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Original article

Metalloporphyrin receptors for histidine-containing peptides

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ABSTRACT

Two new ditopic metalloporphyrin receptors constructed by combining metalloporphyrin with crown ethers have been prepared and characterized. ^{1}H NMR and MS spectra confirmed the complexation of receptor with peptide driven by coordination interaction and hydrogen bonding. UV/vis experiments revealed that the receptors exhibited high binding affinity to histidine-containing peptides. These receptors could differentiate short peptides of C-terminal histidine and N-terminal histidine and formed the most stable complexes with tripeptide.

1. Introduction

Selective recognition of short peptides by synthetic receptors has attracted a great deal of interest in past decade because of their important roles in nature [1–5]. The intrinsic properties of short peptides originated from flexible conformation and irregular topology placed a number of challenges in the design of receptors [6]. In the recently reported examples, one strategy has been proved to be effective for selective recognition of peptides, this is, the binding of peptide N terminus and side chain of peptide in ditopic fashion [7–10].

Crown ethers are ideal binding units for ammonium ions in amino acids [11–14]. On the other hand, metalloporphyrins have been documented to coordinate the nitrogen atom of imidazole [15–18]. Porphyrin platform can further provide a large molecular surface for dispersive interaction with peptide backbone [19–21]. In this context, the aim of this letter is to develop new receptors by combining crown ethers with metalloporphyrin and to investigate their recognition behavior toward histidine-containing short peptides.

The strapped porphyrin developed by our group was served as a scaffold to align peptide backbone in one direction from the N to C terminus [22,23]. The metal cation Zn(II) in the center of porphyrin could provide additional coordination site to bind nitrogen atom of histidine. Aza-crown ethers of varied size were incorporated to porphyrin *via* amide linkage as recognition site of ammonium ion, which allowed the receptor preorganized as the distance between N terminus and side chain of peptides (Scheme 1).

2. Experimental

NMR spectra were recorded on a Varian spectrometer operating at 300 and 400 MHz for ^{1}H and ^{13}C respectively in the indicated solvents. Chemical shifts were expressed in parts per million (δ) using residual solvent protons as internal standards. MALDI-TOF mass spectra were recorded on a Voyager-DE STR mass spectrometer (AB SCIEX, USA). UV/vis absorption spectra were measured with a Cary 100 UV/vis spectrophotometer (Varian, USA). Elemental analysis was carried out at the SIOC analytical center. Unless otherwise indicated, all starting materials were obtained from commercial suppliers and were used without further purification. All solvents were dried before use following standard procedures. All reactions were performed under an atmosphere of dry nitrogen. Compound **5** was prepared following our previous method [24,25]. Column chromatography was carried out using silica gel (300–400 mesh). All of the modified peptides were prepared following standard procedures in the solution (see Supporting information).

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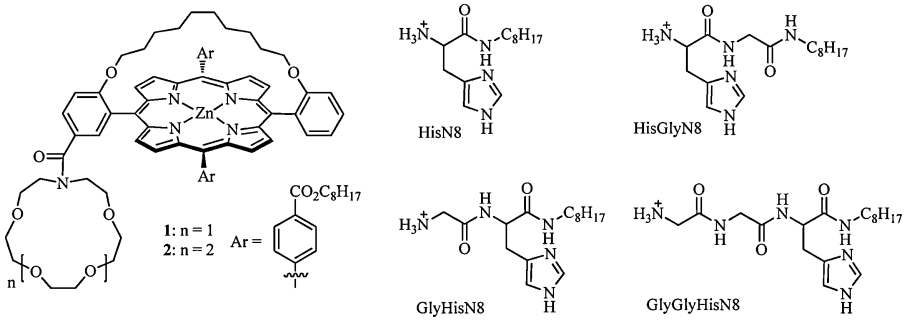


Scheme 1. Metalloporphyrin receptors and modified peptides.

The UV/vis titration experiments were performed according to the following procedure. A solution of receptor (2.0 mL, 1.1×10^{-6} mol/L) in chloroform was titrated with 10 μL of histidine-containing peptide (2.0×10^{-5} mol/L to 5.0×10^{-5} mol/L in chloroform, adjusted with 10 fold HCl). After each addition the solution was allowed to equilibrate for 5 min and the absorption intensity was recorded in the wavelength region of 350–600 nm at 25 °C.

2.1. General procedure for the synthesis of metalloporphyrin receptors

To a solution of porphyrin acid **5** in dichloromethane oxalyl chloride and several drops of DMF were added. The mixture was stirred at room temperature for 5 h. After evaporating the solvents under vacuum, the residue was dissolved in dichloromethane and then aza-crown ether and triethyl amine were added and stirred overnight at room temperature. After removal of solvent, the resulting residue was purified by column chromatography to afford aza-crown ether porphyrin. The free base porphyrin was dissolved in dichloromethane/methanol (3:1) and zinc acetate was added with stirring. The mixture was stirred under reflux overnight. The solvent was removed *in vacuo* and the resulting residue was subjected to column chromatography to afford metalloporphyrin receptor as a purple solid in high yield (Scheme 2).

