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Hydrophobic environment of gable-type bisporphyrin receptors in water promotes binding of amines and oligopeptides

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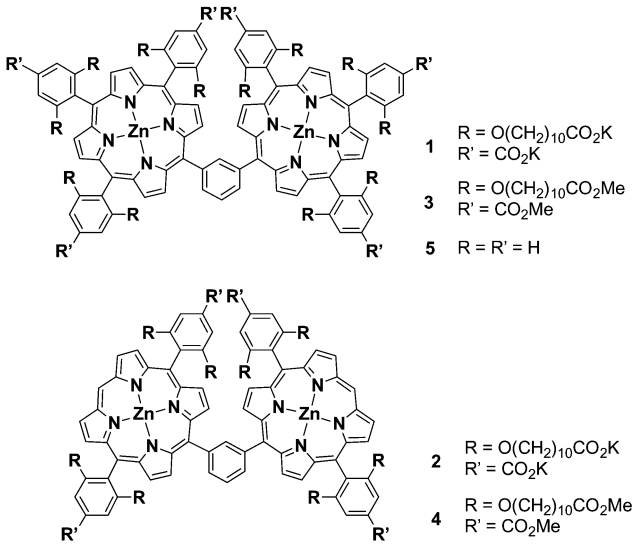
The Lewis acidic site in a hydrophobic environment promoted binding of amines/oligopeptides efficiently: a binding constant of H-His-Leu-His-$NHC_{10}H_7$ to bisporphyrin was 9.4×10^5 M^{-1} in water at 25 °C.

The design and synthesis of receptors for peptides,[1] which have both polar and non-polar groups, are one of the challenges of host–guest chemistry.[2] To date, synthetic receptors for oligopeptides are (1) of cyclodextrin/cyclophane type,[3] where hydrophobic groups of the guest are recognized, or (2) of crown ether/ionic receptor/metalloreceptor type,[4] where ionic or polar groups of the guest are recognized. Receptors of the former type bind to guest more tightly in polar solvents, but the guest is limited to hydrophobic species. Receptors of the latter type bind to guest better in less polar solvents, and poor binding in water is a disadvantage for biological applications. Our receptor design is to construct a polar recognition site in a hydrophobic binding pocket. Such interaction mode is well-known in binding by proteins, while it is less common in binding by synthetic receptors. We report here that bisporphyrin-based synthetic receptors, having Lewis acidic zinc in a hydrophobic environment, bind to amines and histidine-rich oligopeptides where hydrophobic interactions and the zinc–nitrogen interactions in a non-polar environment lead to high affinity binding.

We employed zinc bisporphyrins bearing hydrophobic alkyl chains above and below the porphyrin plane. The zinc will provide polar interaction sites for binding of Lewis basic group(s) of guest.[5] The alkyl chains will provide a hydrophobic recognition site and a hydrophobic environment around the zinc. The carboxylate groups at the terminal of alkyl chains were introduced to provide the receptor with water solubility. Two water-soluble receptors **1** and **2** and their ester derivatives **3** and **4** were prepared. Bisporphyrin **5** having no carboxyalkyl groups was also used as a reference receptor.[6] Receptors **1**–**4** were prepared using palladium catalyzed cross-coupling reactions. Preparation of these receptors will be reported elsewhere.

Receptors **1**–**5** bind to guests having Lewis basic groups such as **6**–**9** tightly both in organic solvents and in water. The binding of diamines and oligopeptides[7] was investigated using fluorescence spectroscopy and UV–vis spectroscopy. When the guest was added to a solution of the porphyrins, the Soret band in the UV–vis spectra was shifted to longer wavelength, and the fluorescence emission of the porphyrin was also shifted to longer wavelength, without quenching of the fluorescence emission. The sharp Soret band of **1** and **2** indicated that these receptors are not aggregated in water at pH 9, which was also supported by a small angle–X-ray scattering experiment. Nonlinear least-squares analysis of the spectral changes as a function of guest concentrations yielded the binding constants (Table 1). For **6** and **9**, binding constants were determined by both fluorescence and UV–vis spectroscopic methods. Binding constants determined by fluorescence titration were in reasonable agreement with those determined by UV–vis titration, although the typical concentrations of porphyrins were 50 nM in the former and 0.5 μM in the latter experiments.

