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Induced circular dichroism by complexation of gadolinium(III) porphyrinates with chiral amino acids and dipeptides: effects of axial β-diketonate ligands on chirality sensing and recognition

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Abstract—Synthetic gadolinium(III)porphyrins with various achiral β-diketonates as axial ligands in benzene solutions extracted chiral α-amino acids and dipeptides from aqueous phases to give intense induced CD peaks in the Soret region via 1:1 supercomplexation. Their CD spectral shapes were dependent on the stereochemistry at the α-positions of amino acids and of the C-terminal components of dipeptides: a reverse S-shape for the L-form and an S-shape for the D-form. When chiral 3-acetylcamphorate was introduced as an axial ligand, Gd(III)porphyrins showed CD spectral changes by supercomplexation with chiral alanylalanine; (+)-acetylcamphorate ligating Gd(III)porphyrin offered larger CD signal with the LL- or DL-form than the corresponding (−)-type Gd(III)porphyrin did, while the former afforded smaller CD peaks by supercomplexation with the DD- or LD-form than the latter Gd(III)porphyrin.
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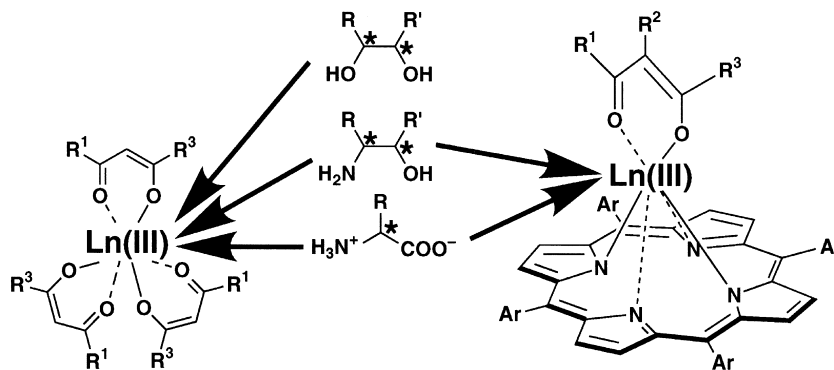
1. Introduction

A series of neutral lanthanide(III) complexes having three anionic ligands are known to also bind various guest species, and the resulting highly coordinated complexes (=supercomplexes) are applicable in several chemical fields.[1] Typically, commercially available europium(III) tris(dipivaloylmethanate) binds polar functions of various molecules in solutions and acts as a proton NMR chemical shift reagent. Achiral praseodymium(III) tris(dipivaloylmethanate) was reported to form CD-active supercomplexes with chiral 1,2-diols in a dry organic solvent (left drawing in Scheme 1).[2] Recently we reported that achiral lanthanide(III) tris(β-diketonates) complexed chiral β-aminoalcohols in a solution to give CD peaks, the sign of which was dependent upon the stereochemistry of the guests.[3] Lanthanide(III)



Scheme 1. Complexation of lanthanide(=Ln(III)) tris(β-diketonates) (left) and porphyrinate β-diketonates (right) with various bidentate analytes.

Keywords: amino acid; CD spectrometry; coordination; dipeptide; gadolinium porphyrin.

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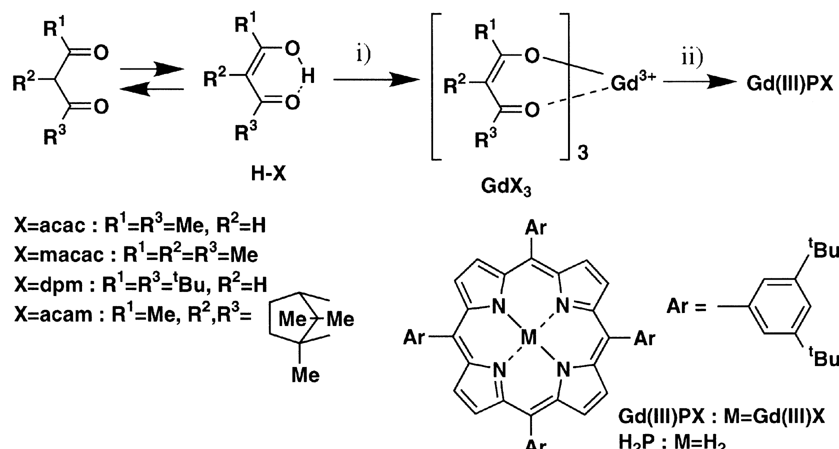


Scheme 2. Synthesis of gadolinium(III) porphyrinate β-diketonate (GdPX); (i) aq. NaOH/EtOH, $Gd(NO_3)_3 \cdot 6H_2O$; (ii) H_2P/1,2,4-$C_6H_3Cl_3$, reflux.

