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'Conjugate strategy' of lanthanide porphyrinate with crown ether toward synergistic binding and chirality sensing of biological substrates

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Abstract

Erbium porphyrinate/benzo-18-crown-6 conjugate extracted amino acids and biogenetic amines more effectively than a mixture of erbium porphyrinate and benzo-18-crown ether, while such an enhancement was rarely observed with gadolinium or ytterbium conjugate. The erbium conjugate further offered 'induced circular dichroism signals' at Soret regions via synergistic binding of zwitterionic amino acids, the sign of which depended on the absolute configuration of the bound substrates.

Keywords: Molecular recognition; Lanthanide porphyrinate; Crown ether; Circular dichroism; Amino acid; Biogenetic amine

1. Introduction

Metalloporphyrin receptors exhibit interesting molecular recognition functions. Since the porphyrin ligands accommodate characteristic metal centers in well-defined geometry, their molecular recognition functions can be finely optimized for a specific substrate [1]. Lanthanide porphyrinates have occupied unique positions in the molecular recognition chemistry. They were employed as NMR shift reagents and UV probes [2,3], but their receptor functions were rarely characterized. We recently demonstrated that lanthanide porphyrinates efficiently extracted unprotected amino acids under neutral pH conditions and acted as circular dichroism (CD) probes capable of chirality sensing of the bound substrates [4,5]. In these systems, the trivalent lanthanide ions were electrically neutralized by coordination from β-diketonate and porphyrinate anions, but had the additional $-CO_2^-$ group of amino acids to form highly coordinated complexes [6]. When hydrophilic and multifunctional biological substrates are chosen as targets, more effective receptors should have multiple binding sites for complementary functional groups of these substrates. Therefore, we connected benzo-18-crown-6 moiety with lanthanide porphyrinates covalently for synergistic binding of zwitterionic amino acids and biogenetic amines (Fig. 1) [7–13]. Because benzo-18-crown-6 catches $-NH_3^+$ cation and since lanthanide porphyrinate acts as a Lewis acid site, the conjugation of these two different kinds of binding sites within a receptor may offer effective binding of biological substrates [14] and sensitive CD probing of their chirality [15].

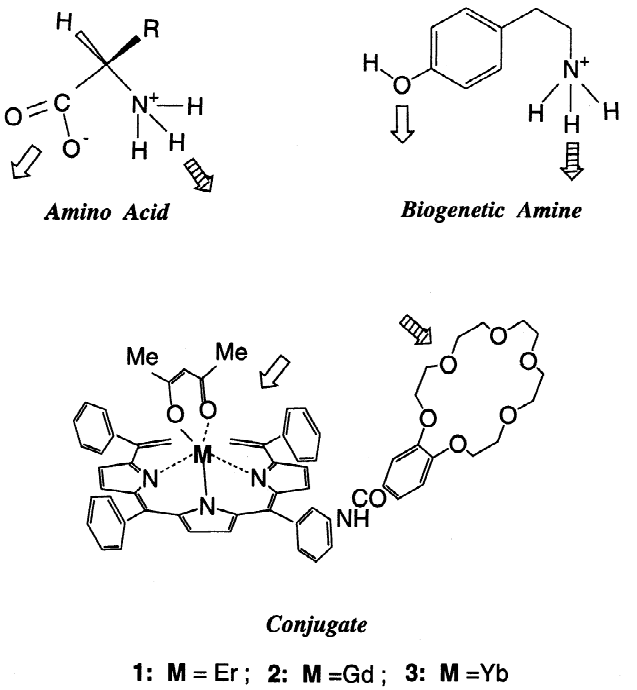

Fig. 1. Structures of biological substrates and conjugate receptors.

