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An efficient recognition motif for an alkyl moiety in water

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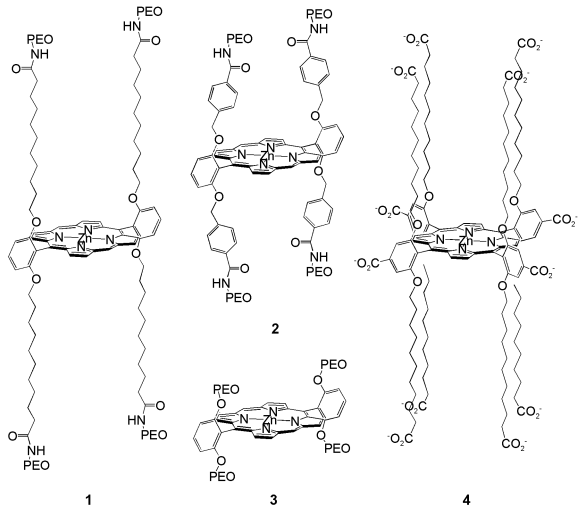
The artificial receptor bearing decamethylene groups bridging the porphyrin framework and the hydrophilic poly(ethylene oxide) auxiliary groups showed incremental binding free energy of 3.5 kJ mol^{-1} per CH_2 for 4-alkylpyridines, demonstrating that an ideal hydrophobic environment for the recognition of an alkyl group is constructed in water.

Hydrophobic interactions perform significant roles in chemistry and biology. Recognition of a hydrophobic moiety of free fatty acid, retinoic acid, and estrogen triggers divergent and significant biological functions.[1] X-ray crystal structure analysis of fatty acid binding protein complexed with stearic acid indicates that 14 hydrophobic amino acid side chains such as Leu, Thr, Phe, and Ala are in van der Waals contact with the folded alkyl chain of stearic acid.[2] Development of a synthetic recognition motif of an alkyl moiety should lead to practical application to adsorbent and sensor as well as provide new insights into the nature of hydrophobic interactions. Here we report three new water-soluble synthetic receptors having a porphyrin scaffold connected to poly(ethylene oxide) (PEO) auxiliary groups *via* hydrophobic alkyl/aryl moieties,[3] with particular attention paid to the evaluation of hydrophobic environment of the binding pocket. We demonstrated that the hydrophobic environment of the binding cavity varied depending on the nature of hydrophobic spacer moieties appended to porphyrin, and also on the auxiliary groups employed to impart water solubility to the receptor. Free energy decrease per an incremental methylene group of the guest was determined to quantitatively estimate the hydrophobicity of the binding pocket. For the binding of 4-alkylpyridines by the PEO-$(CH_2)_{10}$-zinc porphyrin receptor, the free energy decrease per CH_2 was 3.5 kJ mol^{-1}, showing that a well organized hydrophobic binding pocket is constructed *via* induced-fit to the alkyl group of guest.

We prepared three PEO-appended porphyrins **1–3**. The common precursor, 5,15-bis(2,6-dihydroxyphenyl)porphyrin, was prepared by condensation of dipyrromethane and 2,6-dibenzyloxybenzaldehyde, followed by deprotection of the benzyl groups with BBr_3. *O*-Alkylation with either 10-carboxydecyl or 4-carboxybenzyl groups, followed by introduction of PEO *via* amide linkages,[4] and zinc insertion afforded **1** and **2**. Receptor **3** was prepared by the reaction of 5,15-bis(2,6-dihydroxyphenyl)porphyrin with tosyl PEO.[5] The PEO-appended zinc porphyrins were purified by gel permeation chromatography on Sephadex LH-20, and obtained as mixtures of compounds with different PEO chain length since PEO with average molecular weight of 2000 was used. As one of the unique properties of **1–3**, these porphyrins were soluble both in water and most organic solvents including chloroform, THF, ethyl acetate and methanol. They are insoluble in hexane and diethyl ether.

