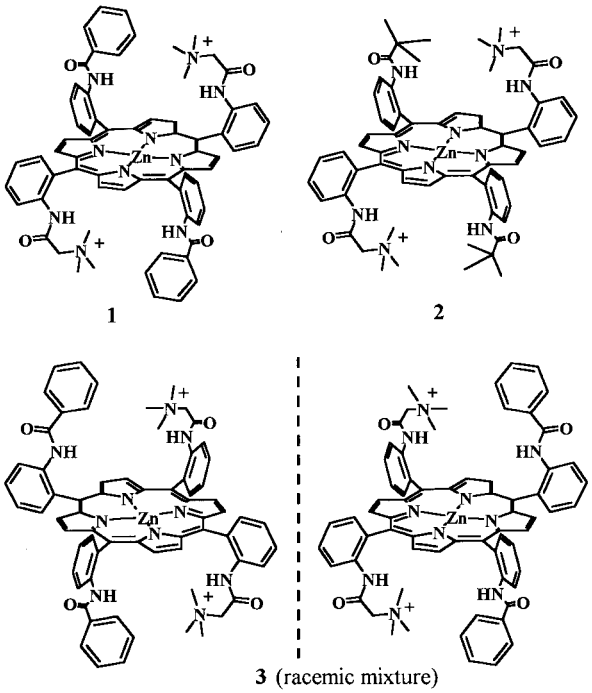
Water-Soluble Zinc Porphyrins as Receptors for Amino Carboxylates

Hiroyasu Imai,* Kensuke Misawa, Hiroki Munakata, and Yoshio Uemori
Faculty of Pharmaceutical Sciences, Hokuriku University, 3 Ho Kanagawa-Machi, Kanazawa 920-1181

(Received April 16, 2001; CL-010339)

Water-soluble zinc porphyrins bearing an ammonium group and a phenyl or tertiary butyl group above each porphyrin plane were designed and synthesized. Binding data for amino carboxylates in aqueous solution suggested that these porphyrins recognize the carboxylates on the basis of coordinative, Coulomb, and hydrophobic interactions and that a chiral recognition phenomenon for glycyl-tryptophan anion is derived from the cooperation of these interactions.

Recognition of biomolecules such as amino acids by receptors plays critical roles in living organisms. To understand the recognition mechanisms in aqueous solution, a variety of artificial receptors have been designed and studied. A class of useful model receptors for amino acids and peptides is zinc porphyrins,[1,2] but not so many studies in aqueous solution[3–5] have been reported due to the lipophilic nature of porphyrins. In this work we have designed and synthesized a new type of water-soluble zinc porphyrin receptors that are capable of revealing three-point recognition for amino carboxylates on the basis of coordinative, Coulomb, and hydrophobic interactions.



The synthesis of 5α,15β-bis(2-benzoylaminophenyl)-10α,20β-bis(2-(trimethylammoniomethylcarbonylamino)-phenyl)porphyrinatozinc(II) (**1**) (chloride) is as follows. To an ice-cold solution of 5α,10α,15β,20β-tetrakis(2-aminophenyl)-porphyrin (2.00 g, 2.96 mmol) and triethylamine (5.0 cm^3) in CH_2Cl_2 (800 cm^3) was added dropwise a solution of triphenylmethyl bromide (2.08 g, 6.44 mmol) in CH_2Cl_2 (400 cm^3). The reaction mixture containing some kinds of partly amino-protected porphyrins was evaporated to dryness and the solid was chromatographed on a silica-gel column (benzene, 4 × 30 cm). The third and forth bands were separated where these were 5α,15β-bis(2-(triphenylmethylamino)phenyl)-10α,20β-bis(2-aminophenyl)porphyrin and the racemic mixture of 5α,20β- and 10α,15β-bis(amino-protected) isomers, respectively. After drying the solution of the third band, the solid was dissolved in CH_2Cl_2 (80 cm^3) containing triethylamine (1.1 cm^3). To the solution, benzoyl chloride (0.35 cm^3, 3.00 mmol) was added then stirred for 12 h at room temperature. The reaction mixture was treated with a mixture of dilute HCl (3 mol dm^{-3}, 10 cm^3) and CH_3COOH (10 cm^3) for 1 h at room temperature, deprotecting the two amino groups. After neutralization with aqueous ammonia, the organic layer was dried over anhydrous Na_2SO_4 then evaporated to dryness. The solid of 5α,15β-bis(2-benzoylaminophenyl)-10α,20β-bis(2-aminophenyl)porphyrin was purified by silica-gel column chromatography ($CHCl_3$, 2.5 × 30 cm), yield 0.249 g (9.6%).