Compound **1**: Purple solid (68%). Mp > 250 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ −1.39 (m, 2H), −1.21 (m, 2H), −1.00 (m, 2H), −0.80 (m, 2H), −0.60 (br, 4H), 0.65 (br, 4H), 0.90 (t, 6H), 1.25–1.56 (m, 20H), 1.89 (p, 4H), 3.35–3.86 (m, 20H), 4.49 (t, 4H, *J* = 6.75 Hz), 7.28 (m, 2H), 7.46 (d-t, 1H, *J* = 0.9, 7.5 Hz), 7.76 (d-t, 1H, *J* = 1.8, 5.1 Hz), 7.86 (d-d, 1H, *J* = 2.25, 8.55 Hz), 8.19 (d-d, 2H, *J* = 1.05, 7.95 Hz), 8.32 (d-d, 1H, *J* = 7.5, 1.8 Hz), 8.37–8.48 (m, 7H), 8.81 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 22.7, 25.1, 26.1, 27.7, 28.3, 28.9, 29.1, 29.3, 29.4, 31.9, 65.5, 70.1, 112.9, 113.8, 116.4, 117.8, 119.5, 120.0, 127.7, 128.0, 129.1, 129.6, 129.8, 131.6, 131.7, 131.9, 132.2, 132.8, 134.4, 134.7, 147.7, 149.5, 150.4, 150.7, 159.8, 160.6, 167.0, 172.0, 172.3. MS (MALDI-TOF) (*m*/*z*): 1407 ($M+H^+$). Anal. Calcd. for $C_{83}H_{97}N_5O_{11}Zn$: C, 70.90; H, 6.95; N, 4.98. Found: C, 70.39; H, 7.04; N, 4.91.

Compound **2**: Purple solid (71%). Mp > 250 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ −1.39 (br, 2H), −1.26 (br, 2H), −1.00 (br, 2H), −0.85 (br, 2H), −0.69 (br, 4H), 0.61 (br, 4H), 0.90 (t, 6H), 1.25–1.55 (m, 20H), 1.88 (p, 4H), 2.29–3.06 (m, 20H), 3.67 (m, 4H), 4.42 (t, 4H, *J* = 6.75 Hz), 7.09 (d, 1H, *J* = 9.0 Hz), 7.29 (s, 1H), 7.46 (t, 1H, *J* = 7.5 Hz), 7.56 (d, 1H, *J* = 5.4 Hz), 7.76 (t, 1H, *J* = 5.1 Hz), 7.99 (s, 1H), 8.22–8.45 (m, 9H), 8.75–8.88 (m, 8H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 14.1, 22.7, 25.1, 26.2, 29.1, 29.3, 29.4, 29.7, 31.9, 65.4, 70.4, 112.8, 113.8, 116.3, 117.7, 119.4, 119.9, 127.6, 128.9, 129.5, 129.8, 131.4, 131.6, 131.8, 132.1, 134.3, 134.7, 147.8, 149.5, 150.5, 150.9, 159.8, 160.5, 167.0, 171.8, 172.0. MS (MALDI-TOF) (*m*/*z*): 1451 ($M+H^+$). Anal. Calcd. for $C_{85}H_{101}N_5O_{12}Zn$: C, 70.40; H, 7.02; N, 4.83. Found: C, 70.61; H, 7.02; N, 4.64.

3. Results and discussion

To investigate the binding modes of the metalloporphyrin receptor and the histidine-containing peptides, ^{1}H NMR experiments were firstly carried out. When 5 equiv. of HisN8 was added to a solution of metalloporphyrin **1** or **2** in $CD_3OD/CDCl_3$ (1/1), the signals of the protons of the imidazole group shifted upfield (*ca.* 0.17 ppm) as a result of the coordination interaction between the imidazole nitrogen atom and the zinc cation (Fig. 1). The strong

