Molecular Recognition of DNA Intercalators at Nanomolar Concentration in Water

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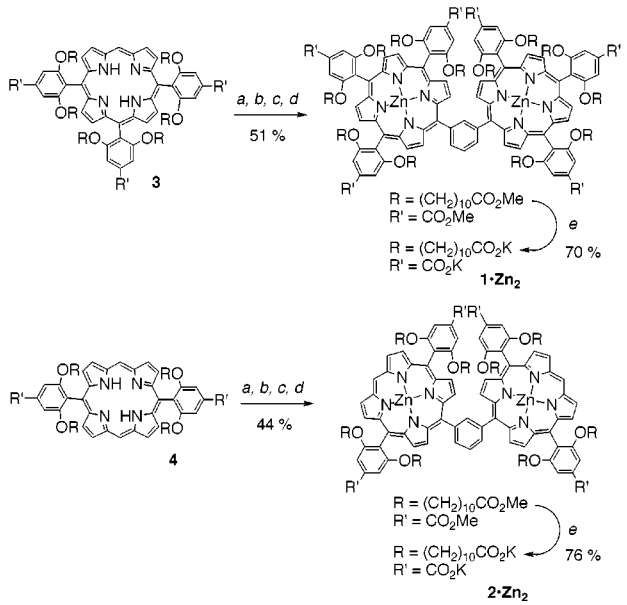
The development of artificial receptors capable of binding to biologically active molecules with high affinity and high selectivity, and understanding the underlying principles of recognition are an intriguing subject of chemistry. For rational design of receptor/ligand interactions,[1,2] there have been several attempts to evaluate contributions of functional groups or constituent atoms to the binding free energy, using compiled data of enzyme−inhibitor, antibody−antigen, and protein−ligand binding.[3] To gain deeper insight into recognition interactions, studies using synthetic receptors are desirable since systematic variation of the functional groups is possible while controlling conformation of the receptors. Although our knowledge of the recognition mechanism is still limited, a number of studies suggested that a larger receptor−guest contact surface area would result in a greater driving force from receptor/guest interactions and desolvation processes. We report here that bisporphyrin-based synthetic receptors, having a large contact surface area with guest, bind to DNA intercalators such as acridine orange, DAPI, and ethidium bromide with unprecedented affinity in water.[4]

Gable-type porphyrins[5] were prepared using the palladium-catalyzed cross-coupling reaction as a key step.[6] We prepared two water-soluble gable porphyrins as shown in Scheme 1. The free base of **1·Zn$_2$**, **1·H$_4$**, was prepared by treatment of the ester of **1·Zn$_2$** with HCl, followed by alkaline hydrolysis. ^{1}H NMR (both in D_2O and in MeOH-d_4) and mass spectroscopic studies confirmed the structure of the receptors.

Receptors **1·Zn$_2$**, **1·H$_4$**, and **2·Zn$_2$** are soluble in methanol and in water (pH >7.0). Binding of various guests was studied by UV−visible and fluorescence spectroscopy. Upon addition of **5**−**11**, the Soret band of **1·Zn$_2$**, **1·H$_4$**, and **2·Zn$_2$** was shifted to longer wavelength.[7] The fluorescence of **5**−**7** was quenched by the addition of **1·Zn$_2$**, while the fluorescence of **1·Zn$_2$** was quenched by the addition of **8** and **9**. Figure 1 shows that the fluorescence of **5** (47 nM) is quenched by the addition of a nanomolar concentration of **1·Zn$_2$**. The binding constants at 25 °C in 0.1 M borate buffer at pH 9 are determined by least-squares curve fitting and are summarized in Table 1.[8] Job plot and the curve fitting showed that both a 1:1 complex and a 1:2 (receptor:guest) complex were formed between the receptors and **5**−**7**, **10**, and **11**. For **8** and **9**, only a 1:1 complex was formed. The binding constant of **9** to **1·Zn$_2$** was also determined to be $10^{8.1}$ M^{-1} by UV−visible spectroscopy by following a red shift of the Soret band of porphyrin, in reasonable agreement with that determined by fluorescence titration.[9] The fluorescence of **6** quenched by the

Scheme 1[a]



[a] Reagents: (a) $Zn(OAc)_2/CHCl_3$, (b) NBS/$CHCl_3$, py, (c) $PdCl_2(PPh_3)_2$, pinacolborane, TEA/$ClCH_2CH_2Cl$, (d) 1,3-diiodobenzene, $Pd(PPh_3)_4$, Cs_2CO_3/DMF, (e) 0.5 M KOH/MeOH-THF.

