Water-Soluble Zinc Porphyrins as Artificial Receptors for Amino Acids

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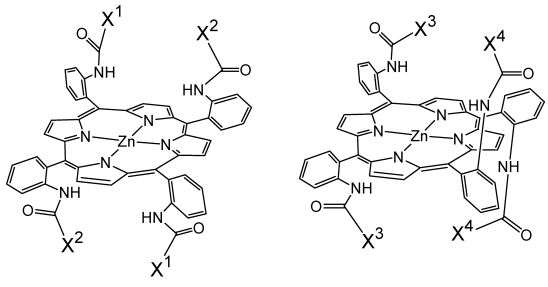
The binding of amino acids to water-soluble zinc porphyrins in basic aqueous solution was spectrophotometrically analyzed. The amino acids were bound to the porphyrins through the coordination of the N atom with the central zinc ion. Additional attractions arise due to Coulomb interactions between the $-COO^-$ anion of the amino acids and the $-N(CH_3)_3^+$ cation of the porphyrin substituents and due to hydrophobic interactions between the porphyrin plane and the hydrophobic substituents of the amino acids. These attractions could be explained based on the binding data. The compensatory relationships of ΔS and ΔH were also discussed.

Key words water-soluble zinc porphyrin; amino acid; molecular recognition; coordination; Coulomb interaction

In living organisms, the process by which small biomolecules such as amino acids and peptides are recognized by receptors plays critical roles. In order to understand molecular recognition mechanisms, various zinc porphyrins have been synthesized as artificial amino acid receptors.[1,2] However, due to the lipophilic nature of porphyrins, only a limited number of water-soluble zinc porphyrins have been synthesized and studied. Recently, we synthesized a series of water-soluble zinc porphyrins that show two or more types of attractions for amino acids and peptides.[3,4] These attractions include the coordination interaction and Coulomb interaction, and along with another interaction, these two interactions facilitate amino acid chiral recognition. Coordination interaction is useful as a recognition factor in supramolecular chemistry.[5] Usually, coordinate bonds are not adequately strong in aqueous solution due to the competitive coordination of water; nonetheless, in porphryin chemical analysis, the coordination interaction is helpful for the spectrophotometric detection with very small amounts of porphyrins. Although the Coulomb interaction decreases with the dielectric constant of solvents and thereby becomes weaker in aqueous solution than in nonpolar organic solvents, it is still very important in biological environments, as is exemplified by the intramolecular salt bridges in proteins. In the present study, to elucidate the details of the attractive interactions between porphyrins and bound amino acids, the binding of amino acids to **1**—**3** in basic aqueous solution (Fig. 1)[6] was examined.



1: $X^1 = X^2 = CH_2N(CH_3)_3 \cdot Cl$
2: $X^3 = X^4 = CH_2N(CH_3)_3 \cdot Cl$
3: $X^3 = C(CH_3)_3$, $X^4 = CH_2N(CH_3)_3 \cdot Cl$

Fig. 1. Water-Soluble Zinc Porphyrins

The bound H_2O molecule has been omitted for clarity.

Results and Discussion

In the absence of amines, zinc porphyrins exist as five coordinated species with a bound H_2O molecule in aqueous solution.[7] The N atoms of amines are not protonated under basic experimental conditions (pH 10.4), and these atoms can therefore coordinate with Zn ions and substitute the bound H_2O molecule of porphyrins. The binding equilibrium between zinc porphyrin (ZnP) and amine (L) is expressed as follows:

$$ZnP \cdot H_2O + L \rightleftharpoons ZnP \cdot L + H_2O$$

where the binding constant is given as

$$K = \frac{[ZnP \cdot L]}{[ZnP \cdot H_2O][L]}$$

Figure 2 shows visible spectra of **1** upon titration of L-Trp. The spectral changes accompanying red shifts correspond to the N coordination of L-Trp instead of the bound H_2O, in accordance with the published spectral assignment for zinc porphyrins.[8] This is well supported by the fact that the addition of oxalate dianion to the porphyrin solutions does not give such spectral changes, where the oxalate dianion may be fixed to the porphyrin by both coordination and Coulomb interaction.