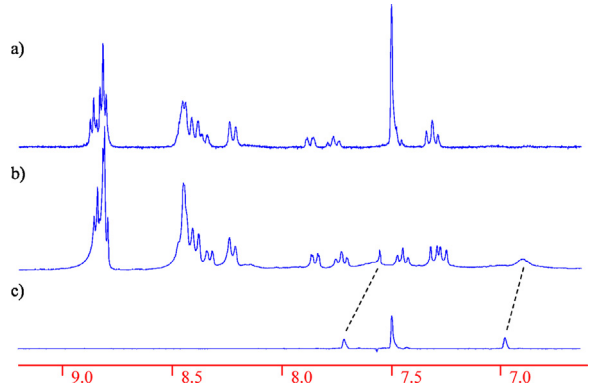
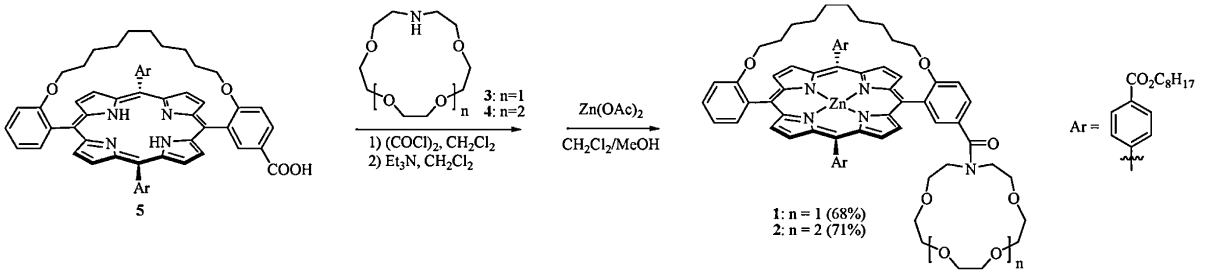


Fig. 1. Partial ^{1}H NMR spectra (300 MHz) in $CD_3OD/CDCl_3$ (1/1) at 25 °C: (a) Receptor **1**; (b) Receptor **1** + **5** equiv. HisN8; (c) HisN8.



Scheme 2. Synthetic route of metalloporphyrin receptors **1** and **2**.

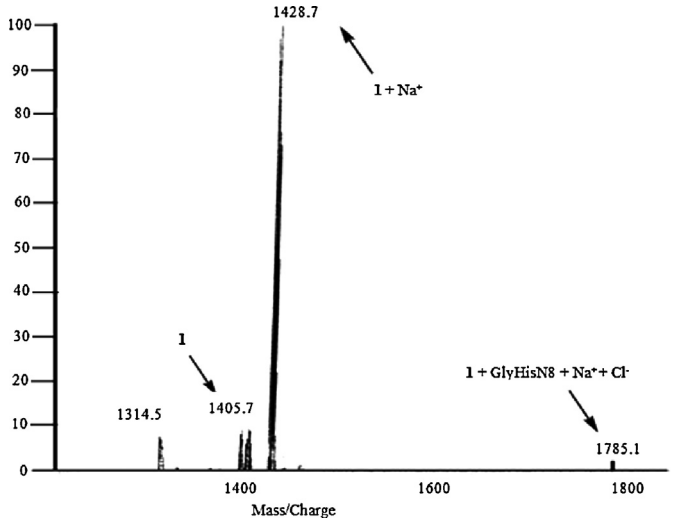


Fig. 2. MALDI-TOF mass spectra of **1**·GlyHisN8 complex.

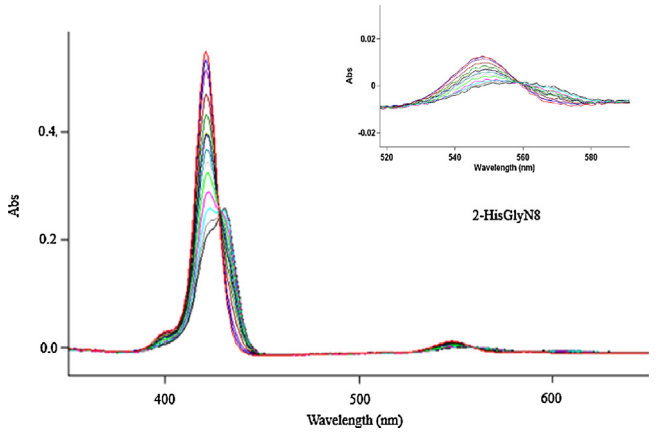


Fig. 3. Absorption spectral changes of 2 (1.1×10^{-6} mol/L) upon addition of HisGlyN8 (1.0×10^{-7}–7.0×10^{-6} mol/L) in chloroform at 25 °C.

upfield shifts were also observed when other histidine-containing peptides were used (Figs. S1–S3 in Supporting information), indicating the placement of the peptide within the ring current of metalloporphyrin. Due to the overlapping of signals in the region of 3.0–4.0 ppm, the changes of the protons of the crown ether were hardly estimated. However, the binding strength between crown ether and ammonium ions should be much weaker than that of Zn^{2+} and imidazole according to previous studies [26–28].

Table 1
Binding constants (L/mol) and the associated free energy change (kcal/mol) of peptides with receptors **1** and **2**.

