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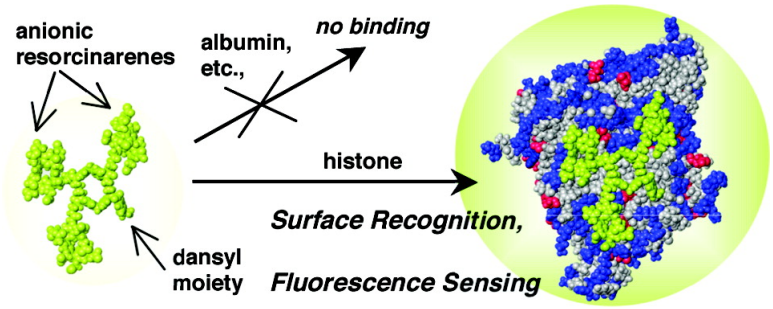

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Surface Recognition and Fluorescence Sensing of Histone by Dansyl-Appended Cyclophane-Based Resorcinarene Trimer

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Abstract: A cyclophane-based resorcinarene trimer (**3**) bearing a dansyl moiety as an environmentally sensitive fluorophore was prepared by stepwise condensation of a tetraaza[6.1.6.1]paracyclophane skeleton with a dansyl moiety and three resorcinarene derivatives having heptacarboxylic acid residues in this sequence. The dansyl-appended cyclophane exhibited the following fluorescence properties regarding solvent polarity dependency and histone surface recognition: With increasing dioxane contents in dioxane/water solvents, the fluorescence intensity originating from the dansyl moiety of **3** increased along with a concomitant blue shift of the fluorescence maximum (λ_{em}). The microenvironmentally sensitive fluorescence properties of dansyl fluorophore were maintained, even when the dansyl moiety was covalently attached to a cyclophane. Most interestingly, the cyclophane-based resorcinarene trimer exhibited recognition and fluorescence sensing capabilities toward histone, a small basic protein of eukaryotic chromatins. The fluorescence intensity originating from **3** increased along with a concomitant blue shift of λ_{em} upon the addition of histone, reflecting the formation of **3**−histone complexes. A relatively large fluorescence polarization (*P*) value was obtained for the **3**−histone complexes (0.15), reflecting highly restricted conformations of **3**, and the obtained *P* value was much larger than that of **3** alone in aqueous medium (0.07). The binding constant (*K*) of **3** with histone (unit basis) was estimated to be 2.1×10^6 M^{-1}. On the other hand, upon the addition of acetylated histone (Ac-histone) to an aqueous solution containing **3**, the extent of change in fluorescence intensity originating from the dansyl group of **3** was almost negligible, indicating that the electrostatic interactions between **3** and Ac-histone were weak. In addition, the fluorescence spectral changes were also small or negligible upon the addition of other proteins such as albumin, ovalbumin, peanut agglutinin, myoglobin, concanavalin A, cytochrome *c*, and lysozyme, having isoelectric points of 4.7, 4.8, 5.7−6.7, 6.8, 7.1, 9, and 11.0, respectively, to an aqueous solution containing **3**.

Introduction

The development of synthetic agents (artificial receptors)[1] exhibiting protein surface recognition capability is of great importance for applications such as sensing, modulating, and inhibiting of specific protein−protein interactions.[2] Organic chemists have made numerous attempts to develop artificial receptors on the basis of macrocycles,[3] porphyrins,[4] and other molecular scaffolds[5] that can recognize and bind to specific protein surface. The molecular recognition behavior of the protein surface receptors depends principally on the size, shape, hydrophobicity, and hydrophilicity of the binding sites; these characteristics complement the distribution of functionality on the protein surface.[6] In their pioneering works on protein surface recognition using artificial receptors, Hamilton and co-workers

