Amine binding to water-soluble zinc porphyrins bearing a hydrophobic binding pocket

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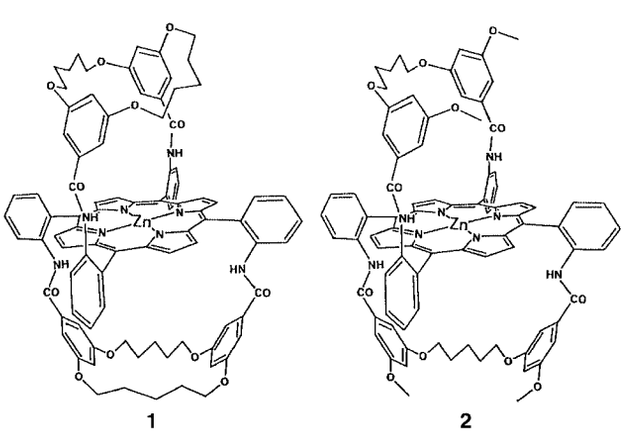
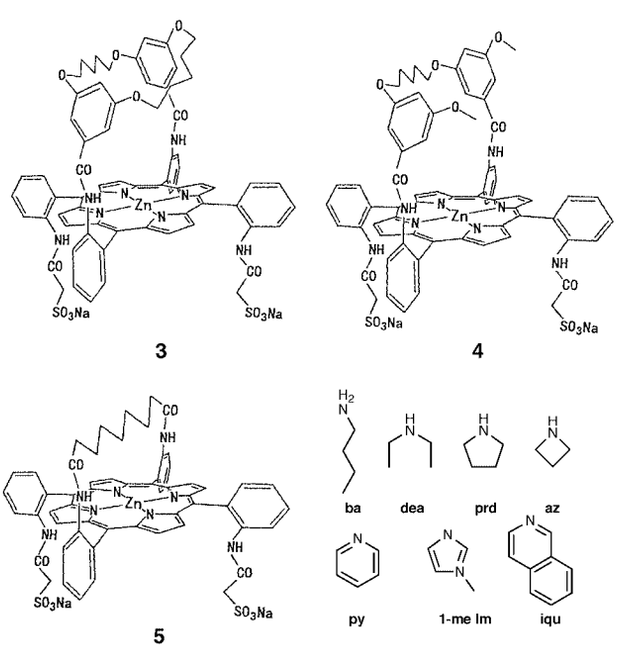
The binding of several amine ligands to water-soluble zinc porphyrins **3**, **4**, and **5** bearing a hydrophobic binding pocket was examined spectrophotometrically in water and in chloroform. In chloroform, substantially decreased binding constants (K) of these porphyrins compared to available data for synthetic zinc porphyrins were observed and this was ascribed to the tightly bound water molecule that must be released upon amine binding. In aqueous solution, the large K values of **3** among these complexes showed that a preorganized structure of the binding pocket is necessary for binding enhancements of the amine ligands. The positive entropy changes in aqueous solution were found to contribute largely to the amine binding to **3** and **4**. These results suggested that hydrophobic interactions would dominantly affect the binding behaviour of these zinc porphyrins and apparently eclipse the direct non-polar attractions between the bound amines and the superstructures of the porphyrins.

Introduction

One of the strategies to better understand host–guest association or molecular recognition phenomena related to biological systems is to explore binding of axial ligands to artificial metalloporphyrins.[1] The ligand selectivity and recognition are driven by simultaneous cooperation of various non-covalent interactions[2] between the porphyrin superstructures and the axial ligand in addition to the coordination bond. Most of these metalloporphyrins are, however, not soluble in water so the experimental conditions are quite different from physiological conditions. Few works[3–7] have been reported concerning water-soluble superstructured porphyrins and little is known about the binding of nitrogenous axial ligands as a guest in aqueous media. Since the binding behaviour of such porphyrin complexes in aqueous solution cannot be presumed from that observed in non-polar organic solvents, to examine the binding in aqueous solution and compare it with that obtained in non-polar environments will be useful for understanding molecular recognition in biological systems.

