Inorg. Chem. **2004**, *43*, 1211–1213

Chiral Recognition of Amino Acids and Dipeptides by a Water-Soluble Zinc Porphyrin

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Received September 22, 2003

A chiral water-soluble zinc porphyrin was optically resolved on a chiral HPLC column, and the binding of chiral amino acids and peptides to each of the enantiomers was examined spectrophotometrically in basic aqueous solution. The binding data apparently indicated that the zinc porphyrin has chiral selectivity for amino acids and dipeptides. This was reasonably explained in terms of the triple cooperation of coordination, Coulomb, and steric interactions of the chiral amino carboxylates with the porphyrin. A compensatory relationship among the thermodynamic parameters for chiral recognition was also shown.

Chiral recognition of amino acids and peptides is a fundamental process to regulate various functions in living systems. One strategy for studying selectivity for amino acids involves the use of zinc porphyrins as artificial receptors.[1] As a result of the lipophilic nature of porphyrins, however, only limited water-soluble zinc porphyrins have been synthesized,[2] and no chiral recognition by porphyrins in aqueous solution has yet been reported. Selectivity of molecules is realized by receptors with the accumulation of noncovalent interactions. Examinations of molecular recognition in aqueous solution provide useful information to elucidate the functions of biomolecules, since these interactions strongly depend on the solvent used due to solvation−desolvation phenomena of the solutes. In an earlier work,[3] we prepared a racemic mixture of water-soluble zinc porphyrin **ZnP** with C_2 symmetry (Figure 1). The porphyrin is capable of interacting multiply with chiral amino carboxylates and can behave as an anion receptor. Such anion receptors have also received much attention in relation to biological systems.[4] In this Communication, **ZnP** was optically resolved, and the binding data with amino carboxylates indicated that this porphyrin shows chiral recognition.

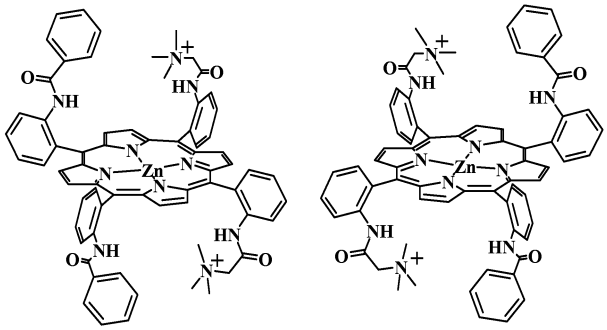

Figure 1. Water-soluble zinc porphyrin **ZnP**. Chloride ions and coordinated H_2O are omitted for clarity.

Chiral zinc porphyrin **ZnP** was resolved into the enantiomers on a chiral HPLC column as shown in Figure 2. Their CD spectra are also included in this figure. Since the absolute configurations of the enantiomers have not yet been determined, these were tentatively classified as **ZnP1** and **ZnP2** according to the elution order on the HPLC. Binding of amino acids and peptides to the enantiomers was examined spectrophotometrically (Figure 3) for coordination of $-NH_2$ to the central zinc under the basic conditions[5] under which

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(1) Chiral recognition by zinc porphyrins, see: (a) Konishi, K.; Yahara, K.; Toshishige, H.; Aida, T.; Inoue, S. *J. Am. Chem. Soc*. **1994**, *116*, 1337. (b) Mizutani, T.; Ema, T.; Tomita, T.; Kuroda, Y.; Ogoshi, H. *J. Am. Chem. Soc*. **1994**, *116*, 4240. (c) Kuroda, Y.; Kato, Y.; Higashioji, T.; Hasegawa, J.; Kawanami, S.; Takahashi, M.; Shiraishi, N.; Tanabe, K.; Ogoshi, H. *J. Am. Chem. Soc*. **1995**, *117*, 10950. (d) Ogoshi, H.; Mizutani, T.; Hayashi, T.; Kuroda, Y. In *The Porphyrin Handbook*; Kadish, K. M., Smith, K. M., Guilard, R., Eds.; Academic Press: San Diego, CA, 2000; Vol. 6, pp 279−340. (e) Ogoshi, H.; Mizutani, T. *Acc. Chem. Res*. **1998**, *31*, 81. (f) Borovkov, V. V.; Lintuluoto, J. M.; Sugeta, H.; Fujiki, M.; Arakawa, R. Inoue, Y. *J. Am. Chem. Soc*. **2002**, *124*, 2993 and references therein. (g) Huang, X.; Fujioka, N.; Pescitelli, G.; Koehn, F. E.; Williamson, R. T.; Nakanishi, K.; Berova, N. *J. Am. Chem. Soc*. **2002**, *124*, 10320 and references therein.

