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Water Accessibility to the Binding Cleft as a Major Switching Factor from Entropy-Driven to Enthalpy-Driven Binding of an Alkyl Group by Synthetic Receptors

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Abstract: Free energy, enthalpy, and entropy changes in the binding of alkyl pyridines to water-soluble zinc porphyrin receptors with varying accessibility of water to the binding cleft were determined to explain why the driving force of hydrophobic effects is enthalpic in some occasions and entropic in others. Zinc porphyrins bearing four alkyl pillars with terminal solubilizing poly(oxyethylene) (POE) chains of molecular weight of 750 (**1**), with eight alkyl pillars with terminal solubilizing POE chains of molecular weight of 350 (**3**), and with eight alkyl pillars with POE of molecular weight of 750 (**4**) had a binding cleft with decreasing water accessibility in this order as revealed by binding selectivity of imidazole/pyridine. Although all these porphyrins showed that the free energy of binding ($-\Delta G^{o}$) increases linearly as the alkyl group of the guest is lengthened ($-\Delta G^{o}$ per CH_2 was 2.6, 2.8, and 2.6 kJ mol^{-1} for **1**, **3**, and **4**, respectively), the origin of the free energy gain was much different. Receptor **1** with the most hydrophilic binding site bound the alkyl group by an enthalpic driving force (4-pentylpyridine favored over 4-methylpyridine by $\Delta\Delta H^{o} = -16.4$ kJ mol^{-1}), while receptor **4** with the most hydrophobic binding site by an entropic driving force (4-pentylpyridine favored over 4-methylpyridine by $\Delta\Delta S^{o} = 39.6$ J K^{-1} mol^{-1}). Receptor **3** showed intermediate behavior: both enthalpic and entropic terms drove the binding of the alkyl group with the enthalpic driving force being dominant. The binding site of the four-pillared receptor (**1**) is open and accessible to water molecules, and is more hydrophilic than that of the eight-pillared receptor (**4**). We propose that the alkyl chains of **1** are exposed to water to produce a room to accommodate the guest to result in enthalpy-driven hydrophobic binding, whereas **4** can accommodate the guest without such structural changes to lead to entropy-driven hydrophobic binding. Therefore, accessibility of water or exposure of the binding site to the water phase switches the driving force of hydrophobic effects from an entropic force to an enthalpic force.

Keywords: hydrophobic effect · molecular recognition · porphyrinoids · receptors · thermodynamics

Introduction

Hydrophobic interactions[1] are key to a number of biological functions such as binding of organic molecules to proteins or DNAs, and structural stabilization of the cell membrane. An understanding of hydrophobic effects is essential in diverse applications such as the rational design of drugs[2] as well as chromatographic separation of biomolecules.[3] However, the driving force of hydrophobic effects has been ascribed to an enthalpic force[4,5] or to an entropic force[6,7] depending on the supramolecular systems. It thus is an open question: what is the real mechanism of hydrophobic effects?

Thermodynamics of hydrophobic effects can be rationalized by considering the solubilization of hydrocarbons in water. Solubilization of gaseous hydrocarbons in water is characterized by negative entropy changes,[8] which can be explained by a mechanism through which water molecules on the surface of a hydrocarbon are strongly hydrogen bonded and lose motional freedom compared with those in the bulk phase. Enthalpy changes of solubilization of gaseous hydrocarbons are positive or negative depending on the solute molecule, but close to zero, and the entropy term is dominant in determining the free-energy changes.[9] For

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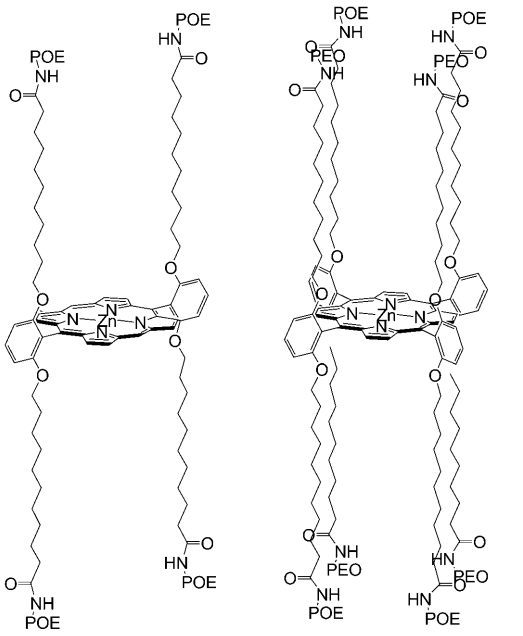
an incremental increase in the number of methylene groups in the dissolution of gaseous hydrocarbons in water, the free-energy change (ΔG^{o}), the enthalpy change (ΔH^{o}), and the value of $-T\Delta S^{o}$ were $+0.75\ kJ\,mol^{-1}$, $-2.8\ kJ\,mol^{-1}$, and $3.56\ kJ\,mol^{-1}$, respectively, at 298.15 K.[1c,8b] These values indicate that the unfavorable free-energy change was determined by the cancellation of larger contributions from favorable enthalpy and unfavorable entropy, and the origin of the lower solubility of alkanes in water as a result of the additional methylene group was ascribed to the fact that the unfavorable entropic term dominates the favorable enthalpic term. Association of hydrophobic molecules in water involves the reverse process of solubilization of hydrocarbons in water, since the solvent-accessible surface area is reduced upon association of hydrophobic moieties. This mechanism can account for positive entropy changes in binding by hydrophobic effects. In addition to the entropic term, solute–solute attractive interactions such as attractive van der Waals interactions, including London's dispersion interactions, make a favorable enthalpic contribution. Homans and co-workers demonstrated that attractive van der Waals interactions between the hydrophobic moieties of protein and ligand made a significant contribution to the binding thermodynamics: the binding of alcohols to the major urinary protein becomes enthalpically favorable as the alkyl chain of the guest is longer.[10] Because the enthalpy and entropy changes of binding of a signal molecule to a protein or of a guest molecule to a synthetic receptor involve not only hydrophobic effects but also hydrogen bonding and conformational and translational entropy,[11] it is generally difficult to extract the contribution from hydrophobic effects.[12]