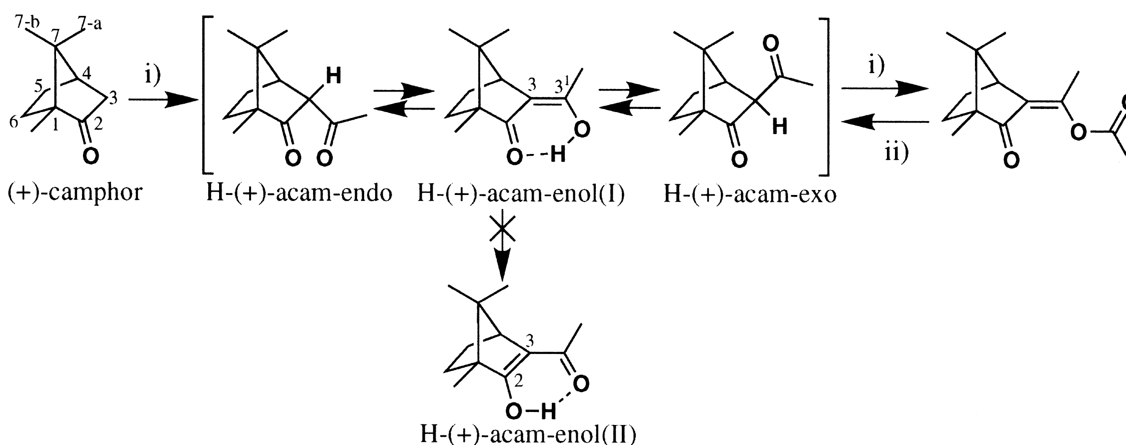
porphyrinate β-diketonates (especially, gadolinium(III) *meso*-arylporphyrinate acetylacetonate) also worked as unique host molecules for unprotected zwiterionic α-amino acids, and their supercomplexes in a water-saturated organic solvent gave intense chirality-specific CD peaks in the visible region (right drawing in Scheme 1).[4,5] Several structural modifications of the lanthanide porphyrin complexes successfully offered specific recognition of some α-amino acid related polyions.[6] But there have been no systematic investigations of how molecular structures of β-diketonates as axial ligands affect the supercomplexation and subsequent sensing properties.

Here, we prepared a series of gadolinium(III) *meso*-arylporphyrinates possessing various chiral and achiral β-diketonates. Their supercomplexation properties with α-amino acids and dipeptides are characterized by liquid–liquid extraction experiments between an organic solvent and water. Since the resulting supercomplexes in an organic phase exhibited the induced CD spectra, their applications in the chirality sensing process[7] were investigated. The present type of gadolinium(III)porphyrins was confirmed as efficient CD receptors in chirality sensing and recognition of amino acid and dipeptide substrates.

2. Results and discussion

2.1. Preparation of gadolinium(III)porphyrins possessing various β-diketonates as axial ligands

According to reported procedures,[5] gadolinium(III) 5,10,15,20-tetrakis(3,5-di-*tert*-butylphenyl)porphyrinate β-diketonates (GdPX, see Scheme 2) were synthesized by refluxing 1,2,4-trichlorobenzene solution of the metal-free *meso*-tetraarylporphyrin (H_2P) and gadolinium tris(β-diketonates) (GdX_3) prepared from gadolinium nitrate hexahydrate and corresponding β-diketone in the presence of sodium hydroxide. As symmetrical (R^1=R^3) and achiral β-diketones (H–X), 2,4-pentanedione (acetylacetone, H-acac), 3-methyl-2,4-pentanedione (methylacetylacetone, H-macac) and 2,2,6,6-tetramethyl-3,5-heptanedione (dipivaloylmethane, H-dpm) were used, while 3-acetyl-(+)- and (−)-camphors (H-(+)/(−)-acam) were used as unsymmetrical ($R^1 \neq R^3$) and chiral β-diketones. The chiral camphor-derived β-diketones were prepared as shown in Scheme 3. (1*R*)-(+)-Camphor was anionized by 2 equiv. of sodium hydride and then acetylated with excess acetyl chloride.[8] The reaction mixture containing diacetylated product was saponified and purified by extraction and vacuum distillation to give pure H-(+)-acam. In $CDCl_3$,



Scheme 3. Synthesis of 3-acetyl-(+)-camphor (H-(+)-acam); (i) NaH/$MeOCH_2CH_2OMe$, MeCOCl; (ii) aq. KOH/MeOH.