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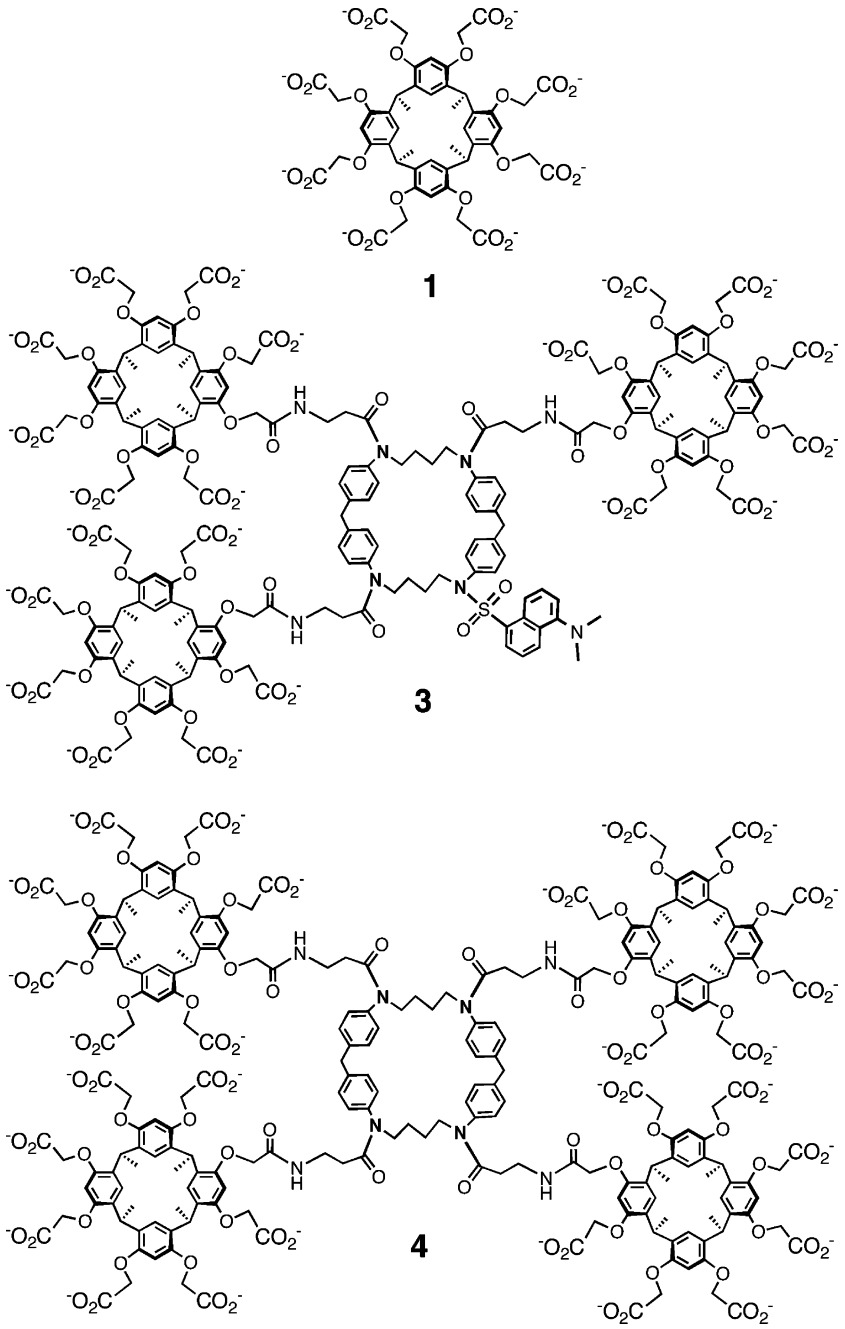

Figure 1. Anionic resorcinarene, dansyl-appended cyclophane-based resorcinarene trimer, and cyclophane-based resorcinarene tetramer.

have successfully developed multiple peptide receptors on the basis of a well-defined calix[4]arene that behaves as a competitive inhibitor of cytochrome *c*.[7] They have also described the use of tetraphenylporphyrin derivatives bearing anionic side chains and functionalized terphenyl scaffolds as binders of cytochrome *c*.[8] For the surface recognition of other specific proteins, Cunsolo et al. have designed calix[8]arene receptors bearing basic amino acid residues to bind human tryptase,[9] while Neri et al. demonstrated the surface recognition of transglutaminase by calix[4]arene that had peptides the authors designed.[10]

Histones, basic nuclear proteins in eukaryotic chromatins, are also attractive as target proteins. That is, histones, which adopt posttranslational modifications such as methylation, acetylation, and phosphorylation, have been suggested to play important roles in diverse biological processes such as gene regulation and chromosome condensation.[11] Therefore, the ability of artificial receptors to detect histones is valuable. Naturally occurring histones have high contents of the amino acids lysine and arginine and show an isoelectric point (pI) of 10.8. On these grounds, we previously revealed that anionic resorcinarene having octacarboxylic acid residues (**1**)[12] is a favorable candidate as a histone receptor[13] (Figure 1). Considering the importance

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of multivalency[14] on the binding sites, we have developed a tetratopic resorcinarene having 28 carboxylate residues (**4**)[15] on the basis of a molecular design that allows the assembly of four anionic resorcinarenes on the tetraaza[6.1.6.1]paracyclophane[16] as a rigid molecular skeleton (Figure 1). We have also clarified that the resulting cyclophane-based resorcinarene tetramer exhibited so-called multivalent effects[14] on the binding with histone according to surface plasmon resonance (SPR) measurements.[15]

On the other hand, fluorescence-probe methods are powerful techniques for studying molecular interactions in analytical chemistry, biochemistry, and cell biology.[17] For instance, environmentally sensitive probes such as 5-(dimethylamino)-naphthalene-1-sulfonyl (dansyl) derivatives have the following characteristic properties on fluorescence emission.[18] With the molecule exercising decreasing microenvironment polarity, its emission intensity increases with a blue shift in its wavelength, while its fluorescence intensity is relatively weak in water. Therefore, the hydrophobicity of the probes enabled the detection and visualization of hydrophobic domains of biomolecules such as cell membranes and lipophilic proteins by fluorescence imaging spectroscopy and microscopy.[18]

In the course of our ongoing research on histone surface recognition, we became interested in developing fluorescent-appended[19−21] cyclophane-based resorcinarene oligomers capable of binding and sensing histone surfaces. We have adopted a simple strategy to develop fluorescent-appended histone surface receptors by conjugating the cyclophane-based resorcinarene oligomers with an environmentally sensitive fluorescence probe. We report here the design and synthesis of a cyclophane-based resorcinarene trimer bearing a fluorophore (**3**) (Figure 1) and its fluorescence properties regarding solvent polarity dependency and histone surface recognition in aqueous medium by fluorescence spectroscopy, with an emphasis on the specificity.