Abstract in Japanese:

タンパク質－基質間の結合や人工ホスト－ゲストの結合などにおいて重要な働きをする疎水相互作用の駆動力が、エントロピーによって説明できる場合と、エンタルピーによって説明できる場合が共に報告されており、疎水相互作用の駆動力が何であるかが明確になっていない。この問題を解明するために、4本のアルキル鎖、もしくは8本のアルキル鎖により疎水空間を構築した亜鉛ポルフィリンで、それぞれのアルキル鎖末端に水に可溶化させるためのポリオキシエチレン鎖をもつ人工レセプターに対するアルキルピリジンの結合の熱力学量を測定し、1つのメチレン基当たりのエンタルピー変化・エントロピー変化を比較した。いずれのポルフィリンでも自由エネルギー変化で評価したアルキル基の認識エネルギーは1つのメチレン基当たりの2.6－2.8 kJ mol^{-1} でほぼ同じであるが、アルキル基の認識の駆動力は、前者のポルフィリンではエンタルピーであり（メチルピリジンよりプロピルピリジンの結合がエンタルピー項で、–16.4 kJ mol^{-1} 有利）、後者ではエントロピーであった（メチルピリジンよりプロピルピリジンの結合がエントロピー項で、39.6 J K^{-1} mol^{-1} 有利）。これらのポルフィリンに対するイミダゾール／ピリジンの認識の選択性より、それぞれのポルフィリンホストの結合サイトへの水の近づきやすさを評価すると、前者のポルフィリンの結合サイトには水が接近しやすい環境であり、疎水相互作用によるアルキル基の認識において水が疎水環境に残るために、エンタルピー駆動になると考えられる。一方、後者では、結合サイトには水が入りにくく、脱水和のエントロピーが駆動力になっている。結合サイトへの水の近づきやすさによって、疎水相互作用の駆動力がエントロピーからエンタルピーに切り替わることがわかった。

Jencks[13] has made an interesting proposal that hydrophobic bonds can be classified into two categories: a "classical hydrophobic bond" forms between nonpolar molecules and a "nonclassical hydrophobic bond" between partially polar molecules. The former hydrophobic bond is characterized by negative entropy changes, whereas the latter hydrophobic bond is characterized by negative enthalpy changes. Schneider et al. suggested that discrimination of solvophobic forces and solute–solute intermolecular forces, such as London's dispersion forces, is important to elucidate the binding mechanism of nonpolar molecules in water, particularly to explain the enthalpic and entropic contributions to the free-energy changes in the binding.[14] If we determine differential enthalpy and entropy changes for a series of guests with systematic variation in hydrophobicity, they should be a good estimate of enthalpy and entropy owing to the hydrophobic effects. Similar perturbation studies to elucidate the binding mechanism of organic molecules to carbonic anhydrase have been reported by Whiteside and co-workers.[15]

In previous papers,[7,16] we reported that the entropy term is favorable and the enthalpy term unfavorable as the alkyl group of 4-alkylpyridines is longer for binding of 4-alkylpyridines to water-soluble zinc porphyrin **4**. The entropic driving



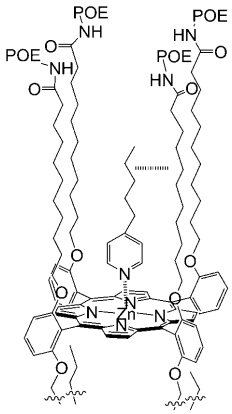
1: POE average mol. wt. 750
2: POE average mol. wt. 2000
3: POE average mol. wt. 350
4: POE average mol. wt. 750
5: POE average mol. wt. 2000

force is consistent with the picture of hydrophobic effects described above, whereby the water molecules around the hydrophobic alkyl chain obtain motional freedom upon contact of the hydrophobic alkyl chain of the guest with those of the receptors. We report herein that a similar receptor (**1**) having a less hydrophobic binding site, which allows more water molecules to penetrate into the binding cleft, showed enthalpically driven binding of the alkyl group of guest. Re-

ceptor **3**, which has a binding site of intermediate hydrophobicity, showed both enthalpically and entropically driven hydrophobic effects, intermediate behavior between **1** and **4**. Thus, the hydrophobicity of the binding cleft—the extent that water molecules are excluded from the binding cleft—is strongly correlated with the driving force of hydrophobic effects.[17]