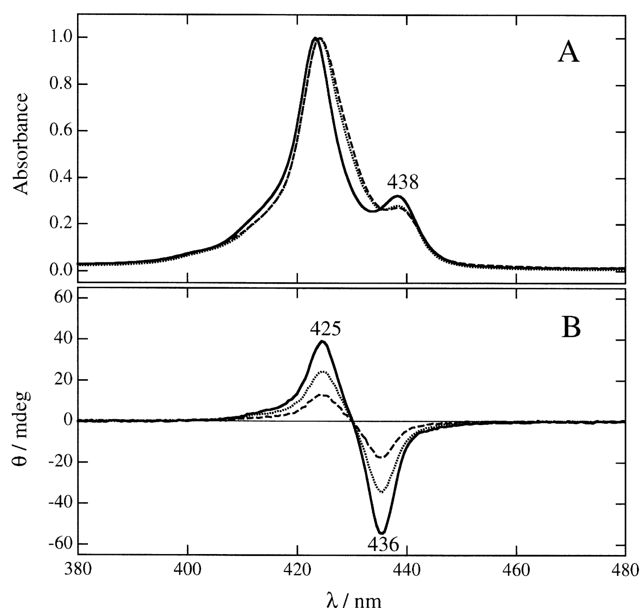


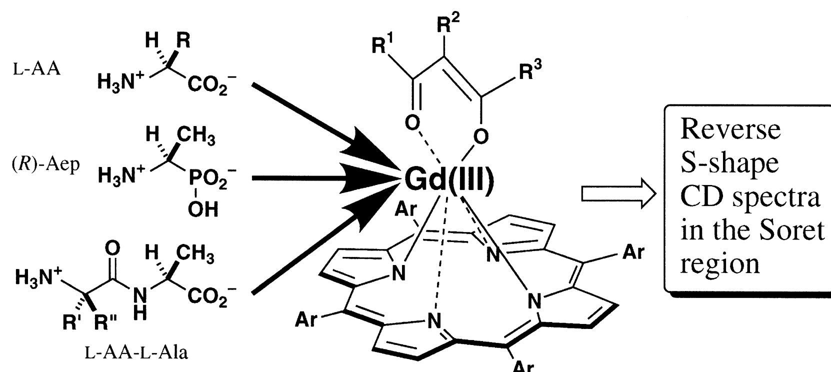
Figure 1. Visible (A) and CD spectra (B) of GdPX in benzene (10 mL, 1.6×10^{-5} M) after stirring with an aqueous solution of L-Pgl (0.6 mL, 2.7×10^{-4} M); X=acac (—), macac (···) and dpm (- - -).

H-(+)-acam was an equilibrium mixture (30:32:38) of endo, enol(I) and exo forms (Scheme 3).[9] Their stereochemical structures were confirmed by 1D/2D-^{1}H/^{13}C NMR and FT-IR spectral analyses (see Section 4.2.1). Especially, the molecular structure of the enol form was determined by long-range correlation of several ^{1}H and ^{13}C nuclei to be the (I)-type possessing a double bond at the 3- and 3^1-positions rather than the (II)-type with C2=C3: apparent correlation between protons of the 1-methyl group and the carbonyl carbon and also between protons of the 3^1-methyl group and the ene carbons, and no correlation of 1-C*H*$_3$ with *C*=*C* and of 3^1-C*H*$_3$ with *C*=O. H-(−)-acam was similarly derived from (1*S*)-(−)-camphor.

2.2. Complexation between achiral GdPX and L-phenylglycine

When a benzene solution (10 mL) of GdP(acac) (0.16 μmol) was mixed with an aqueous solution (0.6 mL) of L-phenylglycine (L-Pgl, 0.16 μmol, 1 equiv.) and the biphasic solution was stirred at room temperature for 40 min in the dark, the upper benzene phase was red and the lower aqueous phase was colorless. From visible spectral analysis, no GdP(acac) moved to the aqueous phase, but a new absorption band appeared at 438 nm in the benzene solution (solid line of Figure 1A). Before stirring, the benzene solution gave no CD peaks because it contained no chiral components. After stirring, the benzene solution afforded a reverse S-shape CD spectrum with a negative peak at 436 nm and a positive peak at 425 nm (solid line of Figure 1B), because achiral GdP(acac) complexed 1 equiv. of chiral L-Pgl as previously reported (see R=C_6H_5 at upper part of Scheme 4).[5] HPLC analysis of the aqueous solutions before and after stirring showed that 0.13 μmol of L-Pgl was extracted from the aqueous phase to the benzene phase. Due to formation of the 1:1 supercomplex,[5] 81% of added GdP(acac) complexed L-Pgl under the employed experimental conditions.