Results and Discussion

Design and Synthesis of Dansyl-Appended Cyclophane-Based Resorcinarene Trimer. A fluorescent-functionalized tetraaza[6.1.6.1]paracyclophane (**3**) bearing three binding side branches and a fluorophore was designed for histone surface recognition and fluorescent sensing. The side branches capable of binding histone were derived from a resorcinarene derivative and were chosen so as to contain heptacarboxylic acid residues. We have previously clarified that the binding affinity of cyclophane-based resorcinarene tetramer **4** with histone was 31-fold larger than that of an untethered resorcinarene octacarboxylic acid **1**,[22] reflecting the multivalent effects on binding sites.[14] The fluorescent cyclophane-based resorcinarene trimer **3** is also expected to exhibit potent multivalent effects in the binding with histone. A dansyl group, whose emission is sensitive to change in microenvironmental polarity, was adopted as a fluorophore and introduced directly into the cyclophane skeleton through a sulfonamide linkage.

We have focused on partially Boc-protected tetraaza[6.1.6.1]-paracyclophane as a macrocyclic framework on which to assemble three resorcinarene derivatives and a dansyl moiety. The fluorescent cyclophane **3** was prepared by following the reaction sequence given in Scheme 1. Tris(Boc-β-alanyl)-tetraaza[6.1.6.1]paracyclophane (**2**)[22] was dansylated with dansyl chloride to obtain the dansyl-appended cyclophane (**5**). Precursor **6** was subsequently obtained by condensation of resorcinarene derivative (**7**) bearing a carboxylic acid residue[14] with a triamine derivative of cyclophane, which was prepared by removal of the Boc-protecting groups of **5**, in the presence of BOP as a coupling agent. The final step was then the alkali hydrolysis of the precursor **6** to give the dansyl-appended cyclophane-based resorcinarene oligomer **3** (4 steps, 48% overall yield).

Molecular mechanics studies on fluorescent cyclophane-based resorcinarene trimer **3**, followed by molecular dynamics simulations (MacroModel 7.5, OPLS 2005, water, 2000 steps; MD, 300 K, 10 ps), gave us useful information about the conformations. For the optimized conformation of **3**, the molecular size of **3** in the extended conformation is 3.3−5.1 nm in the *xy* plane, as shown in Figure 2, while resorcinarene monomer **1** is about 1.5 nm. The hydrophobicity of the cyclophane cavity is relatively masked by negatively charged resorcinarene derivatives surrounding the macrocycle. The exterior charged resorcinarene residues could confer the advantage of enhanced solubility in aqueous, neutral media at biological pH. In addition, the dansyl moiety of **3** was rigidly introduced into the macrocyclic skeleton through sulfonamide linkage, while the triple branches of resorcinarene derivatives have conformational flexibility because the alkyl spacer has an ether bond, as suggested by molecular dynamics simulations (Figure 2b). The three heptaanionic resorcinarene derivatives of **3** were expected to afford negatively charged binding sites adjustable for lysine-rich domains of histone.

Fluorescent Properties of Dansyl-Appended Cyclophane-Based Resorcinarene Trimer. First, the concentration dependency of the fluorescence spectra was investigated for an aqueous 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) buffer (0.01 M, pH 7.4, with 0.15 M NaCl) of **3** at various concentrations at 298 K. At least at the concentrations below 1.0×10^{-5} M of **3**, the fluorescence intensity originating from the dansyl group of **3** increased in a linear fashion as its concentration increased without any changes in its maximum

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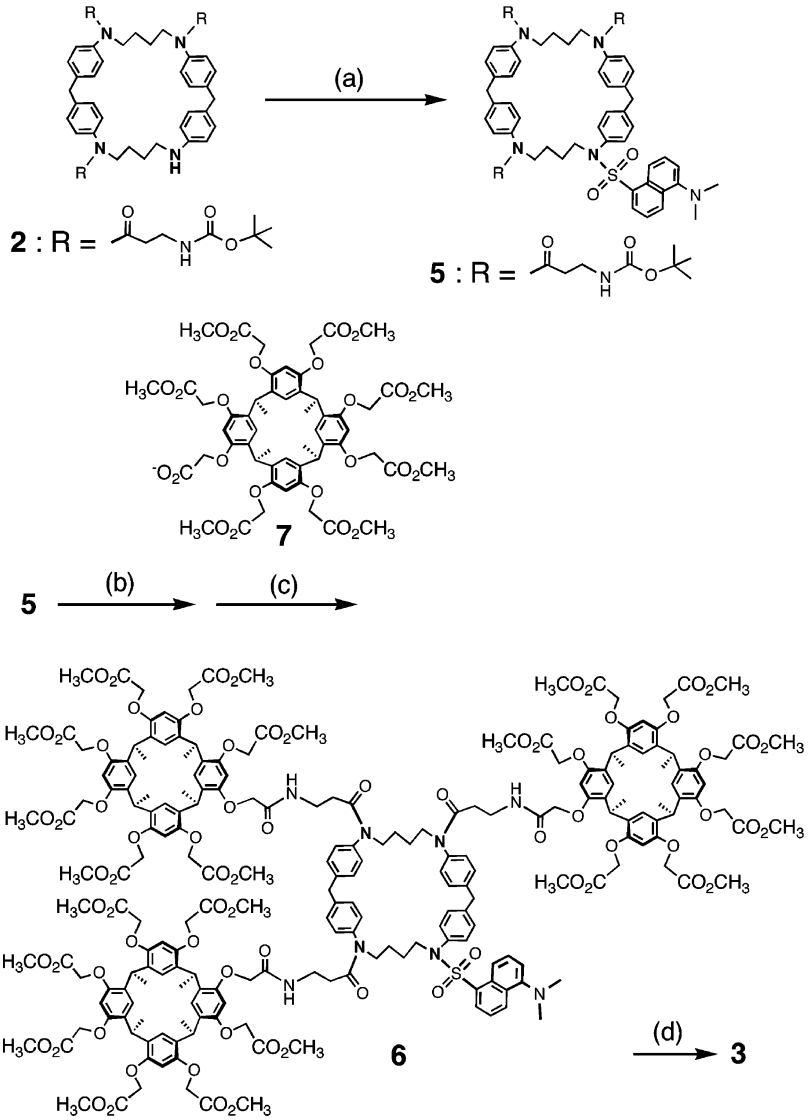
Scheme 1. Preparation of Dansyl-Appended Cyclophane-Based Resorcinarene Trimer