Treatment of the porphyrin (0.500 g, 0.566 mmol) with $ZnCl_2$ (1.00 g, 7.34 mmol) in tetrahydrofuran (100 cm^3) containing 2,6-lutidine (0.50 cm^3) for 4 h at room temperature afforded the corresponding zinc porphyrin, yield 0.291 g (54.3%).

To a solution of the above zinc porphyrin (0.250 g, 0.276 mmol) and triethylamine (10 cm^3, 72 mmol) in CH_2Cl_2 (100 cm^3) in an ice bath was added dropwise a solution of *N*,*N*-dimethylaminoacetyl chloride (0.871 g, 7.16 mmol) in CH_2Cl_2 (100 cm^3). After stirring for 6 h at room temperature, the mixture was washed twice with water. The organic layer was dried over anhydrous Na_2SO_4 then evaporated to dryness. The solid of 5α,15β-bis(2-benzoylaminophenyl)-10α,20β-bis(2-(*N*,*N*-dimethylaminomethylcarbonylamino)phenyl)porphyrinatozinc(II) was purified by a silica-gel column ($CHCl_3$, 2.5 × 20 cm), yield 0.0894 g (30.3%).

To a solution of the above zinc porphyrin (0.0800 g, 0.0716 mmol) in *N*,*N*-dimethylformamide (10 cm^3) was added methyl iodide (0.800 cm^3, 10.3 mmol) with stirring at room temperature. After 7 h, addition of ether to the mixture precipitated a solid of the iodide of **1**. The iodide was converted to the corresponding chloride by ion-exchange column chromatography (CH_3OH, 3 × 30 cm (Amberlyst A-21)), yield 0.0323 g (37.0%).[6]

5α,15β-Bis(2-(*t*-butylcarbonylamino)phenyl)-10α,20β-bis-(2-(trimethylammoniomethylcarbonylamino)phenyl)porphyrinatozinc(II) (**2**) (chloride) was similarly prepared by using pivaloyl chloride instead of benzoyl chloride. 5α,20β-Bis(2-benzoylaminophenyl)-10α,15β-bis(2-(trimethylammoniomethylcarbonylamino)phenyl)porphyrinatozinc(II) (**3**) (chloride) was similarly prepared as the racemic mixture from the mixture of 5α,20β-bis(2-(triphenylmethylamino)phenyl)-10α,15β-bis(2-aminophenyl)porphyrin and its enantiomer.

In aqueous solution a nitrogenous axial ligand (L) such as amino carboxylates can substitute the coordinated water on a zinc porphyrin (ZnP) and this reaction is explained as follows:

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

where the N atom of L coordinates to the zinc ion.[7] Since the pK_a values of the coordinated H_2O of these zinc porphyrins were estimated to be larger than 12 and the pK_a values of amino acids ($-NH_3^+$) are smaller than 9.6, the binding experiments were carried out at a pH value between them. Binding constants (K = [ZnP·L]/[ZnP·H_2O][L]) for the equilibrium were determined spectrophotometrically by a usual method.[8]

Table 1. Binding constants of amines to zinc porphyrins[a]

	2	**1**	**3**
Butylamine[b]	6	27	28
Gly	57	87	89
DL-Phe	270	360	350
DL-Trp	830	1000	1100
L-Trp	810	1000	1100
DL-Asp[c]	300	310	340
Gly-DL-Trp[d]	230	500	560
Gly-L-Trp[d]	240	480	460

[a] K/dm^3 mol^{-1}; at 25 °C. In $NaHCO_3$–Na_2CO_3 buffer (pH 10.4, I = 0.02) except for butylamine. Errors in K were smaller than 10% unless otherwise noted. [b] In 0.01 mol dm^{-3} K_2CO_3 (pH 11.5, I = 0.03). [c] Errors in K were smaller than 23%. [d] Errors in K were smaller than 6%.