In earlier works, we[8,9] have reported that non-polar interactions between the superstructures of zinc porphyrins (**1** and **2**) and nitrogenous axial ligands substantially enhance the ligand binding even in non-polar organic solvents and that the preorganized structure of **1** is required for the axial–ligand recognition. However, the effectiveness of the selectivity revealed by these porphyrins may not be predicted in aqueous media, since the chemically specific character of water as a solvent must strongly affect the binding behaviour.[2] In a preliminary report,[10] in order to create a similar environment to that of natural systems by use of artificial and low molecular weight compounds, we have designed and synthesized amphiphilic zinc porphyrin **3**. We report here the binding of several nitrogenous ligands to water-soluble porphyrins **3**, **4**, and **5** in detail. The binding pocket of **3** is sufficient in size to accommodate an axial ligand and preorganized in the absence of the axial ligand whereas that of **4** is less preorganized. Contrary to this, the strap in **5** does not seem useful in forming a





hydrophobic binding pocket and rather may prevent the coordination of axial ligands because of steric repulsion.[11] Thus, comparisons of the binding data among these complexes will provide information on the ligand-recognition ability of the binding pocket in aqueous media. Since **3** is amphiphilic, the binding data in chloroform were also compared to those of lipophilic zinc porphyrins **1** and **2**.

Experimental

Materials

Amines, except for az † and iqu, were purified by distillation from KOH. Azetidine (Aldrich) was dried over molecular sieves (4 Å). Isoquinoline was purified by vacuum distillation. Chloroform for spectral measurements was ethanol-free (HPLC grade, Merck) and was used without further purification. Zinc porphyrins **3** and **5** were prepared according to the literature.[10] Zinc porphyrin **4** was prepared by a similar method to that for **3** and **5** by use of 5,5′-dimethoxy-3,3′-(pentane-1,5-diyldioxy)-dibenzoyl chloride[9] for constructing the hydrophobic binding pocket: δ_H (400 MHz; CD_3OD; Me_4Si) 0.34 (2H, m, OCH_2-CH_2CH_2), 0.48 (4H, m, OCH_2CH_2), 2.55 (6H, s, OCH_3), 2.80 (4H, t, *J* 6, OCH_2), 2.96 (4H, s, $NHCOCH_2$), 5.48 (2H, s, strap Ph), 5.57 (2H, s, strap Ph), 5.66 (2H, s, strap Ph), 7.3–8.4 (20H, m, Ph and NH), 8.69 (4H, d, *J* 5, pyrrole CH), 8.83 (4H, d, *J* 5, pyrrole CH); λ_{max} (0.01 mol dm^{-3} K_2CO_3)/nm 430, 561, 600 (Found: C, 53.66; H, 3.83; N, 6.98%; M^+, 1394. $C_{69}H_{54}N_8O_{14}S_2Na_2Zn\cdot CHCl_3\cdot 3H_2O$ requires C, 53.62; H, 3.92; N, 7.15%; *M*, 1394).

Measurements

Proton NMR spectra were recorded on a JEOL GSX-400 spectrometer. Infrared spectra were recorded on a Perkin-Elmer Spectrum 2000 spectrometer. Visible absorption spectra were measured with a Hitachi 340 or Hitachi U-3000 spectrophotometer. The equilibrium data for amine binding were determined by using the analyzing method[12] for the visible spectral changes upon titration of zinc porphyrins with amine solutions. The aqueous solutions were adjusted at pH 11.5 with K_2CO_3 (0.01 mol dm^{-3}) to prevent pH changes upon amine titration. Thermodynamic values for amine binding were estimated from the temperature dependence of equilibrium constants ranging from 8 to 44 °C.