Comparison of the binding of dipyridylethane **6** to **1**–**5** in various solvents indicated that zinc–nitrogen and hydrophobic



interactions provide the major driving force of binding. The binding affinity of **6** was high in non-polar solvents such as benzene, AcOEt and CH_2Cl_2,[8] and also high in water ($>10^6$ M^{-1}) but low in methanol (10^3 M^{-1}). The donor number (0 in CH_2Cl_2, 0.1 in benzene, 17.1 in AcOEt, 18 in water and 19 kcal mol^{-1} in MeOH) appears to be correlated with the binding affinity since we observed diminished binding affinity in AcOEt having large DN (*i.e.*, a strong Lewis base). This trend implies that the Lewis acid (Zn)–Lewis base (nitrogen) interaction

Table 1 Binding constants (K/M^{-1}) of various diamines and oligopeptides **6**–**9** at 25 °C[a]

In organic solvents

Guest	3	4	5	Solvent
6	$>10^8$	$>10^8$	$>10^8$	Benzene
6	6.4×10^5	1.6×10^6	9.6×10^5	AcOEt
6	$>10^8$	$>10^8$	$>10^8$	CH_2Cl_2

In MeOH and in 0.1 M borate buffer (pH 9.0)

Guest	1	2	Solvent
6	1860	1450	MeOH
6	1820	1120	MeOH–borate[c]
6	3.4×10^7 (3.7×10^7)[b]	2.4×10^5 (2.4×10^5)[b]	Borate
7	5.1×10^6[b]	1.7×10^5[b]	Borate
8	610	320	Borate
9	9.4×10^5 (1.2×10^6)[b]	1.0×10^5 (1.4×10^5)[b]	Borate

[a] Binding constants determined by UV–vis titrations, unless otherwise noted. Error limits: ±5%. Typical experimental conditions: [porphyrin] = 500 nM for UV–vis titration and 50–80 nM for fluorescence titratration. [b] Determined by fluorescence emission titration. [c] MeOH–borate = 9 : 1 (v/v).

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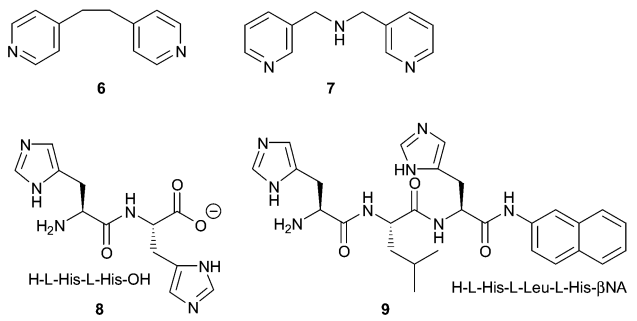

provides the major driving force of binding in organic solvents, and this interaction is reduced in AcOEt, which is capable of coordinating to the zinc. Considering that DN of water is similar to that of AcOEt, the values of DN alone cannot account for the solvent effects. Thus the strong binding in water can be ascribed to the hydrophobic interaction between the non-polar moiety of **6** and that of receptors, and possibly to the enhanced zinc–nitrogen interactions in the non-polar environment. The trend that the binding is enhanced in non-polar solvents and in water but diminished in the intermediate solvent, methanol, was also observed for the binding of various amino acid derivatives by monomeric analogues of **1** and **2**[9] as well as other hosts.[10]

We then focused on the effects of the number of alkyl groups of the receptors on the binding affinity to **6**. In AcOEt, the binding constants of **6** increased in the order, **3** < **5** < **4**. Compared to the reference receptor **5**, having no alkyl groups, the 8 alkyl groups in **4** appear to afford some van der Waals stabilization for guest binding, but the 12 alkyl groups in **3** give rise to weak steric repulsion as seen in the lowest affinity of **3** in AcOEt. Interestingly, the order of binding constants is reversed in water. The binding constant of **1** was two orders of magnitude larger than that of **2**, suggesting that the 12 alkyl groups of **1** provide a hydrophobic binding pocket for guest binding. Therefore, steric repulsion dominates in the organic solvents, while hydrophobic attraction dominates in water.

