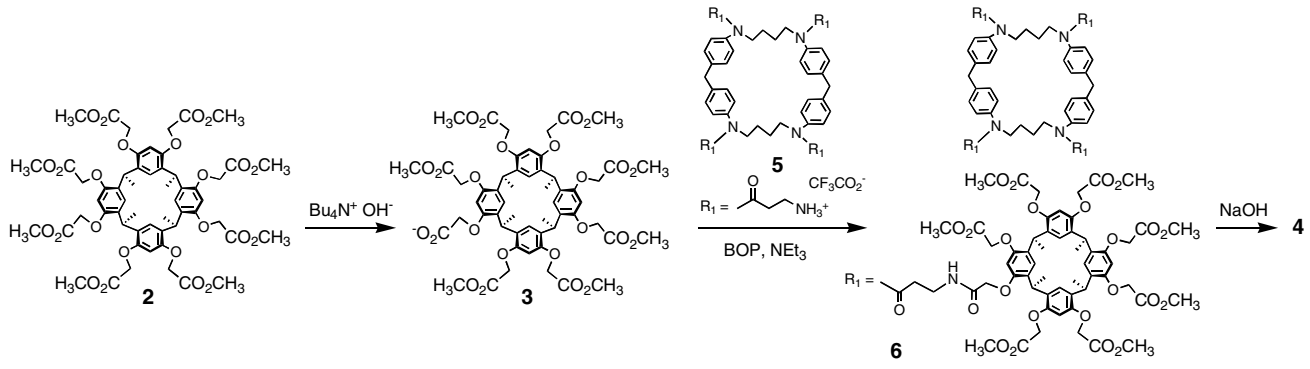


Figure 1. Probable computer-generated CPK model for cyclophane-based tetra(resorcinarene) **4**.

of the solution became almost negligible under identical conditions. Furthermore, we examined the binding interactions of **4** and resorcinarene monomer **1** to an immobilized histone on a sensor chip by SPR[8] measurements. First, immobilization of histone[9] to the carboxylated dextran sensor chip surface (CM5), which was set in BIAcore X (Pharmacia Biotech), was performed by utilizing the EDC–NHS coupling protocol.[10] The amount of immobilization of histone was given as a resonance signal of 6112 RU (resonance units). This value corresponds to an occupation density of 6.1 ng mm^{-2} for histone on the chip, because a 1000 RU[11] corresponds to a surface concentration change of 1 ng mm^{-2}. Second, when a solution of **4** in HEPES buffer was injected over surfaces of immobilized histone, the association was observed as shown in Figure 2A (a, 0.78 μM) and 2B (d, 0.20 μM). Then, by changing the HEPES buffer to wash away the noncovalently bound **4**, the dissociation was initiated and observed as shown in Figure 2A(b) and B. The immobilized histone surface was regenerated with an injection of aqueous sodium hydroxide (50 mM) (see Fig. 2A(c)). The binding constant (*K*) of cyclophane-based resorcinarene **4** with immobilized histone was determined to be 1.3×10^7 M^{-1} on the basis of kinetic analysis in a manner similar to that reported previously.[12] On the other hand, cyclophane-based resorcinarene **4** was hardly adsorbed on the surface of immobilized ovalbumin



Scheme 1. Preparation of cyclophane-based tetra(resorcinarene) **4.**

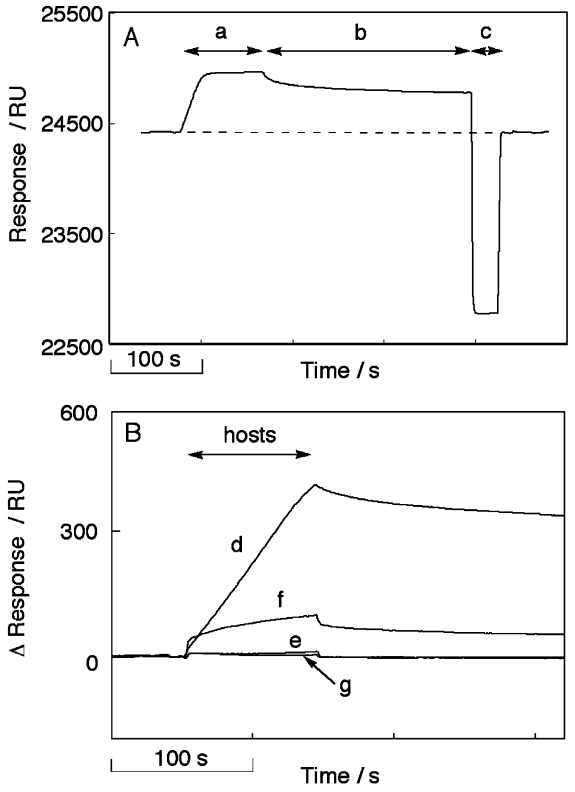


Figure 2. (A) Response curve obtained during (a) and after (b) injection of **4** (0.78 μM) on the immobilized histone surfaces. An aqueous sodium hydroxide (c, 50 mM) was used to regenerate. (B) Oberlay sensorgrams of **4** (d, 0.20 μM) and **1** (e, 0.20 μM; f, 13 μM) with immobilized histone surface. Sensorgram of **4** (g, 0.20 μM) with immobilized ovalbumin surface. Flow rate: 20 μL min^{-1}, in HEPES buffer.