Results and Discussion

We prepared water-soluble synthetic receptors **1** and **3**. The receptors consist of three components, a zinc porphyrin, alkyl pillars, and polyoxyethylene (POE) with an average molecular weight of either 750 or 350 at each terminal of the alkyl pillars. The zinc porphyrin moiety binds a nitrogeneous base through Lewis acid–Lewis base interactions,[18] and alkyl pillars provide a hydrophobic environment to the binding site.[19] The POE groups were attached to the terminal of alkyl pillars to solubilize the whole molecule in water and to protect the binding cleft from water.[20] Because the coordinating interaction between the pyridyl nitrogen atom and the zinc atom is relatively strong, the orientation of the guest in the host–guest complex is well-defined. The alkyl group of 4-alkylpyridines should be forced to align with the alkyl pillars when bound to the receptor (Scheme 1). By sys-



Scheme 1. Schematic representation of orientation of alkylpyridine in the binding cleft of receptor **3**.

tematic variation of the alkyl chain length in 4-alkylpyridines, we can estimate the hydrophobic interaction between the alkyl chain and the hydrophobic wall of the receptor. We determined the free-energy changes, the enthalpy changes, and the entropy changes in the binding of 4-alkylpyridines in water to evaluate the mechanism of hydrophobic effects, by using UV/Vis spectrophotometry and isothermal titration calorimetry.[21] We also determined the binding constants for imidazoles and hydroxyalkylpyridines that favor a hydrophilic binding site because of hydration of the nitrogen atom and the hydroxy group, respectively. Comparison of the binding affinity of imidazoles and pyridines gives a measure of hydrophobicity of the binding site. These data are compared with those determined for a similar receptor **4**,[7] in which eight alkyl pillars with terminal POE of average molecular weight of 750 are used to construct a hydrophobic binding site.

UV/Visible Absorption Spectroscopic Studies on the Aggregation of 1 in Water

A solution of **1** in dichloromethane showed a sharp Soret band at 413 nm. A freshly prepared solution of **1** in a pH 7 potassium phosphate buffer at 25 °C showed splitting of the Soret band at 421 and 416 nm, as shown in Figure 1. The

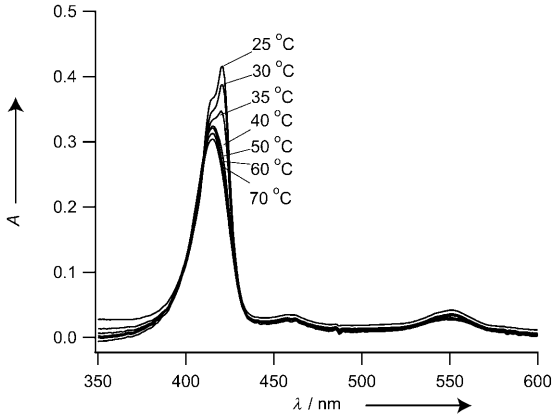


Figure 1. Variable temperature UV/Vis spectra of a solution of **1** in phosphate buffer at pH 7. The solution was heated from 25 °C to 70 °C.

splitting of the Soret band was not observed for a similar receptor **4**. We ascribe the splitting of the Soret band to formation of aggregates of **1** as a result of the intermolecular hydrophobic effects of the porphyrin core and the alkyl chains. When the solution was warmed up to 70 °C, the peak at 421 nm decreased and disappeared, leaving a sharp band at 416 nm. The Soret band remained sharp for a while after the solution was cooled to room temperature. When the solution was left overnight at room temperature, however, the splitting of the Soret band was again observed. A plot of the absorbance at 421 nm against temperature is shown in Figure 2. The heating and cooling curve showed hysteresis, revealing that aggregate formation is a kinetically slow process. To avoid any errors in binding constants owing to aggregation, we started the titration of the receptor solution with guest just after the host solution was annealed at 70 °C and cooled to the designated temperature.