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(4) (a) Beer, P. D.; Gale, P. A. *Angew. Chem., Int. Ed*. **2001**, *40*, 486. (b) Gale, P. A. *Coord. Chem. Rev*. **2001**, *213*, 79.

(5) At pH 10.4 ($NaHCO_3/Na_2CO_3$ buffer, $I = 0.02$). Since the pK_a value of the coordinated H_2O of **ZnP** was estimated to be higher than 12, binding of the amino carboxylates corresponds to a ligand-exchange reaction with the H_2O. For the method used to evaluate K values, see ref 3.

10.1021/ic0302837 CCC: $27.50 © 2004 American Chemical Society
Published on Web 02/03/2004

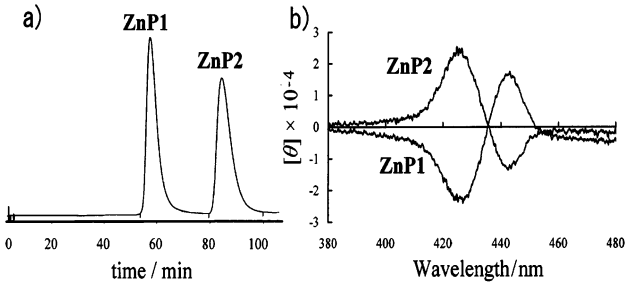

Figure 2. (a) Chiral separation of the **ZnP** enantiomers on HPLC. Conditions: column, Daicel Chiralpak AD (Ø1.0 × 25 cm); eluent, hexane/ethanol/diethylamine/trifluoroacetic acid (85/15/0.1/0.1, v/v); detection, at 420 nm. (b) Circular dichroism spectra of the enantiomers in aqueous solution (pH 10.4, $I = 0.02$).

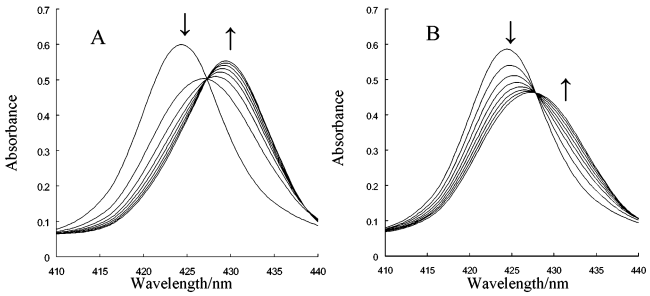

Figure 3. Visible spectral changes of **ZnP** upon titration of Gly-L-Trp anion. At 25 °C, pH 10.4 ($I = 0.02$). (A) **ZnP1**; [Gly-L-Trp] = 0, 8.75×10^{-4}, 1.74×10^{-3}, 2.59×10^{-3}, 3.43×10^{-3}, 4.46×10^{-3}, 5.47×10^{-3}, and 6.47×10^{-3} M. (B) **ZnP2**; [Gly-L-Trp] = 0, 8.52×10^{-4}, 1.69×10^{-3}, 2.52×10^{-3}, 3.34×10^{-3}, 4.35×10^{-3}, 5.34×10^{-3}, and 6.31×10^{-3} M.