Reagents and conditions: (a) dansyl chloride, DCM; (b) 30% TFA; (c) **7**, BOP, DMF; (d) NaOH, H_2O, THF.

(λ_{em}, 538 nm) (see SI (Supporting Information)). These results indicate that **3** is in a monomeric state under these conditions. Even though molecular a modeling study indicates that compound **3** has a somewhat amphiphilic nature due to a hydrophobic cyclophane and hydrophilic branches, the high negative charge density of **3** seems to inhibit the intermolecular complexes (self-aggregates). From a practical standpoint, the cyclophane-based resorcinarene oligomers **3** had good water-solubility values of >0.4 g mL^{-1}.

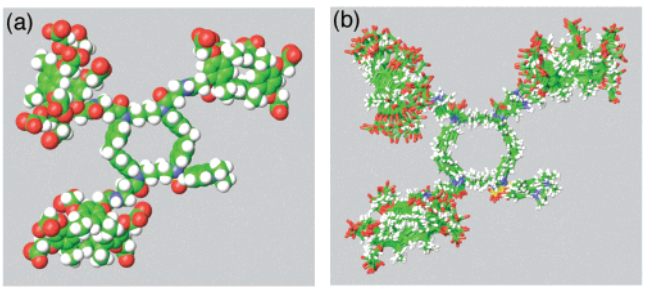


Figure 2. Computer-generated CPK model (a) and molecular dynamics calculations (b) for the dansyl-appended cyclophane-based resorcinarene trimer **3.** Carbon, hydrogen, oxygen, nitrogen, and sulfur atoms are shown in green, white, red, blue, and yellow, respectively.

Second, solvent polarity's dependence on the emission spectra of **3** (0.5 μM) was also examined by fluorescence spectroscopy. It is well-known that placement of dansyl fluorophore in a hydrophobic environment causes a significant increase in quantum yield and a blue shift of the fluorescence emission spectra.[23] With increasing dioxane contents in dioxane/water solvents, the fluorescence intensity originating from the dansyl moiety of **3** increased along with a concomitant blue shift of the fluorescence maximum (λ_{em}), as shown in Figure 3. Additionally, the fluorescence quantum yield of **3** was evaluated to range from 0.15 in water to 0.73 in dioxane, which was an expected trend for dansyl fluorophore.[23] The microenvironmentally sensitive fluorescence property of **3** was maintained even when a dansyl moiety was covalently attached to the cyclophane skeleton. In other words, the dansyl moiety of **3** was exposed

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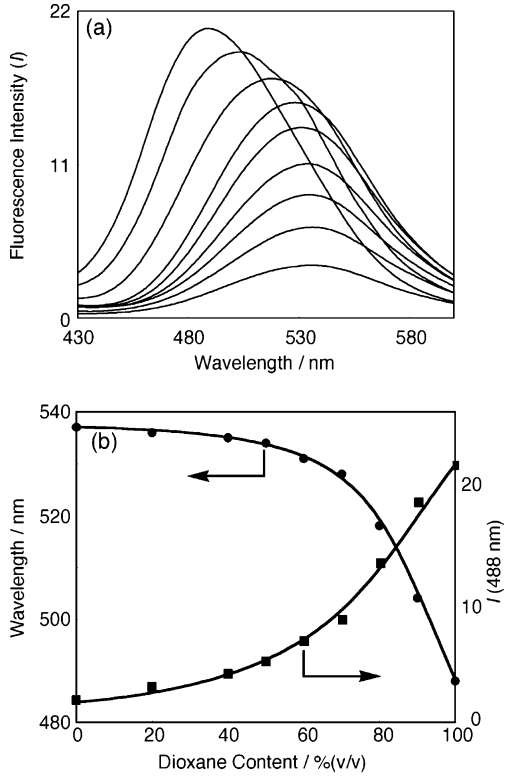

Figure 3. Solvent polarity dependence of fluorescence spectra of **3** (0.5 μM) in dioxane/water solvents at 298 K: dioxane content (%, v/v) in dioxane/water mixtures of 0, 20, 40, 50, 60, 70, 80, 90, and 100 (from bottom to top) (a); correlations of the maximum wavelength (λ_{em}) of fluorescence spectra of **3** and its intensity at 488 nm with a dioxane content (%, v/v) (b).

to the bulk solvent phases, whose emission was sensitive to changes in the microenvironmental polarity experienced by the molecule. The structural characteristics of **3** were also suggested from our molecular model studies mentioned above; i.e., the branched dansyl moiety of **3** does not have enough conformational flexibility for self-inclusion because of the rigid sulfonamide linkages.