UV/Visible Absorption Spectroscopic Studies on the Aggregation of 3 in Water

A solution of **3** in water (0.1 M potassium phosphate buffer at pH 7.0) showed a sharp Soret band at 25 °C. Upon heating the solution to 45 °C, the Soret peak was red-shifted from 425 to 428 nm. When the solution was heated above

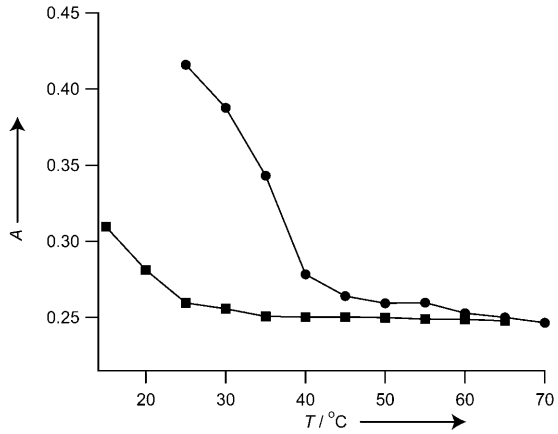


Figure 2. Plot of the absorbance at 421 nm against the temperature of the solution of **1** in pH 7 phosphate buffer.

55 °C, the Soret band became broadened, and on further heating to 85 °C the Soret peak became diminished, indicating that some of **3** was phase-separated. Therefore, **3** forms aggregates at a higher temperature, which is contrasting behavior to that of **1**, which aggregates at lower temperature. The aggregation of **3** at higher temperature is similar to lower critical solution temperature behavior observed for neutral surfactant containing POE groups such as Triton-X[22] and water-soluble polymers with a hydrophobic side chain such as poly(*N*-isopropylacrylamide).[23]

Hydrophobic Interaction Free Energy

The binding constants of 4-alkylpyridines to receptors **1** and **3** were determined by UV/Vis spectroscopic titration experiments. Addition of stock solutions of 4-alkylpyridines to an aqueous solution of **1** in pH 7.0 phosphate buffer caused a red shift in the Soret band. In the difference spectra, the absorbance at 427 nm increased with decreasing absorbance at 410 nm. Similarly, the addition of 4-methylpyridine to **3** in pH 7.0 phosphate buffer resulted in a difference spectrum with decreased absorbance at 425 nm and increased absorbance at 438 nm. The spectral changes were similar to those observed for coordination of amines to zinc porphyrins.[24] Curve fitting to the 1:1 binding isotherm was performed using the least-squares method to obtain binding constants (Table 1). The values of the binding free energy of receptors **1** and **3** are plotted against the number of methylene groups (n) in the guest in Figure 3. From the least-squares fit to the data, we obtained the slope of the line, which gives the free-energy stabilization for an additional CH_2 group on the guest. For receptors **1** and **3**, the free-energy stabilization for an additional CH_2 group on the guest, $-d(\Delta G^o)/dn$, was 2.6 and 2.8 kJ mol^{-1}, respectively. The free-energy increments per methylene group (namely, the slope of the lines) for receptors **1**–**5** are listed in Table 2. The values of $-d(\Delta G^o)/dn$ were in the range of 2.2–3.4 kJ mol^{-1}. Similar behavior was observed for the binding of alkylpyridines to zinc porphyrins incorporated in liposomal bilayer membrane, and the value of $-d(\Delta G^o)/dn$ was in the range of 2.4–3.2 kJ mol^{-1}.[25] Assuming the solvent accessible surface area of a CH_2 group is 31.8 Å^2, the values of 2.2 kJ mol^{-1} and 3.4 kJ mol^{-1} for $-d\Delta G^o/dn$ correspond to the hydrophobic interaction free

Table 1. Binding constants (K) of alkylpyridines, ω-hydroxyalkylpyridines, and imidazoles to receptors **1**–**4** in water at 25 °C, 0.1 M potassium phosphate buffer pH 7.0.[a]

Host	Guest	K [M^{-1}]
1	4-methylpyridine	14300
1	4-ethylpyridine	46000
1	4-propylpyridine	130000
1	4-pentylpyridine	960000
1	4-hydroxypyridine	11
1	4-hydroxymethylpyridine	11000
1	4-(2-hydroxyethyl)pyridine	2500
1	4-(3-hydroxypropyl)pyridine	6000
1	*N*-methylimidazole	730
1	*N*-ethylimidazole	920
2	4-methylpyridine	38400[b]
2	4-ethylpyridine	103000[b]
3	4-methylpyridine	7700
3	4-ethylpyridine	21000
3	4-propylpyridine	64000
3	4-pentylpyridine	640000
3	*N*-methylimidazole	80
3	*N*-ethylimidazole	99
4	4-methylpyridine	19000[c]
4	4-ethylpyridine	54200[c]
4	4-propylpyridine	148000[c]
4	4-hydroxypyridine	13
4	4-hydroxymethylpyridine	5200
4	4-(2-hydroxyethyl)pyridine	2000
4	4-(3-hydroxypropyl)pyridine	6300
4	*N*-methylimidazole	150
4	*N*-ethylimidazole	260

[a] [**1**] = 2.8×10^{-6} M. Binding constants are averages of three to four independent determinations, and errors of the mean are estimated to be 5%. [b] Taken from Ref. [16]. [c] Taken from Ref. [7].