Table 1. Binding Constants K (M^{-1}) of **ZnP** with Amines at 25 °C in Aqueous Solution

amine	K(**ZnP1**)[a]	K(**ZnP2**)[a]	amine	K(**ZnP1**)[a]	K(**ZnP2**)[a]
butylamine	28[b]		Gly-Gly	88	
Gly	105	108	Gly-Gly-Gly	24	
L-Ala	89	69	Gly-L-Ala	148	125
L-Val	118	85	Gly-L-Val	142	93
L-Leu	206	67	Gly-L-Leu	189	86
L-Ser	61	41	Gly-L-Ser	124	70
L-Phe	510	261	Gly-L-Phe	261	164
L-Trp	1530	692	Gly-L-Trp	875	266
				61[c]	29[c]
D-Trp	683	1540	Gly-Gly-L-Trp	287	240
L-Asp	280	214	Gly-L-Pro	719	324
L-Glu	264	213	Gly-L-Glu	397	282

[a] Measured at pH 10.4 ($NaHCO_3/Na_2CO_3$, $I = 0.02$), unless otherwise noted. Estimated errors for K values were smaller than 12%. [b] Reference 3; measured for the racemic mixture, pH 11.5, $I = 0.02$. [c] In $EtOH/H_2O$ (pH 10.4, $I = 0.02$) = 1/1 (w/w).

these amino acids and peptides lose H^+ to exist as amino carboxylates.

The binding constants (K = [**ZnP**·amine]/[**ZnP**]·[amine]) are listed in Table 1. On the binding of these amino carboxylates to **ZnP**, various interactions such as Coulomb, cation-π, coordination, and steric interactions, together with solvation−desolvation processes, are possible and each of these may greatly affect the K values.[6] For L-type amino acids and dipeptides, **ZnP** apparently shows chiral selectivity with a ratio of K(**ZnP1**)/K(**ZnP2**) ranging from 1.2 to 3.3. To reveal chiral selectivity for amino carboxylates, two-point fixation of the carboxylates by attractive interactions and a third interacting site are required.[1a−c] For the two-point fixation of the amino carboxylates by **ZnP**, coordination and Coulomb interactions are utilized as follows. Amine coordination to zinc porphyrins in aqueous solution is usually not strong[2] but is sufficient for the fixation. We previously reported that Coulomb interaction between the carboxylate anion and the ammonium cation enhances binding of amino carboxylates to the *meso* isomer of **ZnP** on the basis of the K-dependence on ionic strength of solution.[3] Comparisons of the K values among butylamine, Gly, Gly-Gly, and Gly-Gly-Gly suggest that good complementarity for two-point fixation with the $-NH_2$ coordination and the Coulomb interaction has been achieved for Gly and Gly-Gly with **ZnP**. In contrast, the two interacting sites of Gly-Gly-Gly do not act additively, probably due to the elongation or increased flexibility of the two sites. That Coulomb interaction with **ZnP** functions for the fixation is supported by the fact that the chiral selectivity for tripeptide Gly-Gly-L-Trp compared to that for Gly-L-Trp or L-Trp has almost disappeared.

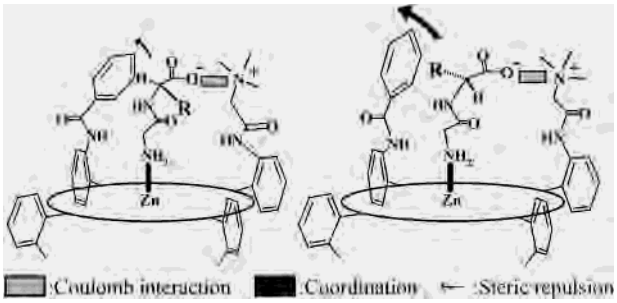

Figure 4. Plausible interactions between an enantiomer of **ZnP** and L-type (left) and D-type (right) amino carboxylates. Hydrophobic interactions between the porphyrin plane and the R group are omitted for clarity.