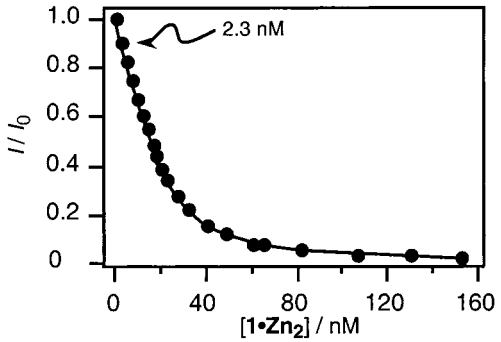


Figure 1. Quenching of fluorescence of **5** upon addition of **1·Zn$_2$**. [**5**] = 47 nM, 0.1 M borate buffer, pH 9.0, at 25 °C, excitation at 491 nm, and emission at 530 nm. The simulated curve is also shown (see text).

$$\mathrm{R} + \mathrm{G} \underset{}{\overset{K_1}{\rightleftharpoons}} \mathrm{R{\cdot}G} \qquad \mathrm{R{\cdot}G} + \mathrm{G} \underset{}{\overset{K_2}{\rightleftharpoons}} \mathrm{R{\cdot}G_2} \qquad \left(\begin{array}{l}\mathrm{R = receptor}\\ \mathrm{G = guest}\end{array}\right)$$

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(2) For recent studies on water-soluble receptors, see: (a) Jain, R. K.; Hamilton, A. D. *Org. Lett.* **2000**, *2*, 1721−1723. (b) Anzenbacher, P., Jr.; Jursikova, K.; Sessler, J. L. *J. Am. Chem. Soc.* **2000**, *122*, 9350−9351. (c) Kano, K.; Kitae, T.; Shimofuri, Y.; Tanaka, N.; Mineta, Y. *Chem. Eur. J.* **2000**, *6*, 2705−2713. (d) Kikuta, E.; Murata, M.; Katsube, N.; Koike, T.; Kimura, E. *J. Am. Chem. Soc.* **1999**, *121*, 5426−5436. (e) Ngola, S. M.; Kearney, P. C.; Mecozzi, S.; Russell, K.; Dougherty, D. A. *J. Am. Chem. Soc.* **1999**, *121*, 1192−1201. (f) Inouye, M.; Fujimoto, K.; Furusyo, M.; Nakazumi, H. *J. Am. Chem. Soc.* **1999**, *121*, 1452−1458. (g) Haino, T.; Rudkevich, D. M.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1999**, *121*, 11253−11254. (h) Niikura, K.; Metzger, A.; Anslyn, E. V. *J. Am. Chem. Soc.* **1998**, *120*, 8533−8534.

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(4) (a) Mizutani, T.; Wada, K.; Kitagawa, S. *J. Am. Chem. Soc.* **1999**, *121*, 11425−11431. (b) Mizutani, T.; Wada, K.; Kitagawa, S. *J. Org. Chem.* **2000**, *65*, 6097−6106.

(5) Tabushi, I.; Sasaki, T. *Tetrahedron Lett.* **1982**, *23*, 1913.

(6) (a) Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457−2483. (b) Hyslop, A. G.; Kellett, M. A.; Iovine, P. M.; Therien, M. J. *J. Am. Chem. Soc.* **1998**, *120*, 12676−12677.