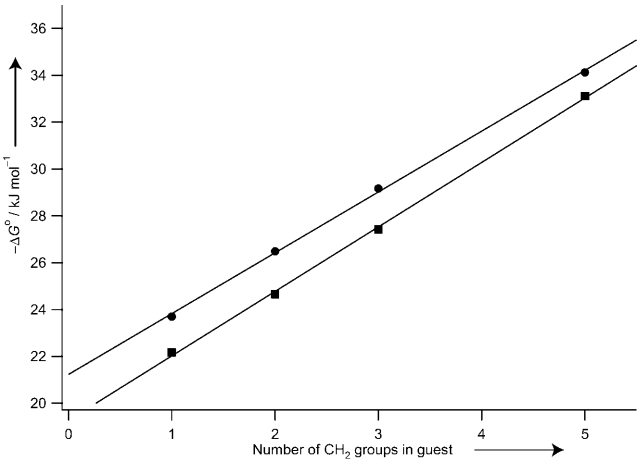


Figure 3. Plot of the binding free energy of 4-alkylpyridines to **1** (●) and **3** (■) against the number of CH_2 groups (n) in the guest. The lines obtained by the least-squares fitting is shown: $-\Delta G^o$/kJ mol^{-1} = 2.6 n+21.2 for **1** and $-\Delta G^o$/kJ mol^{-1} = 2.8 n+19.3 for **3**.

Table 2. The binding free energy increment per CH_2 group ($-d(\Delta G^o)/dn$, kJ mol^{-1}) and the binding free energy extrapolated to $n=0$ ($-\Delta G^o$ ($n=0$)) for POE–Zn porphyrin conjugates **1**–**5** having either four or eight POE alkyl chains and POE molecular weight of 350, 750, or 2000.

Receptor	Number of alkyl pillars	Mol. Wt. of each POE group	$-d(\Delta G^o)/dn$ [kJ mol^{-1}]	$-\Delta G^o$ ($n=0$) [kJ mol^{-1}]	Mol. Wt. POE/Mol. Wt. whole mol.
1	4	750	2.6	21.2	0.70
2	4	2000	3.4	20.2	0.86
3	8	350	2.8	19.3	0.55
4	8	750	2.6	21.8	0.73
5	8	2000	2.2	24.4	0.88

energy per Å^2 contact area of 0.069 and 0.107 kJ mol^{-1} per Å^2, respectively.

The hydrophobic free energy per unit contact surface area has been evaluated on the basis of 1) solubility of alkanes and amino acids, 2) the binding data of ligand–protein complexes, 3) the binding data of host–guest complexes, and 4) partition coefficients between organic solvent and water. Hermann,[26] Tanford and co-workers,[27] and Sharp et al.[28] estimated the hydrophobic surface free energy of alkanes in water to be 0.14, 0.10, and 0.2 kJ mol^{-1} per Å^2, respectively, based on solubility data of hydrocarbons in water. Richards[29] stated in his review that 0.084 kJ mol^{-1} per Å^2 has a fair chance of being an appropriate value for the hydrophobic free energy. Boehm[30] reported that the lipophilic contact between protein and a ligand results in 0.1 and 0.17 kJ mol^{-1} per Å^2, respectively. Cohen and Connors[31] reported the lipophilic free energy per Å^2 contact area is 0.38 kJ mol^{-1} on the basis of the binding free energies of host–guest systems, although the analysis was performed for relatively simple organic host–guest systems such as benzene derivative–anthracene derivative complexes, and the binding constants were in the range 2–230 M^{-1}. Higuchi and co-workers[32] reported that there was a constant increment of 3.8 kJ mol^{-1} in the free energy for each additional increment in methylene group for anions based on the ion pair extraction experiments. This value corresponds to 0.12 kJ mol^{-1} per Å^2. The hydrophobic free energies per Å^2 are thus in a rather broad range, depending on what host–guest complexation or solubility data were employed to derive the energy. The hydrophobic free energy observed in our receptor–ligand complexes is comparable or somewhat smaller than these reported values.

The ratios of the molecular weight of the POE moieties to the molecular weight of the whole molecule are shown in Table 2. On the basis of this ratio, **5** has the largest proportion of POE and this value decreases in the order **5**>**2**>**4**>**1**>**3**. The largest value of $-d(\Delta G^o)/dn$ for receptor **2** implies that the receptor with an intermediate ratio of POE and other moieties had the best binding site for an alkyl group; that is, hydrophilic/hydrophobic balance is important for the design of synthetic receptors.