Histone-Binding by Dansyl-Appended Cyclophane-Based Resorcinarene Trimer. The binding behavior of **3** with histone was conveniently monitored by fluorescence spectroscopy. Upon the addition of histone to an aqueous HEPES buffer (0.01 M, pH 7.4, 0.15 M with NaCl) containing **3** (0.5 μM), the fluorescence intensity originating from the dansyl group of **3** increased by about 5-fold along with a concomitant blue shift of the λ_{em} (from 538 to 502 nm), showing a saturation behavior for the complexation of **3** with histone as shown in Figure 4. This color change can be also visually monitored by the naked eye as the light-yellow solution turns into light-green by the histone addition. The quantum yield of **3** was increased from 0.15 to 0.6 in the absence and presence of histone, respectively, reflecting the complexation. The emission originating from the dansyl group is sensitive to change in microenvironmental polarity and possibly to that in microviscosity. On the basis of the correlation between λ_{em} and solvent polarity (Figure 3b), the microenvironmental polarity parameter,[24] E_T^N, experienced by the dansyl moiety of **3** bound to histone was estimated to be 0.58 equivalent to a value of 90% (v/v) dioxane−water (E_T^N = 0.58). These results suggest that the dansyl moiety of **3** was located on nonpolar surfaces of histone, which was shielded from the bulk aqueous phase, upon complexation with histone. In addition, a relatively large fluorescence polarization (*P*) value was obtained for **3** bound to histone surfaces (0.15). The obtained *P* value was much larger than that of **3** alone in aqueous medium (0.07) and was comparable to those of dansyl−histone conjugates[25] (0.16). These results indicate that the tight **3**−histone interaction, which brings about a marked motional repression of the dansyl moiety of **3**, becomes effective. Such increase in apparent microviscosity around the dansyl moiety of **3** upon complexation with histone reflects the change in fluorescence spectra (i.e., fluorescence intensities and fluorescence maxima).

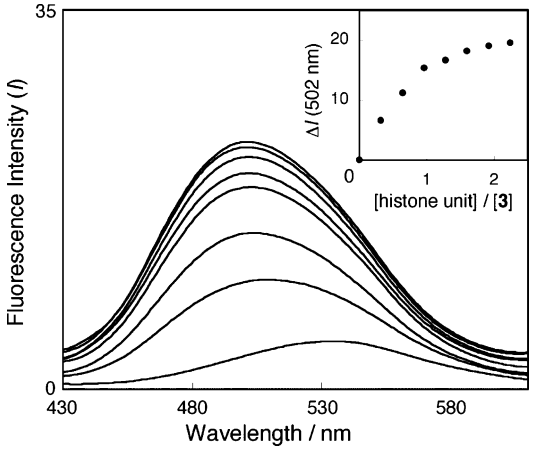

Figure 4. Fluorescence spectral changes for aqueous solution of **3** (0.5 μM) upon addition of histone in HEPES (0.01 M, pH 7.4, 0.15 M with NaCl) at 298 K: [histone] = 0, 0.02, 0.04, 0.06, 0.08, 0.10, 0.12, and 0.14 μM (from bottom to top). Inset: Corresponding titration curve. Excitation = 333 nm.

The stoichiometry for the complexes of **3** with histone was confirmed to be 1:1 host:histone (unit basis) by a Job plot (see SI). The 1:1 binding constant (*K*) of **3** toward histone (unit basis) was evaluated on the basis of the computer-aided least-squares curve-fitting methods applied to the fluorescence titration data as shown in Figure 4 (*K*; 2.1×10^6 M^{-1}). The electrostatic attraction between **3** and histone was a major driving force for molecular recognition, because the *K* value was subjected to an increase (1.7×10^7 M^{-1}) in a lower ionic strength HEPES buffer (0.01 M, pH 7.4, 0.05 M with NaCl). Clearly, negatively charged resorcinarene moieties of **3** were responsible for the histone binding because neither the dansyl amide nor a precursor of **3** lacking only the three resorcinarene moieties showed meaningful fluorescence spectral changes upon the addition of histone (see SI). In addition, replacements of the heptaanionic resorcinarene derivatives of **3** with poly(acrylic acid)[26] having an average molecular weight of 2100 or saccharide branches having terminal galactose residues resulted in the disappearance of the affinity toward histone (see SI), reflecting the importance of the rigid macrocyclic nature as well as the negative charges of resorcinarene moieties of **3** for histone binding.

We previously clarified the binding affinity of resorcinarene tetramer **4** and single octacarboxylated resorcinarene **1** with

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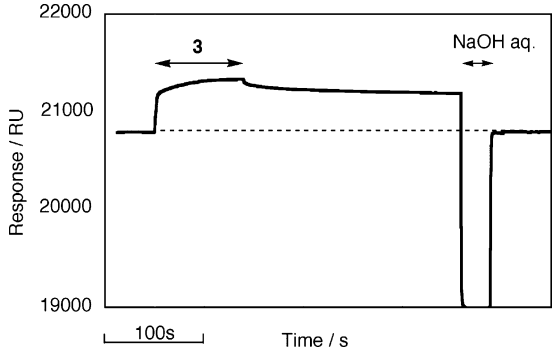

Figure 5. Response curve obtained by the injection of **3** (13 μM) on immobilized histone surfaces. Aqueous sodium hydroxide (50 mM) was used to regenerate the surface. Flow rate: 20 μL min^{-1}, in HEPES buffer.