(7) Porphyrin−guest $\pi-\pi$ stacking induced a red shift of the Soret band, see: (a) Schneider, H.-J.; Wang, M. *J. Org. Chem.* **1994**, *59*, 7464−72. (b) Teulade-Fichou, M.-P.; Vigneron, J.-P.; Lehn, J.-M. *Supramol. Chem.* **1995**, *5*, 139−147. (c) Sirish, M.; Schneider, H.-J. *J. Am. Chem. Soc.* **2000**, *122*, 5881−5882.

(8) The fluorescence quenching of **5**−**7** by receptors and of receptors by **8** and **9** was monitored for the binding constant determinations.

(9) The concentrations of porphyrin were typically 50 nM and 1.0 μM for fluorescence and UV−visible titrations, respectively.

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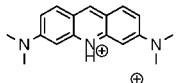
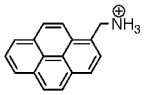
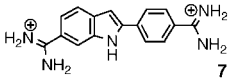
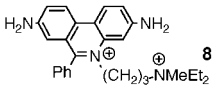
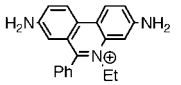
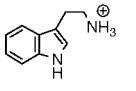
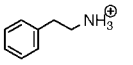
Table 1. Binding Constants (K_1, K_2/M^{-1}) in Borate Buffer at 25 °C, pH 9.0[a]

	1·Zn₂		**1·H₄**		**2·Zn₂**	
	log K_1	log K_2	log K_1	log K_2	log K_1	log K_2
 **5**	8.5	7.7	8.3	7.8	15.3[c]	
 **6**	7.0	7.0	6.0	6.3	13.2[c]	
 **7**	6.5	6.3	6.5	6.8	13.0[c]	
 **8**	8.4	*b*	7.1	*b*	6.4	*b*
 **9**	8.4	*b*	6.9	*b*	6.9	*b*
 **10**	3.5	2.4	*d*	*d*	2.0	1.0
 **11**	2.9	2.3	*d*	*d*	2.1	1.5

[a] Binding constants are averages of 10−50 independent determinations. Estimated error of the binding constant is 10%. Fluorescence spectroscopy was used for **5**−**9** and UV−visible spectroscopy for **10** and **11**. [b] The binding isotherm was fitted to 1:1 complex formation. [c] Determined as log K_1K_2. [d] Not determined.

addition of **1·Zn₂** was recovered by the addition of 1,2-di(4-pyridyl)ethane, whose binding constant to **1·Zn₂** was independently determined to be 3.7×10^7 M^{-1}, showing that the binding of **6** is reversible.

The following characteristic features are noteworthy. (1) Cationic guests are bound with high affinity while neutral guest (acridine) or anionic guest (acridine-9-carboxylate) are not bound. (2) Monocation (**9**) and dication (**8**) are bound with similar affinity. The binding affinity was increased as the ionic strength of the solution was lowered, with the dependence fitted to (1/2) $\log(K_1K_2) = \alpha I^{1/2} + \beta$, or $\log(K_1) = \alpha I^{1/2} + \beta$, where $\alpha = -2.33$, -2.97, and -3.92, and $\beta = 8.81$, 9.38, 9.68, for **5**, **8**, and **9**, respectively.[10] The dependence on the ionic strength is similar between **8** and **9**. (3) The strong binding of **5**−**9** is driven by the enthalpic term (Figure 2). The Soret band of **1·Zn₂** was significantly varied upon addition of **5** (see Supporting Information), showing that there are some electronic attractive interactions such as charge-transfer interactions between **1·Zn₂** and **5**.[7] (4) Both free base (**1·H₄**) and the zinc complex (**1·Zn₂**) showed similar affinity for **5** and **7**. (5) Receptor **1·Zn₂** having more alkyl groups than **2·Zn₂** showed larger binding constants for **5**, **6**, and **8**−**11**. The affinity of **1·Zn₂** to **5** was nearly diminished in MeOH-borate (9:1, v/v): no UV−visible spectral change was observed upon addition of 5 μM of **5** to 0.23 μM of **1·Zn₂**. (6) The value of log K_1K_2 for binding of **5** by a monomeric analogue of **1·Zn₂**[4] was 11.4, indicating that the values of K_1K_2 for the bisporphyrins are 4 or 5 orders of magnitude larger. These findings indicate that the binding is driven by hydrophobic, charge transfer, and electrostatic forces.