Enthalpy and Entropy Changes Associated with Hydrophobic Effects

Although the recognition free energy of the alkyl chain by receptor **1** is similar to that of receptor **4**, the enthalpic and entropic contributions are much different. The enthalpy changes and the entropy changes were determined by van't Hoff analysis of the binding constants in the temperature range 15–45 °C as well as isothermal titration calorimetry. The values of the enthalpy changes and the entropy changes determined by isothermal titration calorimetry were close to those determined by the van't Hoff analysis. However, reproducibility of data obtained by the isothermal titration calorimetry was poorer, presumably because aggregation of the receptors occurs for higher concentrations of **1**. Thus, we used the data obtained by the van't Hoff analysis, in which low concentrations of the receptors can be used to avoid any aggregation. The values of the enthalpy changes and the entropy changes of binding of 4-alkylpyridines to **1**, **3**, and **4** are summarized in Table 3. The values of $T\Delta S^o$ are plotted against ΔH^o for **1**, **3**, and **4** in Figure 4, and the arrows indicate the direction of the increase in the guest alkyl chain length. Interestingly, the enthalpy term becomes favorable and the entropy term slightly unfavorable as the alkyl chain of the guest is longer for receptor **1**, whereas the entropy term becomes favorable and the enthalpy term becomes unfavorable as the alkyl chain is lengthened for receptor **4**. It is noteworthy that the hydrophobic effects are characterized by an increase in entropy in eight-pillared receptor **4** but by a decrease in enthalpy in four-pillared receptor **1**. Receptor **3** showed intermediate behavior: both enthalpy and entropy terms become favorable as the alkyl chain is lengthened, and the enthalpy term changes more significantly.

As shown in the plots in Figure 4, the entropy changes are relatively constant for **1** and the enthalpy changes are rela-

Table 3. Enthalpy changes and entropy changes of binding of 4-alkylpyridines to porphyrin receptors **1**, **3**, and **4**.[a]

Porphyrin	Guest	ΔH^o [kJ mol^{-1}]	ΔS^o [J K^{-1} mol^{-1}]	ΔG^o [kJ mol^{-1}][c]
1	4-methylpyridine	-23.6 ± 2.4[b]	0.6 ± 7.9[b]	−23.8
1	4-propylpyridine	-33.7 ± 0.9	-15.1 ± 2.9	−29.2
1	4-pentylpyridine	-40.0 ± 1.7	-19.3 ± 5.3	−34.3
3	4-methylpyridine	-15.0 ± 2.3	23.7 ± 7.6	−22.2
3	4-ethylpyridine	-17.5 ± 2.4	24.4 ± 8.1	−24.7
3	4-propylpyridine	-19.4 ± 1.0	26.7 ± 3.3	−27.4
4	4-methylpyridine	-24.5 ± 1.2[d]	-0.6 ± 4.7[d]	−24.4
4	4-propylpyridine	-23.3 ± 0.5[d]	19.8 ± 1.7[d]	−29.4
4	4-pentylpyridine	-22.8 ± 0.2[d]	39.0 ± 0.3[d]	−34.4

[a] Standard deviations of ΔH^o and ΔS^o were calculated according to literature.[33] [b] Isothermal calorimetry gave the following data: $\Delta H^o=-25.5$ kJ mol^{-1}, $\Delta S^o=-5.31$ J K^{-1} mol^{-1}, [**1**]$=4.2\times10^{-5}$ M. [c] Calculated from ΔH^o and ΔS^o with $T=298$ K. [d] Taken from Ref. [7].

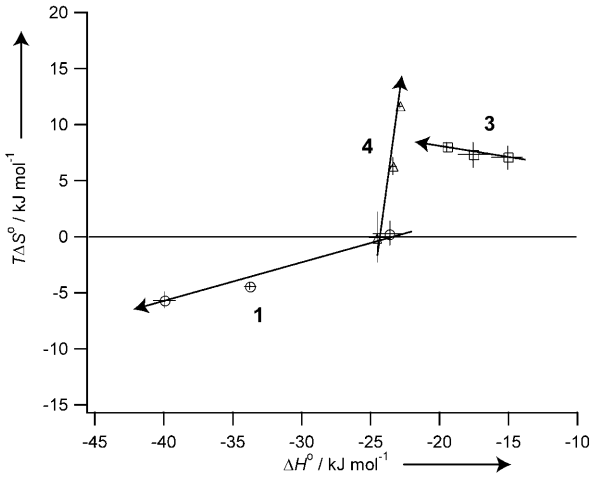


Figure 4. Plot of $T\Delta S^o$ ($T=298$ K) against ΔH^o for binding of 4-alkylpyridines to **1**, **3**, and **4**. Arrows indicates the direction of changes as the length of the alkyl group, and thus the binding free energy $-\Delta G^o$, increases. Bars indicate standard deviations.

tively constant for **4**, showing that there is little enthalpy–entropy compensation.[34] If enthalpy–entropy compensation was observed, the free-energy changes should be almost constant, even if there were large changes in enthalpy and entropy. In the present case, the changes in enthalpy or entropy were directly reflected in the free-energy changes. As a component of the free energy, the enthalpy term is predominant in the alkyl recognition free energy for receptors **1** and **3**, whereas the entropy term is predominant for receptor **4**. Receptor **3** showed intermediate behavior between **1** and **4**, whereby recognition of the alkyl group was driven by an enthalpic term to a lesser extent than **1**. As discussed in the next section, the binding cleft of **1** is accessible to water, whereas that of **4** is protected from water. The binding cleft of **3** showed intermediate accessibility of water. Therefore, we suggest that the hydrophobicity of the binding cleft—the extent of exclusion of water molecules from the binding cleft—is the major factor that determines whether binding is driven by an enthalpic term or by an entropic term. The suggested mechanism is different from the classical versus nonclassical hydrophobic bond as suggested by Jencks.[13] According to his mechanism, the partially polar nature of the solute molecules would result in enthalpy-driven nonclassical hydrophobic bond. In the present systems, we compared the binding thermodynamics for the same guest molecules and we focused on the interaction with an alkyl group, so that the difference in the driving force should be ascribed to the difference in solvation.