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2. Experimental details

2.1. Synthesis and characterization of lanthanide porphyrinates

2.1.1. Synthesis of porphyrin/crown ether ligand

5-(3-Aminophenyl)-10,15,20-triphenylporphine (*m*-APTPP) [16] was combined with benzo-18-crown-6. After treatment with oxalyl chloride, 4-carboxybenzo-18-crown-6 (0.70 mmol) was reacted in CH_2Cl_2 (70 ml) with *m*-APTPP (0.89 mmol) in the presence of triethylamine (1.4 mmol). After washing with water, drying over $MgSO_4$, and silica gel chromatography (CH_2Cl_2/MeOH, 20/1), the ligand was collected from CH_2Cl_2/hexane: yield, 72%; purple powder; mp 176–180°C; R_f=0.56 (CH_2Cl_2/CH_3OH, 5/1); IR (Nujol) 1654, 1604, 1543, 1509, 1271 and 1120 cm^{-1}; ^{1}H–NMR (400 MHz, $CDCl_3$) δ 8.91 (d, J=4.6 Hz, 2H), 8.85 (d, J=4.6 Hz, 2H), 8.84 (s, 4H), 8.31 (t, J=1.7 Hz, 1H), 8.25–8.15 (m, 7H), 8.16 (s, 1H), 7.99 (d, J=7.6 Hz, 1H), 7.8–7.7 (10H), 7.48 (d, J=2.2 Hz, 1H), 7.33 (dd, J=8.3, 2.2 Hz, 1H), 6.70 (d, J=8.3 Hz, 1H), 4.2–4.1 (m, 2H), 4.1–4.0 (m, 2H), 3.85–3.75 (m, 4H), 3.70–3.60 (m, 4H), 3.65–3.55 (m, 4H), 3.55 (s, 4H) and −2.78 (br s, 2H); FAB–MS (*m*-nitrobenzyl alcohol) m/z 990 ($M+Na^+$). Anal. Calcd. for $C_{61}H_{53}N_5O_7\cdot 0.5H_2O$: C, 74.98; H, 5.57; N, 7.17. Found: C, 74.97; H, 5.45; N, 7.15.

2.1.2. Synthesis of lanthanide porphyrinate/crown ether conjugates ***1–3***

Porphyrin/crown ether ligand (0.50 mmol) and lanthanide tris-2,4-pentanedionate (1.25 mmol) were refluxed in trichlorobenzene (23 ml) for 6 h under the N_2 atmosphere. The incorporation of lanthanide metal ion into the porphyrin ligand was confirmed by monitoring the Soret and Q-band absorptions of each conjugate [17]. The reaction mixture was chromatographed (aluminum oxide): free ligand was first eluted with acetone, and the desired conjugate was obtained using dimethyl sulfoxide (DMSO) and then DMSO/H_2O (10/1) as eluents. After extraction with CH_2Cl_2, washing with water and drying over $MgSO_4$, the conjugate was collected from CH_2Cl_2/hexane.

Erbium conjugate **1**: yield, 43%; purple powder; mp 249°C (dec) (CH_2Cl_2/hexane); IR (Nujol) 1664, 1602, 1548, 1511, 1270, 1131 and 1067 cm^{-1}; FAB–MS (*m*-nitrobenzyl alcohol) m/z 1230 (M^+). Anal. Calcd. for $C_{66}H_{58}N_5O_9Er\cdot H_2O$: C, 63.39; H, 4.84; N, 5.60. Found: C, 63.33; H, 4.69; N, 5.80.

Gadolinium conjugate **2**: yield, 52%; purple powder; mp 253°C (dec) (CH_2Cl_2/hexane); IR (Nujol) 1648, 1600, 1546, 1512, 1270, 1131 and 1067 cm^{-1}; FAB–MS (*m*-nitrobenzyl alcohol) m/z 1222 (M^+). Anal. Calcd. for $C_{66}H_{58}N_5O_9Gd\cdot 0.5\cdot H_2O\cdot 1.5$DMSO: C, 61.45; H, 5.08; N, 5.19. Found: C, 61.12; H, 4.69; N, 5.55.

Ytterbium conjugate **3**: yield, 60%; mp 242°C (dec); IR (Nujol) 1638, 1602, 1551, 1514, 1271, 1131 and 1068 cm^{-1}; FAB–MS (*m*-nitrobenzyl alcohol) m/z 1238 (M^+). Anal. Calcd. for $C_{66}H_{58}N_5O_9Yb\cdot$DMSO: C, 62.04; H, 4.90; N, 5.32. Found: C, 61.70; H, 4.50; N, 5.72.

2.1.3. Synthesis of lanthanide porphyrinates ***4–6***

Lanthanide porphyrinates **4–6** were similarly prepared from tetraphenylporphyrin and lanthanide tris-2,4-pentanedionates and employed for comparison (Fig. 2) [17].

Erbium porphyrinate **4**: yield, 52%; purple powder; mp 300°C (dec) (CH_2Cl_2/hexane); IR (KBr) 1596 and 1516 cm^{-1}; EI–MS m/z 778 ($[M\text{-acac}]^+$). Anal. Calcd. for $C_{49}H_{35}N_4O_2Er\cdot H_2O$: C, 65.60; H, 4.16; N, 6.25. Found: C, 65.69; H, 4.06; N, 6.52.