Results and discussion

Equilibrium in solution

The central zinc ion in porphyrin complexes generally has four or five coordination in solution.[1] The visible spectral data showed that, in the absence of amine ligands, zinc porphyrins **3**, **4**, and **5** bind a water molecule to be five coordinate both in chloroform ‡ and in water, while **1** and **2** are four coordinate in chloroform.[8,9] Then, the binding of amines (L) to the zinc porphyrins (ZnP) prepared in this work can be explained by eqn. (1). In this work, the binding constant for eqn. (1) is

$$ZnP\cdot H_2O + L \rightleftharpoons ZnP\cdot L + H_2O \qquad (1)$$

defined by eqn. (2), and allows us to neglect the difference in

$$K = \frac{[ZnP\cdot L]}{[ZnP\cdot H_2O][L]} \qquad (2)$$

† Abbreviations: az, azetidine; ba, butylamine; dea, diethylamine; iqu, isoquinoline; py, pyridine; prd, pyrrolidine; 1-MeIm, 1-methylimidazole.

‡ Compounds **4** and **5** were not sufficiently soluble in chloroform to allow determination of binding constants with amines.

H_2O activity between chloroform and water and to compare the amine binding of this system with that of other systems containing no H_2O. The aqueous solutions for spectral measurements were considerably basic (pH 11.5) but the visible spectra of these zinc complexes were almost the same as those obtained at pH 6. This indicates that ligation of OH^- did not occur, since substitution of H_2O by OH^- should cause substantial red shifts of the absorption maxima.[13]

Amine binding in chloroform

Table 1 lists the binding constants of amines to zinc porphyrins. In chloroform, the binding constants *K* for **3** are two to four orders smaller than those for **1** and **2**. Although the binding pocket of **1** is the same as that of **3**, the former has two pockets on the porphyrin plane but the latter has only one. This difference should make the *K* values twice that for **1** as an entropy factor, if binding for **3** occurs only at the binding pocket site. This factor, however, can not account for the observed large differences in *K*. Another factor comes from the coordinated water as shown in eqn. (1). Since **1** and **2** are four coordinate in the absence of amines, the small *K* values for **3** in chloroform can be ascribed to the release of the bound water molecule upon amine binding as stated above. Coordination of H_2O to a few zinc porphyrins in toluene or chloroform has been reported[8,9,14] but this does not affect amine binding as much as in this case. Judging from Corey–Pauling–Koltun (CPK) modeling, it is reasonable to suggest that the bound water molecule in **3** would be highly stabilized by intramolecular hydrogen bonding with the two sulfonate groups. The presence of the hydrogen bonds was confirmed by IR spectral measurements. The observed SO band at 1202 cm^{-1} (overlapped with another signal) of **3** is shifted to 1236 cm^{-1} upon az addition, in which the hydrogen bonded SO group becomes free because of the release of the coordinated water.

Binding site on the porphyrin plane

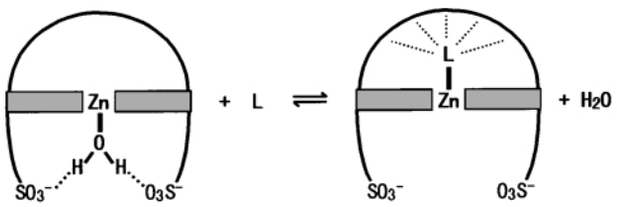
For the amine adducts of zinc porphyrins **3**, **4**, and **5**, two regioisomers are possible owing to the asymmetry on the porphyrin plane, suggesting that the observed *K* values are the sum of the two different equilibrium constants. In chloroform, iqu binding to **1** is substantially weaker than that to **2**, indicating that iqu is too large in size to be accommodated into the binding pocket of **1**.[9] On the other hand, a model building (CPK) study suggested that bound iqu undergoes little steric repulsion from the sulfonate groups. However, for amine binding to **3** appending the same binding pocket as **1**, the *K* value for iqu is also remarkably small among the amines examined in chloroform. Consequently, binding of amines from the sulfonate-group side on the porphyrin is considerably restricted. Further information on the binding site is definitively provided by ^1H NMR data. Upon addition of pyridine-d_5 to **3** in $CDCl_3$, the methylene signals of the strap at 1.00 (m, 4H) ppm and 1.25 (m, 8H)[10] exhibit substantial low-field shifts to 1.11 and 1.46 ppm, respectively, due to the ring current of the bound pyridine in the binding pocket, whereas the other signals show smaller or little shifts. Therefore, the amine binding to **3** occurs dominantly on the binding pocket side in chloroform.