Table 1 lists the binding constants of amino acids. The value of the binding constant reflects the various attractive and repulsive intramolecular interactions in the porphyrin complexes. Examples of the attractions are the coordination, Coulomb, and van der Waals interactions, and in aqueous so-

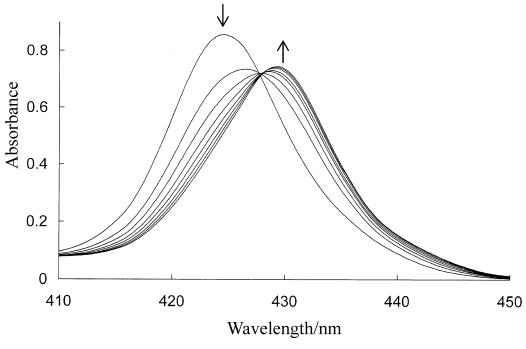


Fig. 2. Visible Absorption Spectra of **1** upon Titration of L-Trp

At 25.0 °C in aqueous solution buffered with $NaHCO_3$–Na_2CO_3 (pH 10.4, I=0.02). [**1**]=2.56×10^{-6} mol/l; [L-Trp]=0, 4.00×10^{-4}, 7.99×10^{-4}, 1.20×10^{-3}, 1.59×10^{-3}, 1.98×10^{-3}, 2.38×10^{-3}, 2.77×10^{-3} mol/l.

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Table 1. Binding Constants (mol/l) for Zinc Porphyrins[a)]

	1	2	3
aet[b)]	9.9	10	10
Gly[b)]	110	150	66
L-Val	110	100	55
L-Leu	140	130	83
L-Phe	320	180	110
L-Trp	1300	770	350
L-Asp	780	770	180
L-Glu	390	540	150
L-Ser	100	110	51
Gly-Gly	96	91	65
Gly-L-Phe	200	340	240
Gly-L-Trp	770	1100	780

a) 25 °C, pH 10.4 (I=0.02). *b*) Ref. 17.

lution, strong solvation-desolvation phenomena such as hydrophobic interactions probably occur.

The values of the binding constants of aminoethanol (aet), which is less likely to strongly interact with the porphyrin substituents, are low, and this indicates that the coordination ability of the N atom itself is weak. The binding of amino acids is apparently stronger than that of aet, and this is reasonably explained by the additively cooperated Coulomb interaction between the cation substituent(s) of porphyrins and the carboxylate anion of amino acids. This explanation is also well supported by the fact that the K values increase as the number of possible Coulomb interactions increases; the K values of Gly for **1** and **2** are greater than those for **3**, and the binding of L-Asp and L-Glu is enhanced compared to that of Gly. In this study, we further analyzed the Coulomb interactions by examining the effects of the ionic strength (I) of the solution. According to the Debye–Hückel limiting law, the relationship between K and I is $\log K = \log K_0 - 1.018|z_a z_b|\sqrt{I}$ (in H_2O, 25 °C), where K_0 is the binding constant at I=0, and z_a and z_b are the effective charges of the ions required to induce a Coulomb interaction.[9,10)] Figure 3 shows the plots of $\log K$ *vs.* $\sqrt{I}$, and Table 2 lists the slope values. If z_a is given as the effective positive charge of the porphyrin, its maximum value will be 2 for **1** and **2** and 1 for **3**; if z_b is given as the effective negative charge, its maximum value will be 0, 1, or 2 for the amines. In accordance with this relationship, the slope values increase as the number of interacting ions increases; the magnitude of possible Coulomb interactions is well correlated.

With regard to the coordination of hydrophobic amino acids, *e.g.*, Phe and Trp, with a water-soluble zinc porphyrin, it has been reported that hydrophobic interactions between the phenyl or indole group of the amino acids and the porphyrin plane strengthen the binding.[11,12)] As shown in Table 1, a similar result was obtained in the present work, *i.e.*, the K values of L-Phe and L-Trp were found to be greater than those of Gly. We have previously reported[4)] that, in a similar zinc porphyrin, some steric interaction between the side chains of the bound amino acids and the porphyrin substituents may exist. The subtle differences in K among Gly, L-Val, and L-Leu with **1**—**3** would be addressed to the sum of the hydrophobic attraction with the porphyrin plane and the steric repulsion with the porphyrin substituents.