These receptors bind pyridine derivatives *via* coordination of the pyridyl nitrogen to the zinc.[6] The binding constants were determined spectrophotometrically by observing the absorbance changes in the Soret band as a function of guest concentrations in pH 7.0 phosphate buffer (25 °C, I = 0.1 M). Typically, a red shift of the Soret band from 410 to 425 nm was observed. We determined the binding constants for a series of



4-alkylpyridines with varying the methylene chain length,[7] as listed in Table 1. Binding constants to receptor **4** were also determined to examine the effect of the charge on the hydrophilic auxiliary groups of the receptors on the hydrophobic interactions.

For the quantitative evaluation of the hydrophobic interaction with the 4-alkyl moieties, we plot the binding free energy, $-\Delta G°$, against the methylene length n (where n is the number of $-CH_2-$ in the 4-alkyl group, py–$(CH_2)_n$–H) of the guests in Fig. 1. The plot is linear up to at least n = 4 for receptors **1–4**. From the slope of the line, we estimated the decrease in the free energy per incremental methylene, $d(-\Delta G°)/dn$, as listed in Table 2.

It is interesting to note that receptor **1** bearing flexible methylene chains to accommodate the alkyl pyridine guest showed the largest free energy decrease of 3.5 kJ mol^{-1} per CH_2, showing that the hydrophobic interaction with guest alkyl groups occurs effectively. The free energy changes of transfer of alkane from water to organic solvent have been used as a

Table 1 Binding constants K of 4-alkylpyridines to receptors **1–4** in water (pH 7, phosphate, 25 °C)[a]

		$\text{Log}_{10}\ K/\text{M}^{-1}$			
Entry	Guest	**1**	**2**	**3**	**4**
1	Pyridine	3.8	3.3	2.5	3.9
2	4-Methylpyridine	4.2	3.8	2.9	4.4
3	4-Ethylpyridine	4.7	3.9	3.0	4.7
4	4-Propylpyridine	5.3	4.1	3.2	4.9
5	4-Butylpyridine	6.0	4.3	3.2	5.1
6	4-Pentylpyridine	> 6	4.2	3.1	5.5
7	4-*tert*-Butylpyridine	5.8	4.3	3.1	[b]
8	4-Phenylpyridine	6.6	4.8	3.4	[b]
9	4-(Hydroxymethyl)pyridine	3.6	3.5	2.7	[b]
10	4-(2-Hydroxyethyl)pyridine	3.6	3.5	2.9	[b]

[a] Error limits of K: ±5%. [b] Not determined.

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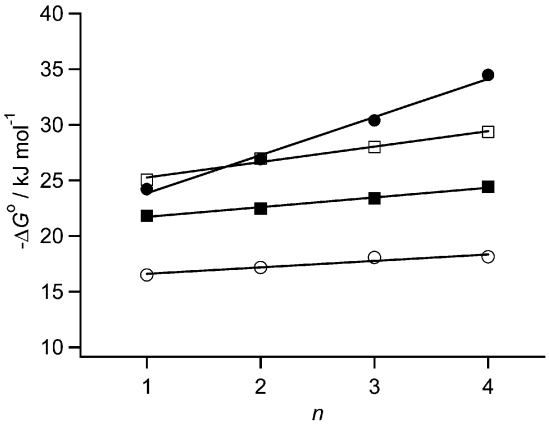

Fig. 1 Plot of $-\Delta G°$ against the methylene length n for **1** (●), **2** (■), **3** (○), and **4** (□).

Table 2 The free energy decrease per CH_2 for the binding of 4-alkylpyridines to receptors **1–4**