Gadolinium porphyrinate **5**: yield, 82%; purple powder; mp>300°C (CH_2Cl_2/hexane); IR (Nujol) 1595 and 1515 cm^{-1}; EI–MS m/z 869 (M^+). Anal. Calcd. for $C_{49}H_{35}N_4O_2Gd\cdot 1.5$DMSO: C, 63.33; H, 4.50; N, 5.68. Found: C, 63.05; H, 4.19; N, 5.91.

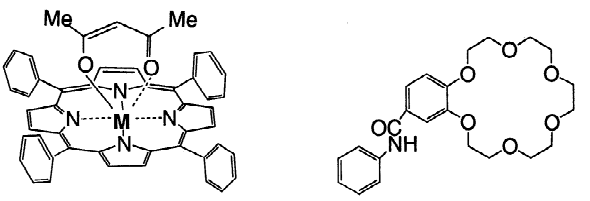
Ytterbium porphyrinate **6**: yield, 26%; mp 280°C (dec); IR (Nujol) 1595 and 1515 cm^{-1}; EI–MS m/z 885 (M^+). Anal. Calcd. for $C_{49}H_{35}N_4O_2Yb\cdot 0.5CH_2Cl_2\cdot 1.5H_2O$: C, 62.30; H, 4.12; N, 5.87. Found: C, 62.28; H, 3.73; N, 6.35.

2.1.4. Synthesis of 4-(*N*-phenylcarbamoyl)benzo-18-crown-6 ***7***

After treatment with oxalyl chloride, 4-carboxybenzo-18-crown-6 (0.60 mmol) was reacted in dry CH_2Cl_2 (20 ml) with aniline (1.2 mmol) in the presence of triethylamine (1.2 mmol), and chromatographed (silica gel: CH_2Cl_2/MeOH=100/1 to 100/5). The crude product was washed with H_2O, dried over $MgSO_4$ and crystallized from CH_2Cl_2/hexane: yield; 35%; white powder; mp 140°C; Rf=0.53 (CH_2Cl_2/CH_3OH, 5/1); IR (Nujol) 1646, 1600, 1543, 1510, 1268, 1118 and 1047 cm^{-1}; ^{1}H–NMR (400 MHz, $CDCl_3$) δ 7.90 (br s, 1H), 7.64 (dd, J=8.7, 1.1 Hz, 2H), 7.47 (d, J=2.2 Hz, 1H), 7.40–74.3 (m, 3H), 7.13 (tt, J=7.4, 1.2 Hz, 1H), 6.88 (d, J=8.3 Hz, 1H), 4.24–4.19 (m, 4H), 3.96–3.91 (m, 4H), 3.78–3.75 (m, 4H), 3.72–3.70 (m, 4H), and 3.68 (s, 4H). Anal. Calcd. for $C_{23}H_{29}NO_7\cdot H_2O$: C, 61.46; H, 6.95; N, 3.12. Found: C, 61.21; H, 6.98; N, 3.13.

2.1.5. Extraction experiments

(a) *Amino acids*: A mixture of an aqueous solution (2.5 ml) of amino acid (125 nmol) and a CH_2Cl_2 solution (10



4: M = Er ; 5: M =Gd ; 6: M =Yb **7**

Fig. 2. Lanthanide porphyrinates and crown ether analog.