(Mw 43 kDa), as an inert protein having an isoelectric point of 4.6 (Fig. 2B(g)), which was also prepared in a manner similar to that used for histone immobilization: 5900 RU, 5.9 ng mm^{-2}. This, coupled with the above results, suggests that polyanionic cyclophane-based tetra(resorcinarene) **4** interacts with the immobilized histone, reflecting formation of the polyion complexes, but not with ovalbumin. However, cyclophane-based resorcinarene **4** was hardly interacted with the immobilized lysozyme as confirmed by SPR measurements, even though lysozyme was a basic protein having an isoelectric point of 11.0. Therefore, **4** exhibited potent recognition capability toward histone. In addition, the cluster effect achieved by multiplying of resorcinarenes[13] seems to be reflected in the binding ability of **4**, because resorcinarene octaacid **1** shows poor RU responses under identical conditions (0.20 μM) (Fig. 2B(e)). An association curve was observed (Fig. 2B(f)); however, when a solution of **1** was injected at higher concentration (13 μM), the binding affinity of **1** toward histone was much weaker than that of **4**: K, 4.2×10^5 M^{-1}. Therefore, the binding constant of **4** with immobilized histone was enhanced by 31-fold relative to that of **1**. The enhancement observed for **4** was one of the cluster effects.

Fluorescence spectroscopy at 298 K was used to examine the guest-binding ability of **4** as a host toward well-known fluorescent guests such as 6-*p*-toluidinonaphthalene-2-sulfonate (TNS), 2-anilinonaphthalene-6-sulfonate (2,6-ANS), and dansylamide, whose emission is extremely sensitive to change in the microenvironmental polarity experienced by the molecules. The fluorescence intensity originated from TNS (0.25 μM) increased upon addition of the cyclophane, as shown in Figure 3. On the other hand, the fluorescence spectral changes were also negligible upon addition of resorcinarene octacarboxylic acid **1** to the aqueous HEPES buffer containing TNS (data not shown). The micro-environmental polarity experienced by the entrapped guest molecule was evaluated on the basis of the correlation between λ_{max} and solvent polarity parameter (E_T^N)[14] as described previously.[15] The E_T^N value for TNS placed in **4** was estimated to be 0.62 (425 nm), which was equivalent to the value for 1-propanol. Furthermore, relatively large fluorescence polarization values (P) were obtained for the identical guest incorporated into **4** (P, 0.15). The obtained P value was somewhat larger than that for TNS bound to a tetraaza[6.1.6.1]paracyclophane[16] bearing four L-valine residues (P, 0.09). This indicates that the tight host–guest interaction brings about effective motional repression of the entrapped guest. A similar complexation behavior of **4** was also confirmed with 2,6-ANS and dansylamide. The 1:1 binding constants (K) of **4** toward these guests were evaluated on the basis of the Benesi–Hildebrand relationship: K, 4.2×10^3, 2.5×10^3, and 1.7×10^3 M^{-1}, for the complexes of TNS, 2,6-ANS, dansylamide, respectively.

Moreover, upon addition of histone to the solution containing **4** and TNS, insoluble materials were readily formed. After removal of the insoluble materials by filtration, there was a 67% decrease in fluorescence intensity which originated from the entrapped guest molecules, reflecting the fact that most of the complexes were precipitated as ternary complexes with histone. In the case of **1**, however, a certain percentage of the guest molecules were simply trapped on histone surfaces from bulk aqueous solution upon addition of histone.