The third interaction yielding chiral selectivity might be steric repulsion or hydrophobic attraction between the substituent on the amino carboxylates and the phenyl group above the porphyrin plane. The steric interaction[7] decreases the K values whereas the hydrophobic interaction enhances the observed K values. The absolute configurations of **ZnP1** and **ZnP2** are correlated with those two interactions that will dominate the chiral selectivity: If a diastereomer with a closely spaced conformation between the substituent on the amino carboxylates and the phenyl group of the porphyrin (Figure 4, right side) gives a decreased K value compared to that of the other diastereomer (Figure 4, left side), the interaction must be steric repulsion, whereas if the diastereomer with a closely spaced conformation gives an increased K value, the interaction will be hydrophobic attraction. Although NMR data in aqueous solution were not available due to the limited solubility, we concluded that the steric interaction is more plausible in which the structure

(6) (a) For example, a large hydrophobic substituent on amino carboxylates such as Trp, Phe, and Gly-Trp enhances the binding in terms of hydrophobic interaction with the porphyrin plane; see refs 2c and 6b. (b) Verchére-Bèaur, C.; Mikros, E.; Perrèe-Fauvet, M.; Gaudemer, A. *J. Inorg. Biochem.* **1990**, *40*, 127.

(7) The steric interaction could be caused by putting up a phenyl group that could interact with the porphyrin plane in a stacked conformation before the amine binding. This may be correlated to an observation that the CD spectral intensities of both **ZnP** enantiomers become weak upon binding of L-Leu, L-Trp, D-Trp, and Gly-L-Trp.

Table 2. Thermodynamic Parameters for Binding of Amino Carboxylates to **ZnP** in Aqueous Solution

system	K/M^{-1}	$\Delta G°$/ kJ mol^{-1}	$\Delta H°$/ kJ mol^{-1}	$\Delta S°$/ J mol^{-1} K^{-1}
ZnP1 + Gly	105	−11.5	−10.1 ± 2.4	4.9 ± 7.9
ZnP1 + Gly-Gly	88	−11.1	−9.0 ± 2.3	7.4 ± 7.9
ZnP1 + L-Trp	1530	−18.1	−12.0 ± 0.9	20.7 ± 3.1
ZnP2 + L-Trp	683	−16.2	−6.6 ± 0.7	32.3 ± 2.4
ZnP1 + L-Leu	206	−13.2	9.7 ± 1.3	76.6 ± 4.5
ZnP2 + L-Leu	67	−10.4	11.3 ± 1.8	72.8 ± 6.0
ZnP1 + L-Asp	280	−14.0	−9.4 ± 1.5	15.5 ± 4.9
ZnP2 + L-Asp	214	−13.3	−15.2 ± 0.9	−6.3 ± 2.9
ZnP1 + L-Glu	264	−13.8	−14.9 ± 1.4	−3.6 ± 4.7
ZnP2 + L-Glu	213	−13.3	−23.0 ± 0.6	−32.5 ± 2.1
ZnP1 + Gly-L-Leu	189	−13.0	8.7 ± 1.9	72.6 ± 6.3
ZnP2 + Gly-L-Leu	86	−11.0	9.1 ± 2.9	67.5 ± 9.7
ZnP1 + Gly-L-Phe	261	−13.8	−12.0 ± 0.8	5.9 ± 2.7
ZnP2 + Gly-L-Phe	164	−12.6	−9.4 ± 1.4	10.7 ± 4.8
ZnP1 + Gly-L-Trp	875	−16.8	−12.8 ± 1.9	13.5 ± 6.5
ZnP2 + Gly-L-Trp	266	−13.8	−2.5 ± 2.1	37.9 ± 6.8