It would be interesting to compare the thermodynamic data obtained herein with those reported for binding of simple aliphatic alcohols to α-cyclodextrin. Spencer et al.[5c] reported that the binding increment for a methylene group was $\Delta\Delta G^o=-3.0$ kJ mol^{-1} and $\Delta\Delta H^o=-3.83$ kJ mol^{-1}. These values are close to the enthalpy and entropy changes observed for **1** ($\Delta\Delta G^o=-2.6$ kJ mol^{-1} and $\Delta\Delta H^o=-4.1$ kJ mol^{-1}). Therefore, the comparison implies, as discussed in the next section, that water accessibility to the binding cleft of α-cyclodextrin could be similar to that of receptor **1**. The binding site of α-cyclodextrin is rather open, resulting in the enthalpy-driven binding.

Imidazole/Pyridine Selectivity as a Measure of Hydrophobicity of the Binding Site

The hydrophobicity of the binding site can be estimated by comparing the binding constants of imidazoles with those of pyridines. Because imidazoles have two nitrogen atoms, and the nitrogen atom not bound to the zinc center should be strongly hydrated, the binding of imidazoles should be inhibited if the binding site is hydrophobic and resistant to hydration. The ratios of binding constant of 4-methylpyridine to that of *N*-methylimidazole and those of 4-ethylpyridine to that of *N*-ethylimidazole are listed in Table 4. The ratio *K*(4-methylpyridine)/*K*(*N*-methylimidazole) is 124 for receptor **4** whereas it is 20 and 96 for receptors **1** and **3**, respectively. These results suggest that the hydrophilicity of the binding site of the receptors, water accessibility to the binding cleft, decreases in the order: **1**>**3**>**4**. The binding site becomes more hydrophilic as the number of alkyl pillars decreases and the molecular weight of the POE groups is lower. This order of water accessibility of binding sites is not identical to the order of affinity to alkyl groups as measured by $-d\Delta G^o/dn$ (Table 2). The imidazole to pyridine selectivity reflects hydrophobicity near the porphyrin plane, whereas the value of $-d\Delta G^o/dn$ reflects the hydrophobicity at some point distant from the porphyrin plane.

Table 4. Ratios of binding constants of pyridines to imidazoles as a probe for the polar/nonpolar environment of the binding site.

	1	**3**	**4**	**5**
K(4-methylpyridine)/*K*(*N*-methylimidazole)	20	96	124	164
K(4-ethylpyridine)/*K*(*N*-ethylimidazole)	50	212	210	340