The K values of Gly-Gly are slightly less than those of Gly, suggesting that the Coulomb interactions between

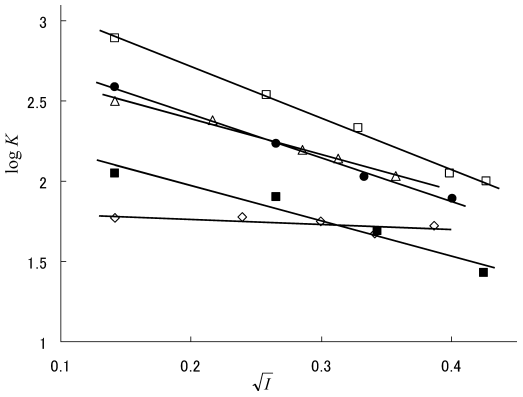

Fig. 3. Correlation of $\log K$ with $\sqrt{I}$

□, **1**+L-Asp; ●, **1**+L-Glu; △, **1**+L-Phe; ■, **1**+Gly; ◇, **1**+1-methylimidazole (1-MeIm).

Table 2. Slope Values for Plotting $\log K$ *vs.* $\sqrt{I}$[a)]

	1	2	3
1-MeIm[b)]	−0.32 (2 : 0)	−0.10 (2 : 0)	−0.06[c)] (1 : 0)
Gly	−2.18 (2 : 1)	−2.25 (2 : 1)	−1.08 (1 : 1)
L-Phe	−2.49 (2 : 1)	−1.95 (2 : 1)	−1.03 (1 : 1)
L-Asp	−3.20 (2 : 2)	−4.42 (2 : 2)	−2.11 (1 : 2)
L-Glu	−2.74 (2 : 2)	−3.02 (2 : 2)	−2.55 (1 : 2)

a) The cation/anion ratios are given within parentheses. *b*) 1-Methylimidazole. *c*) Ref. 17.

dipeptides and porphyrins are comparable to those between amino acids and porphyrins. The K values of Gly-L-Phe and Gly-L-Trp are greater than those of Gly-Gly, indicating that the interactions between these dipeptides and the porphyrins are similar to those between L-Phe and L-Trp and porphyrins. Interestingly, the K values of the interactions of Gly-L-Phe and Gly-L-Trp with **1** are two-thirds less than those of the L-Phe and L-Trp interactions, whereas the K values of the same interactions with **2** and **3** are higher by 1.4—2.2 than those of the L-Phe and L-Trp interactions. The observation for **1** may be partly due to the weakened hydrophobic interaction in the dipeptides, since, in ^{1}H-NMR (0.03 mol/l Na_2CO_3 in D_2O), the upfield shifts $\Delta\delta$ of the indole C_2–H upon coordination of L-Trp and Gly-L-Trp to **1** are −2.16 and −1.32 ppm, respectively. However, since the magnitude of each of the various interactions cannot be individually estimated, it is currently difficult to reasonably explain the differences in the K values between the interactions of the dipeptides and amino acids.

Table 3 presents the thermodynamic data corresponding to the binding of the amino acids. Generally, in nonpolar organic solvents such as toluene both the ΔH and ΔS values for the binding of amines to zinc porphyrins are apparently negative, since the binding reaction is the formation of five coordination from four coordination. In contrast, the binding of amines in aqueous solution leads to the release of the bound H_2O, due to which the ΔH and ΔS values are substantially positive; thus, the eventual values are only slightly negative or positive. In fact, the ΔH and ΔS values listed in Table 3 are substantially greater than those reported for the binding of amines to tetrakis(*o*-substituted-phenyl)porphyrinatozinc complexes in toluene[13)] (−43—−54 kJ mol^{-1} for ΔH, and