immobilized histone on a sensor chip surface by means of SPR measurements.[15] The *K* value of **3** toward immobilized histone was also evaluated from the SPR methods in a manner similar to that reported previously. When a solution of **3** in HEPES buffer (0.01 M, pH 7.4, 0.15 M with NaCl) was injected over surfaces of immobilized histone,[27] the association shown in Figure 5 was observed. Then, by our changing the HEPES buffer to wash away the noncovalently bound **3**, the dissociation was initiated and observed as shown in Figure 5. The immobilized histone surface was regenerated with an injection of aqueous sodium hydroxide (50 mM) (Figure 5). The binding constant (*K*) of **3** with immobilized histone was determined to be 5.7×10^6 M^{-1} on the basis of kinetic analysis in a manner similar to that reported previously.[15,28] By combining these results with the fluorescence results, we can see that resorcinarene trimer **3** shows binding affinity toward histones in the order 10^6 M^{-1} in the aqueous HEPES buffer as well as on the sensor chip surface.[29] In comparison with the corresponding value of **1** with immobilized histone (4.2×10^5 M^{-1}),[15] a 14-fold increase[30] in the *K* value of **3** relative to **1** can be regarded as a "multivalency effect" that is exhibited by the resorcinarene trimer. The *K* value of resorcinarene trimer **3** was somewhat smaller than that of **4** (1.3×10^7 M^{-1}),[15] reflecting a decrease in the number of resorcinarene binding sites. Such a difference in histone-binding affinity between **3** and **4** was also confirmed by fluorescence competition experiments. That is, upon the addition of a tetramer of resorcinarene **4** to an aqueous solution containing **3**−histone complexes, the saturated fluorescence intensity originated from **3** bound to histone was subjected to decrease to almost the original position, indicating that **4**, having four resorcinarene moieties, was bound to the histone in place of **3**.

(27) The degree of immobilization of histone was given as a resonance signal of 5340 RU (resonance units).
(28) Hayashida, O.; Mizuki, K.; Akagi, K.; Matsuo, A.; Kanamori, T.; Nakai, T.; Sando, S.; Aoyama, Y. *J. Am. Chem. Soc.* **2003**, *125*, 594−601.
(29) There was a difference in the *K* values estimated by fluorescence and SPR methods. The difference was attributable to the respective complexation behaviors of **3** toward nonimmobilized histone in a solution and immobilized histone on the SPR sensor chip surface.
(30) This was also confirmed by a competitive inhibition experiment. A single octacarboxylated resorcinarene macrocycle (**1**) as an inhibitor competes for the binding sites of histone with **3**. The binding constant (K_I) of **1** toward histone (unit basis) was estimated by the methods reported by Diederich and Dick according to eq 1 (Diederich, F.; Dick, K. *J. Am. Chem. Soc.* **1984**, *106*, 8024−8036.). In eq 1, K_{app} is an apparent binding constant of **3** toward histone, while I_0 is the total concentration of inhibitor **1**. The K_I value was calculated to be 1.8 x 10^5 M^{-1}. On the other hand, the *K* value of resorcinarene trimer **3** toward histone was evaluated to be 2.1 x 10^6 M^{-1} by fluorescence titration experiment as mentioned in the text. Again, we can see that the guest-binding affinity of **3** toward histone was enhanced (12-fold), reflecting the multivalency effect achieved by multiplying the resorcinarene: $K_I = \{K - K_{app}\}/\{K_{app}\, I_0\}$ (eq 1).

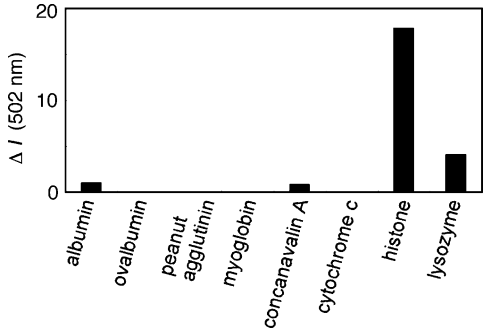

Figure 6. Changes in fluorescence intensity at 502 nm for an aqueous solution of **3** upon addition of proteins (0.1 μM) such as albumin, ovalbumin, peanut agglutinin, myoglobin, concanavalin A, cytochrome *c*, histone, and lysozyme having isoelectric points of 4.7, 4.8, 5.7−6.7, 6.8, 7.1, 9, 10.8, and 11.0, respectively. Excitation = 333 nm.

To examine the binding specificity, we examined the fluorescence spectra of **3** in the presence of acetylated histone (Ac-histone) prepared by the reaction of histone with acetic anhydride in a manner similar to that applied in the literature.[31] The average extent of acetylation was estimated to be ca. 70% of lysine residues of histone by the trinitrobenzene sulfonic acid (TNBS) method.[32] Upon the addition of Ac-histone to an aqueous solution containing **3**, the extent of change in the fluorescence intensity originating from the dansyl group of **3** was almost negligible, indicating that the electrostatic interactions between **3** and Ac-histone were very weak (see SI). In addition, the fluorescence spectral changes of **3** were almost negligible upon the addition of other proteins such as albumin, ovalbumin, peanut agglutinin, myoglobin, concanavalin A, and cytochrome *c*, which had pI values[33] of 4.7, 4.8, 5.7−6.7, 6.8, 7.1, and 9, respectively, to the aqueous HEPES buffer containing **3**, as shown in Figure 6 (see SI). Upon the addition of lysozyme having a pI of 11.0 to the aqueous solution of **3**, the fluorescence spectral change was much smaller than that of histone. Even though lysozyme is also a basic protein like histone (pI = 10.8), the positive charge density originating from the lysine residues on the lysozyme surface[34] was not high in comparison with that in the case of histone. These results suggest that **3** exhibited a fluorescence sensing capability toward histone, reflecting electrostatic interactions.