The critical dependence of the binding affinity on the charge of guest suggests that the positive charge of the guest triggers the conformational change of the receptor to a folded form (Figure 3) to be preorganized for hydrophobic binding. Without guest the folded form of **1·Zn₂** is unstable due to anion−anion repulsion and the extended form is unstable due to exposure of alkyl groups to water. Only the binding of hydrophobic cations can relieve both unfavorable interactions to result in a high-affinity binding. This role of electrostatic interaction can explain the particular preference for hydrophobic cationic guests. As supporting evidence for the conformational change, ^{1}H NMR study of the binding of pyridine derivatives to a monomeric analogue of **1·Zn₂** indicated that the binding of guest induced the conformational change of the porphyrin from an extended form to a folded form.[4]

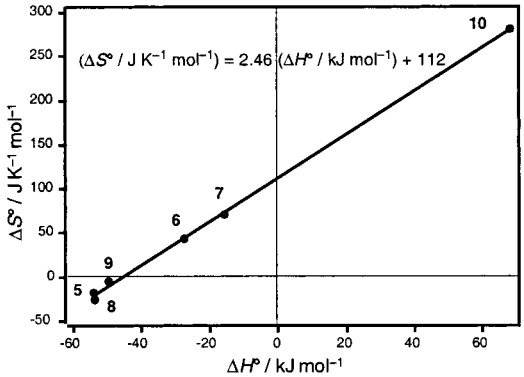


Figure 2. Plot of $\Delta S°$ against $\Delta H°$ for the binding of **5**−**10** by receptor **1·Zn₂** in 0.1 M borate buffer, pH 9.0. Entropy and enthalpy were determined by fitting binding constants to either $\ln K_1 = -(1/RT)\Delta H° + (1/R)\Delta S°$ for **8** and **9** or $(1/2)\ln(K_1K_2) = -(1/RT)\Delta H° + (1/R)\Delta S°$ for **5**−**7** and **10**, where the plot was linear in the range 283 K < T < 333 K.

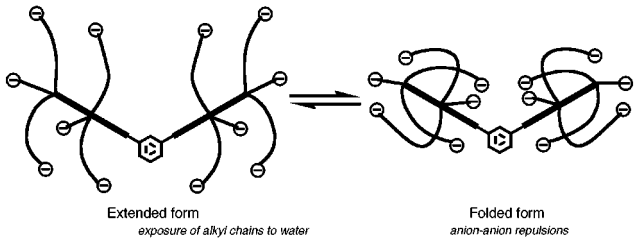


Figure 3. Schematic representation of the two possible conformations of receptors **1·Zn₂** and **2·Zn₂**.

Fluorescence and UV−visible spectroscopic experiments indicated that **1·Zn₂** can extract **8** from a salmon sperm DNA−**8** complex. Thus, the fluorescence emission of the **8**-DNA complex was quenched by the addition of **1·Zn₂** in borate buffer, pH 8 and 9 ([DNA] = 8 μg/L, [**8**] = 0.67 μM, [**1·Zn₂**] = 0−0.8 μM). A concomitant red shift of the Soret band of **1·Zn₂** in the UV−visible spectra confirms that **8** is transferred from DNA to **1·Zn₂**.

In conclusion, the bisporphyrin-based water-soluble receptors bind to hydrophobic cations with nanomolar dissociation constants, where conformational control by an interplay of electrostatic and hydrophobic interactions led to both high affinity and high selectivity.

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Supporting Information Available: UV−visible spectra of complexes of **1·Zn₂** and **5**−**7**, Job plot for binding of **1·Zn₂** to **5**, and plots of log K against $I^{1/2}$ (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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