The sulfonate groups are common among **3**, **4**, and **5** but apparently increased *K* values for **3** are observed in aqueous solution as compared to those for **5**. In earlier studies, we[11] have shown that a heptamethylene strap in metalloporphyrins such as **5** substantially weakens the binding of bulky amines by steric blocking. In contrast, the hydrophobic binding pocket of **1** and **3** is suitable in size for the amines used, except for iqu. Therefore, the amine binding to **3** dominantly occurs at the hydrophobic binding pocket side in aqueous solution as well as in chloroform. Thus, the equilibrium of **3** for eqn. (1) can be illustrated as follows.

Table 1 Binding data[a] for zinc porphyrins

Compound	Solvent	Amine							Reference
		ba	dea	prd	az	py	iqu	1-MeIm	
1	$CHCl_3$	7.7×10^4	5.8×10^4	2.7×10^6	1.4×10^7	1.4×10^4	6.1×10^2		8
2	$CHCl_3$	7.6×10^4	1.2×10^4	1.3×10^6	1.8×10^6	9.0×10^4	1.1×10^5		9
3	$CHCl_3$	9.2×10	2.1×10^2	5.4×10^2	2.1×10^3	2.6×10	<10	1.9×10^2	this work
	H_2O[b]	2.4×10^2	1.9×10^2	1.0×10^3	1.1×10^3	1.5×10^2		2.0×10^2	this work
4	H_2O[b]	4.3×10	<10	1.1×10^2	7.9×10	3.0×10		3.6×10	this work
5	H_2O[b]	2.7×10	<10	7.6×10	5.6×10	2.7×10		6.9×10	this work

[a] K/dm^3 mol^{-1} at 25 °C. [b] 0.01 mol dm^{-3} K_2CO_3 (pH 11.5).



Amine binding in aqueous solution

Although simple flat porphyrins have a tendency to aggregate at an appropriate concentration in aqueous solution, the zinc porphyrins prepared can not aggregate because of the both-face protection. For flat zinc porphyrins, binding of amino acids in aqueous solution has been reported,[15] where the net contribution from the Zn–N coordination-bond formation to the observed binding was weak and hydrophobic interactions between the porphyrin plane and the amino acid residues greatly affected the binding constants. The zinc porphyrins prepared have a structural similarity in the construction of the both-face protection, and the hydrophobic interactions between the porphyrin plane and amines are similar among **3**, **4**, and **5**. Thus, the differences in amine binding reflect the interacting modes between the bound amines and the porphyrin superstructures.

We have reported that the binding pocket of **1** shows selectivity for amine ligands on the basis of non-covalent interactions by which dea, prd, and az exhibit apparently increased binding to **1**.[8] Further, it was found that the preorganized structure of **1** compared to **2** is effective for the binding enhancements in non-polar organic solvents.[9] The binding enhancements are also seen for **3** (Table 1) to which amines bind most strongly among the zinc porphyrins in aqueous solution. Thus, the preorganized structure of the binding pocket is required for binding enhancements in aqueous solution as well as in non-polar solvents. However, the amine selectivity or recognition ability shown by **1** is drastically decreased in aqueous solution; for instance, the K(az)/K(ba) and K(prd)/K(ba) values are 182 and 35 for **1** in chloroform but only 4.6 and 4.2 for **3** in aqueous solution, respectively. Further, the hydrophobic binding pocket of **4** seems to have little effect on amine binding since the binding data for **4** are fairly close to those for **5**. This is also in contrast to what is observed in non-polar organic solvents where **2** showed binding enhancements for amines compared to an unprotected zinc porphyrin.[9] Thus, although hydrophobic binding pockets are predicted to have a role for binding enhancements in polar environments, our results indicate that the hydrophobic pockets in this system are not as effective in aqueous solution as in non-polar organic solvents.