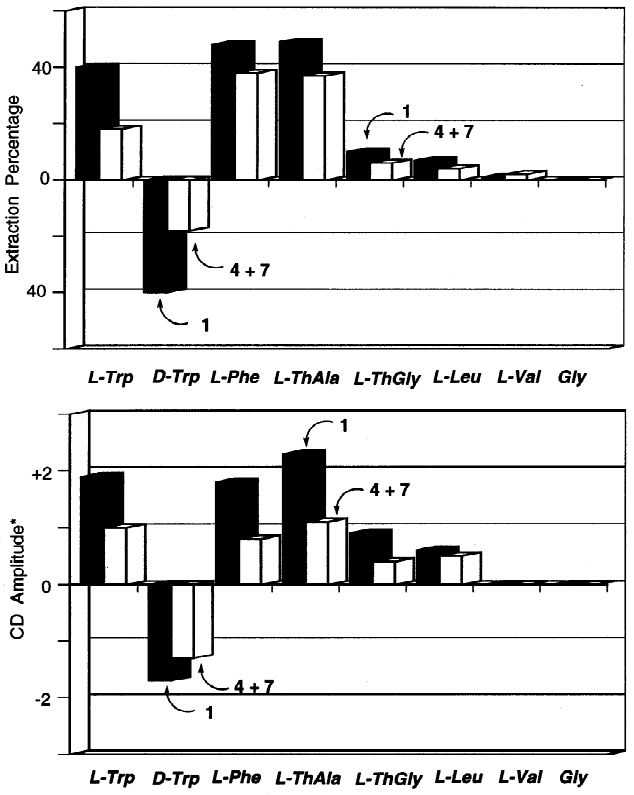
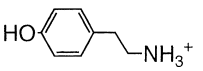
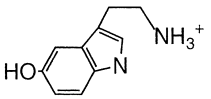
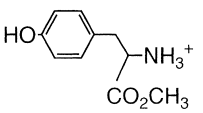
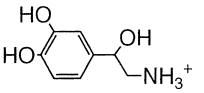


Fig. 3. Extraction percentage of zwitterionic amino acids and amplitude of induced CD signals. *CD amplitude = [θ at 1st λ] − [θ at 2nd λ]/10^5 deg cm^2 $dmol^{-1}$.

ml) of receptor (125 nmol) was shaken for 30 min. Extraction percentages of tryptophan (Trp), 3-(2-thienyl)-alanine (ThAla) and (2-thienyl)-glycine (ThGly) were determined by UV spectroscopy, while the concentrations of phenylalanine (Phe), leucine (Leu), valine (Val) and glycine (Gly) were estimated based on amino acid analysis (ninhydrin colorimetry: HITACHI L-8500). The averaged values of two or three independent experiments are shown in Fig. 3. After extraction experiments, induced CD spectra of the separated organic phases were recorded with a Jasco J-720 spectrometer.

Table 1
Extraction of biogenetic amine salts

Biogenetic amine salts (pH = 6.0)	Extraction (%)	
	Conjugate **1**	Mixture **4+7**
	14	0
	5	1
	13	9
	16	3

(b) *Biogenetic amines*: A mixture of an aqueous solution (0.3 ml) of biogenetic amine (1.5 μmol) and $LiClO_4$ (1.5 μmol) was shaken with a CH_2Cl_2 solution (0.3 ml) of receptor (1.5 μmol) for 2 h. Extraction percentages of biogenetic amines were determined by UV spectroscopy, and the averaged values of two independent extraction experiments are summarized in Table 1.

3. Results and discussion

3.1. Synergistic binding and chirality sensing of amino acids

The receptor/probe abilities of lanthanide porphyrinate/crown ether conjugates **1–3** were characterized by liquid–liquid extraction of amino acids and subsequent CD measurements. Among them, erbium conjugate **1** offered the most efficient 'conjugate effects' of lanthanide porphyrinate with crown ether on the extraction efficiencies of zwitterionic amino acids. It extracted Trp (40%) from an aqueous solution (pH = ca. 6.0) into a CH_2Cl_2 solution more effectively than porphyrinate **4** (18%), crown ether **7** (0%) and their mixture **4+7** (18%). Although **4** and **7** have binding sites for $-CO_2^-$ and $-NH_3^+$ groups respectively, the conjugation of these two binding sites greatly increased extraction ability [18]. In contrast, gadolinium conjugate **2** and ytterbium conjugate **3** exhibited comparable extraction efficiencies with corresponding mixtures: 39% for **2** and 34% for **5+7**; 14% for **3** and 10% for **6+7**. Thus, the lanthanide center should play important roles in the effective conjugation[1]. The extracted amount of Trp by conjugate **1** largely depended on pH value of the aqueous solution: 13% (pH = 3.5) < 40% (pH = 5.7) ≃ 38% (pH = 8.1) > 28% (pH = 9.4) > 8% (pH = 10.1). This probably suggests that the amino acid was bound with conjugate **1** as a zwitterionic form. When the concentration of Trp in the aqueous phase was greatly increased, mole ratio of the extracted Trp to conjugate **1** reached 0.9, suggesting 1:1 complexation. The organic phase (CD_2Cl_2) was also characterized using NMR spectroscopy after extraction experiments, which included the highly coordinated complex between conjugate **1** and Trp. The signals for aliphatic protons of the bound Trp disappeared, while those for aromatic protons broadened but still appeared around 5.5–

[1]The size of lanthanide center may have influences on coordination strength and mode (monodentate or bidentate) from $-CO_2^-$ group of the substrate.