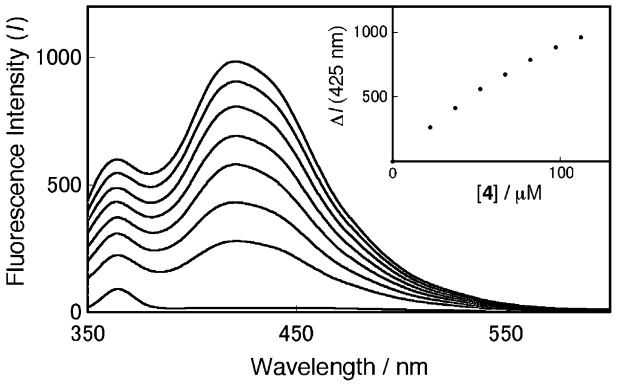


Figure 3. Fluorescence spectral changes for aqueous solution of TNS (0.25 μM) upon addition of **4** in HEPES buffer (0.01 M, pH 7.4, with 0.15 M NaCl) at 293 K: [**4**] = 0, 22.5, 37.5, 52.5, 67.5, 82.5, 97.5, and 125 μM (from bottom to top). Inset: the corresponding titration curve. Ex, 321 nm.

In conclusion, cyclophane-based tetra(resorcinarene) was synthesized on the basis of a molecular design that allows the connection of four resorcinarene derivatives to a cyclophane skeleton. The hydrophobic internal cavity provided by the cyclophane binds guest molecules such as TNS and 2,6-ANS, while the polyanionic external surface of the tetra(resorcinarene) interacts with immobilized histone. The resulting supramolecules formed with cyclophane-based tetra(resorcinarene) as a host and fluorescence probe as a guest are expected to be useful as reagents for detecting histones. In addition, the peripheral resorcinarene of **4** was expected to have the potential to encapsulate tri-methylated lysine of histone and recognize the posttranslational modifications (methylation) of histone. Further studies are currently underway to investigate these predictions.

Acknowledgements

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7. Compound **3**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.49 (d, 12H), 3.69–3.80 (m, 21H), 4.59 (br, 14H), 4.71–4.73 (q, 8H), 6.15–6.30 (m, 4H). ^{13}C NMR (150 MHz, $CDCl_3$, 298 K): δ 20.16, 20.22, 31.08, 31.13, 31.19, 31.23, 52.09, 52.32, 52.34, 52.44, 52.63, 66.86, 67.27, 67.35, 67.40, 67.48, 67.55, 67.64, 67.78, 100.13, 100.86, 101.92, 125.91, 126.03, 126.24, 126.37, 126.49, 129.90, 130.60, 153.56, 154.36, 154.48, 154.78, 154.91, 170.09, 170.18, 170.26. MS (MALDI-TOF) *m*/*z*, 1108 $[M+H]^+$, 1130 $[M+Na]^+$, 1145 $[M+K]^+$. Found: C, 59.23; H, 5.64. Calcd for $C_{55}H_{62}O_{24}\cdot 0.5H_2O$: C, 59.19; H, 5.69. Compound **6**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.2 (m, 8H), 1.4–1.6 (m, 48H), 3.2–3.4 (m, 8H), 3.6–3.8 (m, 8H), 4.0–4.3 (m, 8H), 4.3–4.6 (m, 56H), 4.6–4.8 (m, 16H), 6.0–6.4 (m, 16H), 6.96–6.98 (d, $J = 8$ Hz, 8H), 7.16–7.18 (d, $J = 8$ Hz, 8H). ^{13}C NMR (150 MHz, $CDCl_3$, 298 K): δ 18.65, 18.76, 18.88, 24.10, 29.76, 29.84, 29.87, 29.95, 33.35, 34.29, 40.02, 47.95, 50.04, 50.94, 50.97, 51.01, 51.10, 51.39, 65.90, 65.99, 66.04, 66.86, 98.50, 124.7, 124.9, 127.3, 129.3, 139.3, 139.5, 152.3, 152.9, 153.5, 167.3, 168.5, 168.6, 168.7, 168.8, 169.4. MS (MALDI-TOF) *m*/*z*, 5168 $[M+Na]^+$. Found: C, 60.93; H, 5.79; N, 2.26. Calcd for $C_{266}H_{300}N_8O_{96}\cdot 4H_2O$: C, 61.24; H, 5.95; N, 2.15. Compound **4**: ^{1}H NMR (400 MHz, D_2O): δ 1.4 (m), 1.5 (m), 2.0 (m), 3.5 (m), 3.7 (m), 5.7 (m), 6.0 (m), 6.4 (m), 6.9 (m). ^{13}C NMR (150 MHz, D_2O, 298 K): δ 19.66, 19.97, 20.10, 20.4, 24.5, 30.5, 35.8, 39.1, 49.1, 61.9, 68.9, 100.5, 125.8, 128.1, 130.9, 139.7, 141.3, 154.3, 155.2, 162.4, 171.7, 177.5. IR 1590 cm^{-1} (C=O). MS (MALDI-TOF) *m*/*z*, 4752 $[M-H]^-$. Found: C, 58.39; H, 5.18; N, 2.41. Calcd for $C_{238}H_{244}N_8O_{96}\cdot 7H_2O$: C, 58.59; H, 5.33; N, 2.30.
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