Peptide	**1**		**2**	
	Binding constant	ΔG	Binding constant	ΔG
HisN8	1.1×10^5	−6.9	1.2×10^5	−7.0
HisGlyN8	1.9×10^5	−7.2	2.5×10^5	−7.4
GlyHisN8	4.4×10^5	−7.7	5.5×10^5	−7.9
GlyGlyHisN8	5.2×10^5	−7.8	7.8×10^5	−8.0

[a] Values are the average of two separate measurements and with error of ±15%.
[b] Obtained in chloroform.

Following the NMR study, mass spectrometry was used to probe the non-covalent interactions between the metalloporphyrin receptors and the histidine-containing peptides. The formation of a stable complex from receptor **1** and GlyHisN8 driven by coordination interaction and hydrogen bonding was confirmed by MALDI-TOF analysis (Fig. 2). It gave a peak at m/z 1785.1, which corresponded to 1:1 complex of **1** and GlyHisN8, although the intensity was weak.

The binding constants were determined by UV/vis titration experiments in chloroform. Both receptors **1** and **2** showed the maximum absorption at the wavelength of 421 nm. Upon the addition of a histidine-containing peptide to a solution of receptor **1** or **2**, a pronounced decrease in the absorption for all those peptides was observed. No significant shift was observed for receptors **1** and **2** when a modified histidine (HisN8) was added to their solutions. Upon addition of dipeptide (HisGlyN8 or GlyHisN8) two clear isosbestic points appeared at 432 and 559 nm, respectively. Furthermore, the addition of tripeptide (GlyGlyHisN8) also resulted in a significant bathochromic shift (*ca.* 12 nm and 10 nm) (Fig. 3 and Figs. S4 and S5 in Supporting information).

On the basis of the absorption data obtained at 421 nm, binding constants were calculated using a 1:1 binding model [29]. The results are summarized in Table 1, which reveals that those histidine-containing peptides bind well to the metalloporphyrins. In contrast to receptor **1**, receptor **2** shows slightly higher binding affinity to all the four peptides. It can be attributed to a higher binding ability of aza-18-crown-6 toward ammonium ion than that of aza-15-crown-5 [30].

Receptors **1** and **2** could differentiate short peptides with C-terminal histidine and N-terminal histidine. For example, receptors exhibited higher binding affinity toward GlyHisN8 than HisGlyN8. It might be attributed to a more matched spatial distance between ammonium ion and imidazole group in GlyHisN8. It was also noteworthy that the binding energy increased with the increasing length of the peptides, which was attributed to the dispersive interaction of the amide groups with the porphyrin unit. Receptors **1** and **2** bound best to tripeptide GlyGlyHisN8, which bears three amide oxygen atoms with

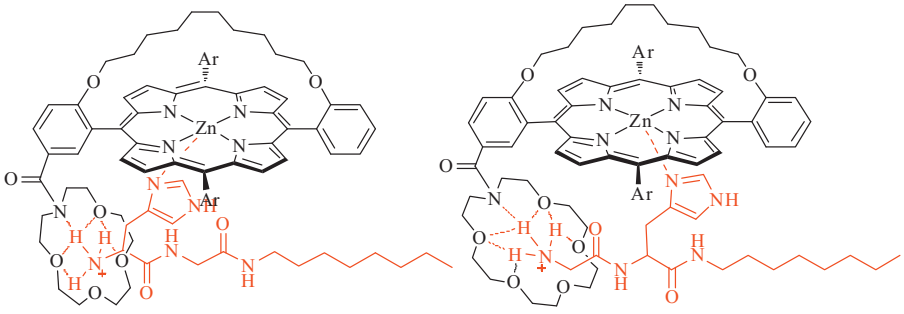


Fig. 4. Proposed structures of the complexes formed from receptors and peptides.

negative charge of high polarizibility. Based on these results and the previous reports, it was proposed that complexes formed by the binding of receptors and peptides in the following modes (Fig. 4).

4. Conclusion

In summary, the combination of a strapped metalloporphyrin with aza-crown ethers resutled in two new synthetic receptors. They exihibited high binding affinity to histidine-containing short peptides and showed higher binding affinity to C-terminal histidine than to N-terminal histidine. As a consequece of additive dispersive interaction of the amide group with porphyrin, both receptors formed the most stable complex with tripeptide. In addition, the binding affinity of receptor **2** to the histidine-containing peptides was slightly higher than **1**, which could be attributed to the stronger binding of ammonium ion by the aza crown ethers of larger ring size in receptor **2**. Current efforts are focusing on exploring selective recognition of short peptide in aqueous media by modifying the metalloporphyrin receptors.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cclet.2014.03.034.

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