Table 3. Thermodynamic Data Corresponding to the Binding of Amino Acids

	System	ΔH (kJ mol^{-1})	ΔS (J mol^{-1} K^{-1})	ΔG^{o} (kJ mol^{-1})
1	+Gly	−5.9±1.2	19.4±4.1	−11.7
	+L-Val	−3.9±2.7	25.7±9.0	−11.6
	+L-Leu	−1.8±0.9	34.9±2.9	−12.2
	+L-Phe	−8.0±0.6	21.1±2.0	−14.3
	+L-Trp	−15.1±1.0	8.8±3.4	−17.7
	+L-Asp	−12.9±1.1	12.3±3.8	−16.5
	+L-Glu	−15.0±1.2	−0.7±3.9	−14.8
	+L-Ser	−23.4±3.3	−39.8±11.1	−11.5
2	+Gly	−13.3±1.0	−2.9±3.2	−12.5
	+L-Val	−14.9±1.1	−11.2±3.7	−11.5
	+L-Leu	−14.5±1.3	−8.1±4.4	−12.1
	+L-Phe	−8.9±1.1	13.3±3.9	−12.9
	+L-Trp	−5.6±1.2	36.5±4.0	−16.5
	+L-Asp	−13.4±1.8	10.4±6.2	−16.5
	+L-Glu	−14.6±0.7	3.4±2.4	−15.6
	+L-Ser	−27.9±2.0	−54.0±6.7	−11.8
3	+Gly	2.4±1.6	42.9±5.2	−9.9
	+L-Val	−2.0±3.9	26.5±13.0	−11.6
	+L-Leu	2.5±2.6	45.0±8.8	−11.0
	+L-Phe	2.3±0.5	46.7±1.8	−11.7
	+L-Trp	−3.1±1.2	38.5±3.9	−14.5
	+L-Asp	−10.4±2.6	8.2±8.6	−12.8
	+L-Glu	−7.3±1.2	16.9±4.0	−12.4
	+L-Ser	−11.7±2.6	−6.5±8.9	−9.7

−55—−72 J mol^{-1} K^{-1} for ΔS). Among the porphyrins examined, **3** exhibited greater ΔH and ΔS values than **1** and **2** did. This may be due to a decrease in the number of Coulomb interactions, which lead to negative ΔH and ΔS values.

With regard to isomerism, both the K values in Table 1 and the slope values in Table 2 are comparable between **1** and **2**. This suggests that the relative configuration of the porphyrin substituents in the two atropisomers might provide similar Coulomb interactions with the amines. The thermodynamic data are somewhat different between **1** and **2**, but there is no apparent tendency. This may be caused by additional interactions such as steric repulsions between the porphyrin substituents and the bound amines, but we cannot give reasonable explanation at present for the difference.

Figure 4 shows the compensation plot of the thermodynamic data corresponding to the equation $T\Delta S=\alpha\Delta H+T\Delta S_0$.[14,15] The slope α reflects the degree of conformational change that occurs on host-guest binding, while the intercept $T\Delta S_0$ can be regarded as a measure of desolvation during the binding. For the association of *p*-quinones with a porphyrin through hydrogen bonds, the slope was reported to be 0.62[16]; where the preorganized porphyrin host and the rigid *p*-quinone guests do not undergo conformational changes. In this study, a high α value close to unity was obtained, suggesting that during the binding of amino acids to porphyrin, appreciable conformational changes occur, where both the flexible amino acids and the porphyrin substituents must reorganize to interact. When crown ethers and cyclodextrins are the hosts in aqueous solution,[14] the $T\Delta S_0$ values usually lie between 8 and 23 kJ mol^{-1} where desolvation of guests accompanies guest binding. The obtained $T\Delta S_0$ value of 12.3 kJ mol^{-1} falls within this range but is slightly low. This

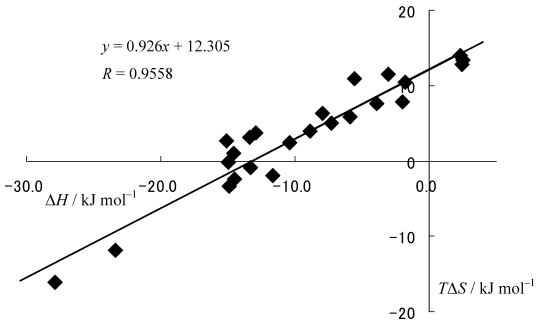

Fig. 4. Plot of $T\Delta S$ against ΔH for the Binding of Amines Based on the Data in Table 3

may be due to the *exo*-receptor-type structure of porphyrins, since the $T\Delta S_0$ values for *endo*-receptor-type hosts are generally high because of the strong desolvation of the guests.

Experimental

Zinc porphyrins were obtained as given in the literature.[3,17] Proton NMR spectra in D_2O were obtained from a JEOL JNM-GSX-400 (400 MHz) spectrometer with sodium 3-trimethylsilyl-propanesulfonate as the internal standard. Visible absorption spectra were recorded on a Hitachi U-3000 spectrophotometer. The binding of amines to porphyrins was examined by spectrophotometric titration of the amines in aqueous solution buffered with $NaHCO_3$–Na_2CO_3 (pH 10.4, I=0.02). The ionic strength I was controlled with NaCl. The binding constants were determined by a published method.[3,18] The thermodynamic data were estimated from the temperature dependence of the binding constants in the temperature range between 11 and 38 °C.

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