Thermodynamics in aqueous solution

Tables 2 and 3 list thermodynamic values for the binding of ba and py, respectively. In most cases, both $\Delta H°$ and $\Delta S°$ values for Zn(II)–N bond formation by amine binding to zinc porphyrins in organic solvents are negative,[1b] indicating that the complex formation was enthalpy driven. In aqueous solution only binding to **5** is actually exothermic. For **3** and **4** in aqueous solution, the $\Delta S°$ values are positive and the $\Delta H°$ values are nearly zero. This suggests that the binding mode of **5** is different from that of **3** and **4**. In addition, the large K values for **3** compared for **4** and **5** come from the positive $\Delta S°$ values and the complex formation is evidently entropy driven. These results are reasonably explained in terms of hydrophobic interactions, since the interactions should give positive $\Delta S°$ values and must be weak for amine binding to **5** compared to **3** and **4**. This may be further supported by the fact that the K value of **3** with az (low C/N atomic ratio, relatively hydrophilic) is large in chloroform whereas that with py (high C/N atomic ratio, relatively hydrophobic) is large in water. Consequently, hydrophobic interactions dominantly contribute to the thermodynamic values in aqueous solution and apparently eclipse the direct non-polar interactions revealed in organic solvents. It is worthwhile noting that the thermodynamic balance (K) for **4** is fairly close to that of **5** despite the fact that the thermodynamic data are consistently different for each other. The reason for this is not understood at present but might be accounted for by the differences in desolvation–solvation processes of each solute and rearrangements of bulk water upon amine binding.§

Table 2 Thermodynamic values for butylamine binding to zinc porphyrins

Compound	Solvent	log K[a]	$\Delta H°$/kJ mol^{-1}	$\Delta S°$/J mol^{-1} K^{-1}
1	toluene	4.18[b]	-33.1 ± 0.4[b]	-32 ± 1[b]
	$CHCl_3$	4.89[b]		
3	$CHCl_3$	1.96	12.1 ± 2.5	78 ± 8
	H_2O	2.38	2.1 ± 1.3	52 ± 4
4	H_2O	1.63	0.8 ± 2.9	35 ± 8
5	H_2O	1.43	-7.5 ± 1.3	2 ± 4

[a] At 25 °C, estimated from van't Hoff plots. [b] Reference 8.

Table 3 Thermodynamic values for pyridine binding to zinc porphyrins

Compound	Solvent	log K[a]	$\Delta H°$/ kJ mol^{-1}	$\Delta S°$/J mol^{-1} K^{-1}
3	$CHCl_3$	1.42	4.2 ± 1.3	41 ± 4
	H_2O	2.16	0.8 ± 1.3	44 ± 5
4	H_2O	1.47	2.1 ± 2.9	36 ± 10
5	H_2O	1.43	-18.8 ± 0.8	-36 ± 3

[a] At 25 °C, estimated from van't Hoff plots.

Comparisons of binding in water and in chloroform

Solvents used or microenvironments in host–guest association appreciably affect various non-covalent interactions and there-

§ Differences in solvation of amine adducts or in reorganization of the binding pockets upon amine binding may account for the differences in thermodynamic data.

by the association phenomena.[2] Since **3** is amphiphilic, binding data were estimated in both chloroform and H_2O. To our knowledge, binding data for the same reaction in the two quite different solvent systems are not available. Interestingly, as can be seen in Table 1, the K values in chloroform are not very different from those in aqueous solution. Furthermore, the thermodynamic data of **3** for ba and py binding (Tables 2 and 3) are quite similar for the two solvent systems, where both $\Delta H°$ and $\Delta S°$ are positive. These results were not predicted since the desolvation–solvation processes of the solutes should be substantially different for the two solvents and the hydrophobic interactions could not exist in chloroform. We interpreted the binding data obtained in chloroform as a result of the release of the tightly bound water molecule upon amine binding; since the water forms hydrogen bonds with the sulfonate groups before amine binding as shown before, the cleavage of the hydrogen bond should substantially decrease the K values and must give rise to positive $\Delta H°$ and $\Delta S°$. In aqueous solution, such hydrogen bonds do not apparently influence amine binding because the strong solvation of the released water molecule by migration into the surrounding bulk water must somewhat compensate for the hydrogen-bond effects on the thermodynamic values.[3b] On the other hand, an increase in $\Delta S°$ is accompanied by the hydrophobic interactions in aqueous solution. As a result of these overall terms, similar thermodynamic data would be obtained between the two solvent systems.

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