6.0 ppm. These observations support that the extracted Trp locates near the erbium center and also above the porphyrinate plane.

Erbium conjugate **1** extracted a variety of amino acids more effectively than mixture **4**+**7** and exhibited chirality-specific CD signals (Figs. 3 and 4) [19,20]. After extraction experiments with L-Trp, L-Phe, L-ThAla, L-ThGly and L-Leu, the resulting organic solutions gave the 'reversed' S-shaped CD bands around the region of the Soret absorption band, while their D-isomers offered the S-shaped CD bands [21–25]. Fig. 4 illustrates the observed CD signals for L- and D-Trp with conjugate **1** and mixture **4**+**7**. The CD amplitudes with conjugate **1** ([θ at 1st λ]−[θ at 2nd λ]/10^5 deg cm^2 dmol^{-1}) were generally larger than those with mixture **4**+**7**, though they gave CD signals with similar shapes: **1** (1.9)>**4**+**7** (1.0) for L-Trp; **1** (1.8)>**4**+**7** (0.8) for L-Phe; **1** (2.3)>**4**+**7** (1.1) for L-ThAla; **1** (0.9)>**4**+**7** (0.4) for L-ThGly; **1** (0.6)>**4**+**7** (0.5) for L-Leu. Since lanthanide porphyrinates exhibit intense Soret bands in the visible spectra, 'conjugate strategy' offers a promising possibility in designing a sensitive CD probe for chirality determination.

3.2. Synergistic binding of biogenetic amines

Erbium porphyrinate/crown ether conjugate **1** was applied in the extraction of biogenetic amine salts which included tyramine, serotonin, tyrosine methyl ester and noradrenaline salts (Table 1). Conjugate **1** typically extracted noradrenaline (16%) from a neutral aqueous solution (pH=6.0) into a CH_2Cl_2 solution, while mixture **4**+**7** rarely extracted it (3%). Its extraction efficiencies for the employed amines were generally modest, but 'conjugate effects' were clearly observed in the extraction of tyramine and serotonin salts. The noradrenaline was also extracted by conjugate **1** (10%) from acidic aqueous solution (pH= 3.6), suggesting that conjugate **1** bound biogenetic amine as a monocationic form. When chiral tyrosine methyl ester and noradrenaline salts were extracted into the CH_2Cl_2 phases, the induced CD signals appeared at the Soret region, but their amplitudes were too small to be used for chirality determination. These biogenetic amines have two kinds of binding sites for conjugate **1**: (i) phenol or catechol moiety for coordination with the erbium center; and (ii) ammonium cation pointing toward the crown ring. Since the former site locates apart from the asymmetric center, erbium conjugate **1** was confirmed to work as an effective receptor but an inefficient CD probe for biogenetic amines (see Fig. 1).

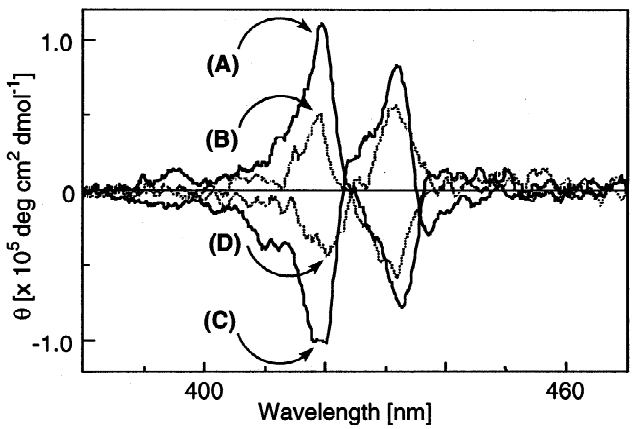


Fig. 4. CD spectra of conjugate **1** or mixture **4**+**7** in CH_2Cl_2 after extraction of L- or D-Trp. (A) L-Trp·**1**; (B) L-Trp·**4**+**7**; (C) D-Trp·**1**; (D) D-Trp·**4**+**7**.

In conclusion, we demonstrated that the conjugation of erbium porphyrinate with 18-crown-6 ring significantly enhanced extraction abilities of biological substrates, and also provided new sensitive CD chirality probing of amino acids. Therefore, our 'conjugate strategy' may have wide application in the development of effective sensing, transport and separation of other biological substrates.

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