Both the solvent effects and the alkyl group effects described above revealed the important role of the hydrophobic interactions. One would then expect that receptors **1** and **2** can generally bind to guest with a hydrophobic group as do cyclodextrins and cyclophanes. However, **1** does not bind to guests such as phenol and *N*, *N*, *N*-trimethyl-2-adamantylammonium, which have a hydrophobic moiety but lack a strongly coordinating group, and to phenethylammonium only weakly in water (K_a = 760 M^{-1}).[9] Therefore, without the zinc–nitrogen interactions, the guest binding is weak. It should be noted, however, that zinc–nitrogen interaction becomes weaker in a polar solvent: the binding constant of pyridine to (5,10,15,20-tetraphenylporphyrinato)zinc is 7720 M^{-1} in CH_2Cl_2, while that of pyridine to [5,10,15,20-tetra(4-carboxyphenyl)porphyrinato]zinc in water is 21 M^{-1}.[9] These observations that hydrophobic forces alone are not strong enough to drive binding, and the zinc–nitrogen interaction is weak in a polar environment, suggest that the polar interaction in the hydrophobic environment leads to high affinity to **6** in water.

The monomeric analog of **1** showed strong binding to imidazole derivatives *via* the zinc–imidazole coordination interactions.[9] Since histidine-rich proteins[11] attract interests for their unique biological functions, we examined the binding of histidine-containing oligopeptides to the receptors. Receptors **1** and **2** formed 1 : 1 complexes with oligopeptides **8** and **9**. The low affinity of **8** can be ascribed to the C-terminal negative charge of this guest. The binding constants for tripeptide **9** were 2–3 orders of magnitude larger than those of **8**. Tripeptide **9** has no negative carboxylate and has two hydrophobic moieties, the leucine side chain and the β-naphthyl group. These two factors, no negative charge and higher hydrophobicity, appear to enhance the binding affinity. It is noteworthy that a bulky guest such as **9** showed higher affinity for the more crowded receptor **1** than receptor **2**.

Table 2 Enthalpy and entropy changes of binding of amines and oligopeptides by **1** in 0.1 M borate buffer pH 9.0[a]

Guest	$\Delta H°$/kJ mol^{-1}	$\Delta S°$/J K^{-1} mol^{-1}
6	−42.9	0.9
7	−28.7	48.8
9	−28.1	21.9

[a] Determined by van't Hoff plot in the temperature range of 283–333 K.

As shown in Table 2, binding of **6**, **7** and **9** in water is driven by both enthalpy and entropy terms. A large entropic contribution to the binding is consistent with a mechanism in which desolvation-driven solvophobic interactions provide one of the driving forces of binding. It is interesting that triamine **7** with high solubility in water showed a positive entropic change. The value of pK_a for the central nitrogen of **7** was reported to be 7.15,[12] suggesting that the salt-bridge type interaction between this nitrogen and the carboxylate of the host is not the major driving force of binding at pH 9.0. Desolvation from the NH group may lead to the positive entropic change.

In conclusion, we have shown that the polar recognition site in the hydrophobic environment provided by flexible alkyl groups leads to high affinity toward amphiphilic molecules such as oligopeptides and amines. The number of alkyl groups of the receptors significantly influences the binding affinity in water. The interaction works better in water than in MeOH, and these systems will find wide applications in the recognition of amphiphilic biomolecules.

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