Receptors	$\frac{d(-\Delta G^o)}{dn}$, kJ mol^{-1}
1	3.5
2	0.9
3	0.6
4	1.5

quantitative measure of hydrophobic interaction.[8] Partition coefficients of alkanes or alkanols between water and 1-octanol or hydrocarbon gave $-\Delta G°$ per CH_2 to be 3.0–3.7 kJ mol^{-1}.[9] The value observed for receptor **1** falls within this range, indicating that the binding site is hydrophobic enough to accommodate nonpolar guest in an effective manner. Receptor **3** with PEO groups directly attached to the *meso*-phenyl groups showed a smaller value of $d(-\Delta G°)/dn$ (0.6 kJ mol^{-1}). A simple water-soluble porphyrin, [tetrakis(4-carboxylatophenyl)porphyrinato]zinc, showed $d(-\Delta G°)/dn$ of 0.0 kJ mol^{-1} in water. These observations indicate that the decamethylene moieties of **1** are necessary for the recognition of the alkyl groups of guest. Receptor **2** with the aryl groups attached to the *meso*-phenyl groups showed an unexpectedly small value of 0.9 kJ mol^{-1}, where the rigid aryl group may not be able to induced-fit to the relatively small alkyl chain moiety of the guest. The different affinity between **1** and **2** implies a possible design of the selective binding pocket for similar hydrophobic guests.

The tighter binding of the long alkyl guest is the characteristic feature of binding in water: the values of log K, where K is the binding constants of 4-Me, Et, Pr, and Bu-pyridine to **1** in CH_2Cl_2, are 4.0, 4.1, 4.0, 4.0, respectively, leading to the free energy increment per CH_2 of 0.0 kJ mol^{-1} for the binding in CH_2Cl_2. Therefore the large incremental free energy of **1** observed in water should be ascribed to the unique role of water, that is, desolvation of the alkyl groups of both the receptor and the guest, and enhanced van der Waals interaction between the alkyl groups in water. Introduction of a hydroxy group at the terminal of the alkyl chain reduced the binding affinity much as shown in entries 9–10 in Table 1.

Receptor **4** with eight $(CH_2)_{10}$ chains but anionic charges at the $(CH_2)_{10}$ terminals is not as effective as receptor **1** for the recognition of the 4-alkyl moiety as shown in a smaller value of $d(-\Delta G°)/dn$ (1.5 kJ mol^{-1}).[10] This poor recognition of the alkyl moieties is ascribed to the anion–anion repulsion of the carboxylates when the alkyl chains are induced-fit to the 4-alkyl groups of guest.[11]

Van't Hoff analysis of the binding constants determined between 10 °C and 60 °C gave linear correlation between log K and T^{-1}: $\Delta H°$ was (−35.1 ± 1.1) kJ mol^{-1} and $-T\Delta S°$ was (5.7 ± 1.0) kJ mol^{-1} for the 4-propylpyridine-**1** complex, while $\Delta H°$ was (−32.3 ± 0.8) kJ mol^{-1} and $-T\Delta S°$ was (11.0 ± 0.8) kJ mol^{-1} for the pyridine–**1** complex (T = 25 °C). Therefore the additional propyl group of guest made favorable contributions to both enthalpic and entropic terms of binding free energy, with the entropic stabilization more significant at 25 °C. Similar trends were observed, although less distinct, for complexation between **4** and 4-alkylpyridines. The value of $\Delta H°$ was (−33.7 ± 1.1) kJ mol^{-1} and $-T\Delta S°$ was (5.5 ± 1.0) kJ mol^{-1} for the 4-propylpyridine-**4** complex, while the value of $\Delta H°$ was (−32.8 ± 0.2) kJ mol^{-1} and $-T\Delta S°$ was (7.7 ± 0.5) kJ mol^{-1} for the 4-methylpyridine-**4** complex (T = 25 °C). The additional $-CH_2CH_2-$ group of guest leads to both enthalpic and entropic stabilization of the complex. Apparently, enthalpy–entropy compensation, which is generally observed for $\Delta H° - T\Delta S°$ of host–guest complexation,[12] does not hold in this system. These thermodynamic parameters are consistent with the generally accepted mechanism of hydrophobic interactions,[13] where desolvation of nonpolar groups leads to positive entropic changes, and van der Waals interaction between the nonpolar groups leads to negative enthalpic changes.

In conclusion, we demonstrated a general strategy for construction of the recognition motif of an alkyl group: flexible alkyl chains with neutral PEO groups attached at the alkyl terminal afforded the efficient hydrophobic binding pocket for the recognition of the alkyl moiety of guest. The free energy change for the incremental CH_2 group of 4-alkylpyridines was 3.5 kJ mol^{-1}, demonstrating the ideal hydrophobic environment for the binding of an alkyl moiety.

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