Conclusion

Cyclophane **3** was developed bearing a dansyl moiety and three heptaanionic resorcinarene derivatives that act as an environmentally sensitive fluorophore and as histone-binding sites, respectively. The dependence of solvent polarity on the emission spectra of the dansyl moiety was retained by the present cyclophane, as demonstrated by changing the solvent systems. A remarkable increase in the emission spectra was observed for the aqueous solution of **3** upon complexation with histone; this cannot be explained without considering the

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microenvironments provided by histone. The binding affinity of **3** toward histone was relatively specific among the adopted proteins and was evaluated to be $2.1 \times 10^6\ M^{-1}$ by fluorescence spectroscopy. To the best of our knowledge, the dansyl-appended cyclophane **3** can be cited as the first example of fluorescence detection of histone. Naturally, histone adopts post-translational modifications such as acetylation, methylation, and phosphorylation, which are thought to be key to important biological processes such as gene regulation and chromosome condensation. The present cyclophane exhibited potent discrimination capability between histone and chemically acetylated histone due to the electrostatic interactions. In addition, in the case of methylation at the histone surface, the resulting trimethylated ϵ-ammonium groups of lysine residues are analogues to good guests for resorcinarene-type hosts. Therefore, we believe that our concept of molecular design by the conjugation of an environmentally sensitive fluorophore and a cyclophane-based resorcinarene trimer will provide a useful guidepost for the development of fluorescent probes capable of sensing post-translational modifications of histone. In addition, **3** has a cyclophane cavity and acts as a host[35] for a hydrophobic guest. When a guest molecule having a dabcyl moiety (quencher)[36] is entrapped by **3**, the emission of **3** is effectively quenched. The resulting host−guest complex (**3**·dabsylated guest) is also expected to be utilized as a supramolecular fluorescence probe, accompanying the recovery of the emission upon complexation with target proteins. These subjects of interest will be explored further in the future.

Experimental Section

General Methods. Elemental analyses were performed at the Microanalysis Center of Kyushu University. ^{1}H and ^{13}C NMR spectra were taken on Bruker DRX 600 and JEOL JNM-EX400 spectrometers. A JEOL JMS-HX110A spectrometer was used for FAB mass spectrometry. Fluorescence spectra were recorded on a JASCO FP-750 spectrophotometer. Surface plasmon resonance (SPR) response curves were recorded on a BIAcore X system docked with a sensor chip (CM5) under the flow of degassed HEPES buffer (0.01 M, pH 7.4, with 0.15 M NaCl).

Materials. Tris(Boc-β-alanyl)tetraaza[6.1.6.1]paracyclophane (**2**) was prepared after a method reported previously.[22] The following proteins were obtained from commercial sources and used without further purification: calf thymus histone, egg white ovalbumin (both from Worthington); egg white lysozyme, horse heart cytochrome *c* (both from Wako Pure Chemical); peanut agglutinin (from Vector); bovine serum albumin (from Sigma), concanavalin A, and myoglobin (both from Nacalai Tesque).

Binding Constants of Dansyl-Appended Cyclophane-Based Resorcinarene Trimer with Histone. To a solution of **3** (0.5 μM) in HEPES buffer (0.01 M, pH 7.4, with 0.15 M NaCl) were added increasing amounts of histone at 298 K, and the fluorescence intensity was monitored after each addition by excitation at 333 nm. The 1:1 binding constant (*K*) of **3** toward histone (unit basis) was evaluated on the basis of computer-aided least-squares curve-fitting methods applied to the fluorescence titration data.

Fluorescence Quantum Yield. The fluorescence quantum yields (Φ) of **3** was measured in the absence and presence of histone in aqueous HEPES buffer as well as in dioxane using the known quantum yield standard of dansyl amide. The excitation wavelength was 333 nm. The following equation[37] was used to determine the Φ's:

$$\Phi_{unk} = \Phi_{std} \frac{F_{unk}}{F_{std}} \frac{A_{std}}{A_{unk}} \frac{{n_{unk}}^2}{{n_{std}}^2}$$

Here, F_{unk} and F_{std} correspond to the fluorescence intensities of the λ_{max}, A_{unk} and A_{std} correspond to the absorbance of the solutions, and n_{unk} and n_{std} are the refractive index values of the solvents used.

SPR Measurements. Preparation of sensor surface: The carboxyl groups on the sensor surfaces of CM5 were activated with an injection of a solution containing 0.4 M *N*-ethyl-*N*′-(3-(diethylamino)propyl)-carbodiimide (EDC) and 0.1 M *N*-hydroxysuccinimide (NHS), at a flow rate of 5 μL min^{-1}. Specific surfaces were obtained by injecting histone or other proteins. These proteins were diluted in 10 mM acetate buffer at pH 4 and used at a concentration of 100 μg mL^{-1}. The immobilization procedure was completed by a 7-min injection of 1 M ethanolamine hydrochloride to block the remaining ester groups. Interaction of **3** with immobilized proteins: HEPES buffer solutions containing **3** were injected for 1.5 min, at a flow rate of 20 μL min^{-1}.