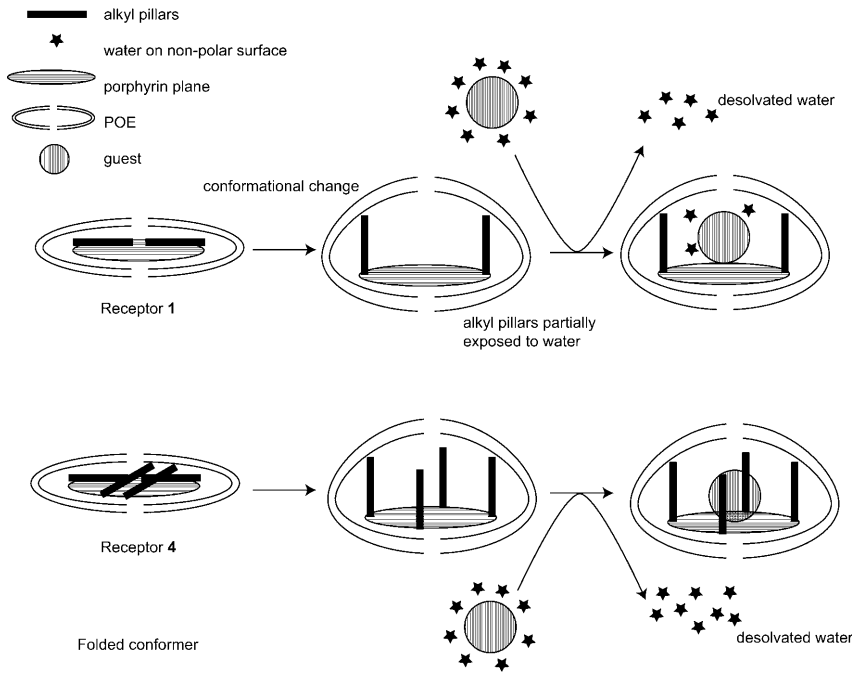
Binding of ω-Hydroxyalkylpyridines

We reported that the presence of a polar substituent in the alkyl chain of alkylpyridines considerably inhibits binding.[16] The ratios of binding constants of alkylpyridines to ω-hydroxyalkylpyridines are 1.3 (**1**–Me-py), 18 (**1**–Et-py), 22 (**1**–Pr-py), 3.7 (**4**–Me-py), 27 (**4**–Pr-py), and 23 (**4**–Pr-py). Thus, the binding constants of 4-(2-hydroxyethyl)pyridine and 4-(3-hydroxypropyl)pyridine become one order of magnitude smaller than those of nonhydroxylated guests. These ratios amount to the free-energy penalty of binding of 7.2 to 8.2 kJ mol^{-1} for ethyl and propylpyridines. A similar thermodynamic penalty was reported for the binding of 1-octanol and 1,8-octandiol to the major urinary protein: a decrease of the binding free energy of 18 kJ mol^{-1} was observed when the polar OH group was forced to enter to a hydrophobic binding site.[35] The effects are insignificant for 4-methylpyri-

dine: 4-hydroxymethylpyridine binds to receptors **1** and **4** with relatively strong affinity. The high affinity of 4-hydroxymethylpyridine is somewhat surprising, and polar OH groups comfortably enter the binding site. Molecular dynamics calculations of the receptor **1**–4-hydroxymethylpyridine complex suggest that the binding site of receptor **1** is rather compact, and the hydroxy group of the 4-hydroxymethyl group is hydrogen bonded to the oxyethylene group of the receptor (see Figure S1 in the Supporting Information).

Conformational Changes, Guest Desolvation, and Receptor–Guest Interactions upon Binding

In an aqueous solution, receptors **1** and **4** should have a folded conformation whereby the alkyl chains are in contact with each other and with the porphyrin plane to form a nonpolar core and the core is surrounded by the POE moieties to avoid direct contact with water. To bind a guest in the binding site of receptors **1**, **3**, and **4**, conformational changes in the alkyl pillar groups should occur to create a space to accommodate the guest (Scheme 2). We propose that the alkyl chains of receptor **1** become more exposed to water when the guest is bound to accommodate and encompass the alkylpyridine in water. The POE auxiliary groups of **1** could be insufficient to cover the increased hydrophobic surface. Thus, the binding of hydrophobic guest caused more water molecules to have contact with hydrophobic surfaces and hence result in a negative entropic change. Because there are some water molecules in the binding site of **1**, only partial desolvation of the guest alkyl chain may occur. In contrast, receptor **4** has enough POE moieties to protect the binding cleft from water, and the conformational changes have little effect on the values of the enthalpy and entropy changes. We expect that desolvation of the guest alkyl chain upon binding to **4** is more extensive. The hydrophobic/hydrophilic balance of the receptors altered the accessibility of water to the binding site, and they significantly affected the enthalpic and entropic contribution to the binding thermodynamics.



Scheme 2. Schematic representation of the binding of guest to receptors **1** and **4**. In receptor **1**, water molecules enter into the binding site to result in enthalpy-driven binding, whereas in receptor **4** complete dehydration occurs to result in entropy-driven binding.

Conclusions

The binding of an alkyl group to the hydrophobic site in a synthetic receptor was driven by either an enthalpic force or an entropic force. Entropically driven binding was explained by a classical picture of hydrophobic effects, where water molecules around the nonpolar molecular surface are released to bulk water phase to lead to increase in entropy. However, attractive van der Waals interactions between the nonpolar surfaces of receptor and ligand also make a significant contribution to the enthalpic driving force, as suggested for nonclassical hydrophobic interactions. Therefore, the overall thermodynamic parameters are determined by a subtle balance of enthalpic and entropic driving forces, and other factors such as conformational changes in the receptor upon binding switch the driving force from being entropy-driven to being enthalpy-driven. In our work, we compared the binding mechanism of an alkyl group among the three receptors. Receptor **1** has only four alkyl pillars and the binding site is open, whereas receptor **4** has eight alkyl pillars and the binding site should be well protected from water. Receptor **3** has also eight alkyl pillars, as well as shorter POE moieties than in **4**. Aggregation behavior and imidazole/pyridine selectivity indicated that receptor **4** has a hydrophobic binding site in which the accessibility of water molecules is low, whereas receptor **1** has some water in its binding site and a less hydrophobic binding site. The hydrophobicity of receptor **3** was intermediate between **1** and **4**. We demonstrated that receptor **4** having a hydrophobic binding site recognizes an alkyl group by an entropic driving force, whereas receptor **1** having a less hydrophobic binding site recognizes an alkyl group by an enthalpic driving force. Receptor **3** showed intermediate behavior between **1** and **4**. Therefore, the

accessibility of water to the binding site switches the driving force from entropy to enthalpy.

Experimental Section

Experimental details are reported in the Supporting Information.

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