of the enantiomer in Figure 4 corresponds to **ZnP1** for the following two reasons. First, even for less hydrophobic amino carboxylates—such as L-Ser, Gly-L-Ser, and L-Asp—that will not give enhanced K by the hydrophobic interaction, a slight but certain chiral selectivity is observed. Second, in a mixed solvent of EtOH/H_2O (1/1) in which the hydrophobic interaction becomes less firm and the K values of hydrophobic amino carboxylates should be drastically decreased,[8] the chiral selectivity for Gly-L-Trp is appreciably retained. Consequently, as shown in Figure 4, chiral recognition of the amino carboxylates by **ZnP** seems to occur through the triple cooperation of coordination, Coulomb, and steric interactions. It is worth noting that a recognition ability for dipeptides comparable to that for amino acids has been achieved in spite of the fact that the coordination site of the dipeptides is apart from the chiral center through flexible chemical bonds. In previous works on chiral recognition for amino acid analogues,[1a−c] the solvents used were nonpolar and thereby hydrogen bonds were effectively utilized as an attractive interaction. To mimic in vivo chiral recognition of amino acids, however, hydrogen bonds seem inappropriate, since water molecules act as competitors. The present work demonstrates that chiral recognition in aqueous solution can be realized based on weak interactions other than hydrogen bonds, with chiral selectivity comparable to that observed for the other models in organic solvents.

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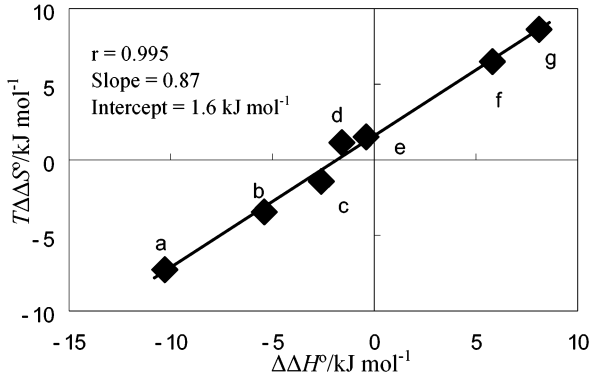

Figure 5. Plot of $T\Delta\Delta S°$ against $\Delta\Delta H°$ for chiral recognition by **ZnP**. $\Delta\Delta H° = \Delta H°$(**ZnP1**) − $\Delta H°$(**ZnP2**); $\Delta\Delta S° = \Delta S°$(**ZnP1**) − $\Delta S°$(**ZnP2**). (a) Gly-L-Trp, (b) L-Trp, (c) Gly-L-Phe, (d) L-Leu, (e) Gly-L-Leu, (f) L-Asp, (g) L-Glu.

Table 2 lists the thermodynamic data[9] for the binding of amino carboxylates to **ZnP**. Figure 5 shows the compensation plot of the thermodynamic data for chiral recognition, and an excellent linear relationship is observed.[10] The intercept can be regarded as a measure of desolvation upon host−guest binding, and usually lies between 8 and 23 kJ mol^{-1} in aqueous solution for crown ethers and cyclodextrins as hosts that accompany desolvation on guest binding.[11] The obtained intercept of 1.6 kJ mol^{-1} is substantially small. Further, the chiral selectivity for the hydrophobic amino carboxylates Gly-L-Trp and L-Trp is apparently not entropy-driven but enthalpy-driven. If hydrophobic interaction functions for chiral recognition, the observed desolvation energy would become larger and the chiral selectivity for hydrophobic amino carboxylates should be entropy-driven. Therefore, the results obtained here support the above suggestion that hydrophobic interaction will not be a dominant factor in chiral recognition by **ZnP**.

Acknowledgment. This work is partially supported by a Grant-in-Aid for Scientific Research (No. 15550154, H.I.) from MEXT, Japan.

IC0302837

(9) The thermodynamic parameters were estimated from the temperature dependence of K between 15 and 40 °C.

(10) A similar result has recently been reported for chiral recognition by β-cyclodextrin; see: Rekharsky, M.; Inoue, Y. *J. Am. Chem. Soc.* **2000**, *122*, 4418.

(11) (a) Inoue, Y.; Wada, T. In *Advances in Supramolecular Chemistry*; Gokel, G. W., Ed.; JAI Press: Greenwich, CT, 1997; Vol. 4, pp 55−96. (b) Rekharsky, M.; Inoue, Y. *Chem. Rev.* **1998**, *98*, 1875.