Dansyl-Appended Tris(Boc-β-alanyl)tetraaza[6.1.6.1]-paracyclophane (5). Dansyl chloride (48 mg, 0.18 mmol) was added to a solution of tris(Boc-β-alanyl)tetraaza[6.1.6.1]paracyclophane (0.1 g, 98 μmol) and triethylamine (0.15 mL, 1.1 mmol) in dry dichloromethane (DCM) (10 mL) under nitrogen at room temperature; the resulting mixture was stirred for 48 h at room temperature. Ethanolamine (0.15 g) and DCM (100 mL) were added to the reaction mixture, and the mixture was then washed with 5% aqueous citric acid (20 mL) and saturated aqueous sodium chloride (20 mL) in this sequence. After being dried ($MgSO_4$), the solution was evaporated to dryness under reduced pressure. The crude product was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol−chloroform (1:1 v/v) as an eluant. Evaporation of the product fraction under reduced pressure gave a white solid (88 mg, 72%): ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ 1.35 (s, 27H), 1.40 (m, 8H), 2.1−2.2 (m, 8H), 2.9 (s, 6H), 3.2 (m, 6H), 3.5−3.6 (m, 6H), 3.88 (s, 4H), 5.3 (m, 3H), 6.9−7.0 (m, 9H), 7.1−7.2 (n, 8H), 7.39 (t, 1H), 7.46 (t, 1H), 8.07 (m, 2H), 8.52 (d, 1H); ^{13}C NMR (150 MHz, $CDCl_3$, 298 K) δ 24.8, 25.3, 28.8, 35.2, 36.7, 41.4, 45.8, 49.0, 79.4, 115.5, 120.4, 123.5, 126.3, 128.1, 128.7, 128.8, 129.7, 130.6, 130.7, 130.9, 131.3, 134.7, 137.5, 140.5, 140.9, 152.0, 156.3, 171.9; HRMS (FAB) calcd for $C_{70}H_{90}N_8O_{11}S$ 1250.6450, found 1250.6442.

Precursor of Dansyl-Appended Cyclophane-Based Resorcinarene Trimer (6). Trifluoroacetic acid (1.0 mL) was added to a solution of compound **5** (88 mg, 70 μmol) in dry DCM (2 mL), and the mixture was stirred for 1 h at room temperature. After the solvent was evaporated off under reduced pressure, DCM (20 mL) was added to the residue and this procedure was repeated 3 times to remove remaining trifluoroacetic acid. Evaporation of the solvent under reduced pressure gave a triamine derivative of cyclophane (73 mg) as the trifluoroacetic acid salt. A solution of resorcinarene derivative bearing carboxylic acid residue (**7**)[15] (343 mg, 310 μmol) in dry *N*,*N*-dimethylformamide (DMF, 2 mL) was added dropwise to a solution of the triamine derivative of cyclophane (73 mg, 70 μmol), (benzotriazol-1-yl)oxytris(dimethylamino)phosphonium hexafluorophosphate (BOP, 205 mg, 464 μmol), and triethylamine (65 μL) in dry DMF (4 mL) under nitrogen at room temperature; the resulting mixture was stirred for 6 days at room temperature. EtOAc (160 mL) was added to the reaction mixture, and the mixture was then washed with 5% aqueous citric acid (40 mL), saturated aqueous sodium chloride (40 mL), and 5% aqueous sodium

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hydrogen carbonate (40 mL) in this sequence. After being dried ($MgSO_4$), the solution was evaporated to dryness under reduced pressure. The crude product was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol−chloroform (1:1 v/v) as an eluant. Evaporation of the product fraction under reduced pressure gave a pale yellow solid (205 mg, 69%): ^{13}C NMR (150 MHz, $CDCl_3$, 298 K) δ 20.2, 25.3, 31.3, 34.7, 35.6, 41.3, 45.8, 49.2, 52.4, 67.4, 99.9, 115.4, 120.3, 123.5, 126.1, 128.0, 129.7, 130.2, 130.6, 143.7, 140.5, 140.8, 151.9, 153.7, 154.5, 170.0, 171.0. Anal. Found: C, 61.75; H, 5.87; N, 2.84. Calcd for $C_{223}H_{251}N_9O_{75}S\cdot 2H_2O$: C, 61.92; H, 5.94; N, 2.91.

Dansyl-Appended Cyclophane-Based Resorcinarene Trimer (3). A mixture of **6** (100 mg, 23 μmol), aqueous sodium hydroxide (1 mL, 1 mmol), tetrahydrofuran (6 mL), and water (6 mL) was stirred for 18 h at room temperature. The reaction mixture was evaporated to dryness under reduced pressure. The residue was purified by gel filtration chromatography on a column of Sephadex G-50 with water as eluant. Evaporation of the product fraction under reduced pressure gave a pale yellow solid (100 mg, 96%): ^{13}C NMR (150 MHz, D_2O, 298 K) δ 21.1, 28.3, 31.0, 33.9, 35.6, 40.6, 45.0, 61.9, 69.0, 100.6, 108.4, 117.8, 126.2, 128.2, 130.7, 139.2, 141.6, 152.8, 154.2, 155.2, 171.2, 173.2, 177.4. Anal. Found: C, 53.13; H, 4.30; N, 2.74. Calcd for $C_{199}H_{183}N_8Na_{21}O_{74}S\cdot 5H_2O$: C, 53.40; H, 4.35; N, 2.50.

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Supporting Information Available: NMR spectra for compounds **3**, **5**, and **6**, concentration dependency of the fluorescence spectra of **3** and a Job plot for histone complexation, and additional titration experiments and fluorescence spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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