Table 1 lists binding data of amino carboxylates and butylamine to zinc porphyrins. It is appeared that amino carboxylates bind more tightly to the zinc porphyrins than butylamine and that the K values for Asp are greater than those for Gly, suggesting that Coulomb interaction between an $-N^+(CH_3)_3$ group of the porphyrins and the $-COO^-$ group(s) of the coordinated carboxylates enhances the binding. This was supported from examining the dependence of K on ionic strength I where slope for plotting log K versus $\sqrt{I}$ can correlate to the magnitude of Coulomb interaction.[4,9] The slope on the binding of butylamine to **2** was found to be 0.09 while those of L-Trp and DL-Asp were –1.26 and –1.63, respectively, indicating that Coulomb interaction strengthens the binding.

On the binding of amino carboxylates to 5,10,15,20-tetrakis(*N*-methyl-4-pyridyl)porphyrinatozinc(II) in aqueous solution, the K values for Phe and Trp anions were increased in terms of hydrophobic interactions between the hydrophobic side chains of the carboxylates and the porphyrin plane.[3] This effect cannot be expected for the amino carboxylates with no hydrophobic side chain such as Gly and Asp. For the zinc porphyrins prepared, similar increments in K are also observed for the binding of Phe and Trp compared to Gly, suggesting the presence of hydrophobic interaction with the porphyrin plane. Another hydrophobic interaction is also possible between the side chain of Phe or Trp and the *t*-butyl or phenyl group of the zinc porphyrins prepared. This can be correlated to the fact that the K values for **1** and **3** with the amines except Asp are apparently larger than those for **2**. This result might be reasonably ascribed to the increased hydrophobic area of the phenyl group compared to the *t*-butyl group.

Thus, compounds **1** and **3** can recognize amino carboxylates on the basis of coordination, Coulomb interaction, and two-point hydrophobic interactions. Since **3** exists as the racemic mixture, chiral recognition for the enantiomers of the amino carboxylates was expected. Our binding data on amino acids, however, do not show chiral recognition evidence; no difference in K is observed between DL- and L-Trp. Contrary to this, a slight but certain difference in K for **3** was obtained between Gly–DL-Trp and Gly–L-Trp.[10] This can be reasonably explained in terms of the relative magnitude of the two hydrophobic interactions produced by the indole group of the amino carboxylates with the porphyrin plane and with the phenyl group of the porphyrin. In the bound Trp the former interaction must be strong to eclipse the latter interaction, whereas in the bound Gly–Trp the relatively decreased former interaction by elongating the separation of the indole group from the porphyrin plane might lead to the appearance of the chiral recognition.

References and Notes

1 T. Mizutani, T. Ema, T. Tomita, Y. Kuroda, and H. Ogoshi, *J. Am. Chem. Soc.*, **116**, 4240 (1994); Y. Kuroda, Y. Kato, T. Higashioji, J. Hasegawa, S. Kawanami, M. Takahashi, N. Shiraishi, K. Tanabe, and H. Ogoshi, *J. Am. Chem. Soc.*, **117**, 10950 (1995).

2 K. Konishi, K. Yahara, H. Toshishige, T. Aida, and S. Inoue, *J. Am. Chem. Soc.*, **116**, 1337 (1994).

3 E. Mikros, A. Gaudemer, and R. Pasternack, *Inorg. Chim. Acta*, **153**, 199 (1988); C. Verchére-Bèaur, E. Mikros, M. Perrèe-Fauvet, and A. Gaudemer, *J. Inorg. Biochem.*, **40**, 127 (1990).

4 T. Mizutani, K. Wada, and S. Kitagawa, *J. Am. Chem. Soc.*, **121**, 11425 (1999).

5 T. Mizutani, K. Wada, and S. Kitagawa, *J. Org. Chem.*, **65**, 6097 (2000).

6 Selected data: Compound **1** (chloride): ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.25 (s, 18H, $N(CH_3)_3$), 6.61 (d, 4H, CO–ph), 6.67 (t, 4H, CO–ph), 6.95 (t, 2H, CO–ph), 7.66 (m, 4H, ph), 7.88 (m, 6H, ph), 7.99 (d, 2H, ph), 8.02 (d, 2H, ph), 8.33 (d, 2H, ph), 8.60 (d, 2H, pyrrole), 8.63 (d, 2H, pyrrole), 8.72 (s, 2H, pyrrole), 8.73 (s, 2H, pyrrole), 8.98 (s, 4H, NHCO), 9.17 (s, 4H, NHCO); vis (H_2O/$NaHCO_3$–Na_2CO_3, pH 10.4) λ_{max} (log ε) 424 (5.44), 557 (4.18), 596 (3.61) nm; Anal. Calcd for $C_{68}H_{60}N_{10}O_4Zn \cdot Cl_2 \cdot 6H_2O$: C, 61.61; H, 5.47; N, 10.57%. Found: C, 61.36; H, 5.38; N, 10.03%; MS *m/z* 1181 (M^+), M^+ calcd for $C_{68}H_{60}N_{10}O_4ZnCl$, 1180. Compound **2** (chloride): ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.07 (s, 18H, $C(CH_3)_3$), 2.30 (s, 18H, $N(CH_3)_3$), 7.58 (t, 2H, ph), 7.61 (s, 2H, NHCO), 7.71 (t, 2H, ph), 7.81 (t, 2H, ph), 7.86 (t, 2H, ph), 7.97 (m, 6H, ph), 8.40 (d, 2H, ph), 8.58 (s, 4H, pyrrole), 8.64 (s, 4H, pyrrole), 9.01 (s, 2H, NHCO); vis (H_2O/$NaHCO_3$–Na_2CO_3, pH 10.4) λ_{max} (log ε) 424 (5.65), 557 (4.25), 596 (3.63) nm; Anal. Calcd for $C_{64}H_{68}N_{10}O_4Zn \cdot Cl_2 \cdot 5H_2O$: C, 60.71; H, 6.02; N, 11.06%. Found: C, 60.69; H, 5.83; N, 11.01%; MS *m/z* 1141(M^+), M^+ calcd for $C_{64}H_{68}N_{10}O_4ZnCl$, 1140. Compound **3** (chloride): ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.34 (s, 18H, $N(CH_3)_3$), 6.67 (m, 8H, CO–ph), 6.96 (m, 2H, CO–ph), 7.66 (m, 4H, ph), 7.88 (m, 6H, ph), 7.99 (m, 4H, ph), 8.32 (d, 2H, ph), 8.52 (s, 2H, pyrrole), 8.62 (d, 2H, pyrrole), 8.75 (d, 2H, pyrrole), 8.84 (s, 2H, pyrrole), 9.02 (s, 2H, NHCO), 9.29 (s, 2H, NHCO); vis (H_2O/$NaHCO_3$–Na_2CO_3, pH 10.4) λ_{max} (log ε) 424 (5.18), 557 (4.20), 596 (3.66) nm; Anal. Calcd for $C_{68}H_{60}N_{10}O_4Zn \cdot Cl_2 \cdot 3H_2O \cdot 2CH_3OH$: C, 62.95; H, 5.58; N, 10.49%. Found: C, 63.23; H, 5.21; N, 10.06%; MS *m/z* 1181(M^+), M^+ calcd for $C_{68}H_{60}N_{10}O_4ZnCl$, 1180.

7 H. Imai, H. Munakata, A. Takahashi, S. Nakagawa, and Y. Uemori, *Chem. Lett.*, **1997**, 819; H. Imai, H. Munakata, A. Takahashi, S. Nakagawa, Y. Ihara, and Y. Uemori, *J. Chem. Soc., Perkin Trans. 2*, **1999**, 2565.

8 A plot of [L] versus [L]/ΔA gave a straight line with intercept $-K^{-1}$, where ΔA is the difference between the absorbance at 424 nm of the solution of ZnP without L and that with a given concentration of free L; J. P. Collman, J. I. Brauman, K. M. Doxsee, T. R. Halbert, S. E. Hayes, and K. S. Suslick, *J. Am. Chem. Soc.*, **100**, 2761 (1978).

9 M. A. Hossain and H.-J. Schneider, *Chem. Eur. J.*, **5**, 1284 (1999).

10 The K values of **3** with chiral amines were estimated from the formation of the Zn–N bond (reference 8) where the two diastereomeric conformations of the amine adducts can not be distinguishable. Therefore chiral recognition of the amines by **3** results a decrease in K for L- (or D-) body of the amines compared with the DL- racemic mixture in terms of a decreased amount of a preferred diastereomeric conformation.