Using GdP(macac) or GdP(dpm), similar new visible absorption bands were observed at 438 nm and CD peaks appeared in the Soret region (dotted or broken lines of Figure 1A and B). All the CD spectra induced by GdPX (Fig. 1B) offered the same reverse S-shape but the intensities (θ) decreased in the order of GdP(acac), GdP(macac) and GdP(dpm). From their HPLC analyses, 0.084 and 0.042 μmol of L-Pgl were extracted by GdP(macac) and GdP(dpm), respectively. As summarized in Table 1, extracted amounts of L-Pgl from the aqueous to benzene solutions decreased with an increase in bulkiness of the axial β-diketonate ligand: extracted amount of L-Pgl=81% (acac)>52% (macac)>26% (dpm). More steric hindrance around the gadolinium would disturb the extraction of L-Pgl through the biphasic interface to give the observed difference in the extracted amounts. In addition to the steric factor, the hydrophobicity of GdPX might be considered for explanation of the order. The CD intensity was estimated on the assumption that supercomplexation was complete. These three GdPX provided almost identical $\Delta\theta_{100\%}$ values (Table 1), indicating that induced CD spectra were not perturbed by the nature of β-diketonate ligand. An α-amino acid is believed to coordinate tightly with the gadolinium center in the CD active supercomplex, irrespective of the molecular structure of the axial β-diketonate ligand.



Scheme 4. Schematic drawing of supercomplexes of GdPX with L-α-amino acids L-AA (R=C_6H_5 for L-Pgl, R=CH_3 for L-Ala, R=$CH_2S-]_2$ for L-cystine, upper), (*R*)-Aep (middle) and dipeptides AA-L-Ala (R′=CH_3/R″=H for AA=L-Ala, R′=H/R″=CH_3 for AA=D-Ala, R′=R″=H for AA=Gly, lower) to give reverse S-shape CD spectra in the Soret region.

Table 1. CD intensity (θ) in benzene solution and amount of L-Pgl extracted to benzene phase[a]

	GdP(acac)	GdP(macac)	GdP(dpm)
Observed $\Delta\theta$ (mdeg)[b]	93	59	30
Extracted amount (μmol)[c]	0.13	0.084	0.042
Extracted amount (%)	81	52	26
Expected $\Delta\theta_{100\%}$[d]	115	114	115
$\Delta\Delta\varepsilon$ (M^{-1} cm^{-1})[e]	2200	2200	2200

[a] GdPX (0.16 μmol) in C_6H_6 (10 mL), L-Pgl (0.16 μmol) in H_2O (0.6 mL), stirring at room temperature for 40 min in the dark.
[b] $\Delta\theta=\theta$(at 425 nm)$-\theta$(at 436 nm), 1-mm cell.
[c] Determined by HPLC (see Section 4.3).
[d] $\Delta\theta_{100\%}=\Delta\theta$/extracted amount (%).
[e] Molar circular dichroism $\Delta\varepsilon$ in 1:1 supercomplex of GdPX with L-Pgl was calculated from expected $\theta_{100\%}$ and $\Delta\Delta\varepsilon=\Delta\varepsilon$(at 425 nm)$-\Delta\varepsilon$(at 436 nm).

2.3. Complexation between achiral GdP(acac) and chiral amino acids and oligoalanines

Under the conditions described in Section 2.2, GdP(acac) in benzene extracted L-alanine (L-Ala) from the aqueous phase to give a CD active supercomplex with the same reverse S-shape spectrum (solid line of Figure 2) (see R=CH_3 at upper part of Scheme 4), while D-Ala offered a mirror image CD spectrum with an S-type shape. The CD spectra in the visible region were sensitive to the stereochemistry of α-amino acids. GdP(acac) also complexed an α-amino phosphoric acid as well as an α-amino carboxylic acid to form similar CD active supercomplexes. Using an aqueous solution of (*R*)-1-aminoethylphosphoric acid (Aep), the CD spectrum had a similar reverse S-shape to that as in L-Ala which had the same stereo-configuration as (*R*)-Aep (see middle part of Scheme 4), though the peak positions moved to slightly shorter wavelengths than those as in L-Ala and the intensity was almost the same (broken line of Figure 2). Since the supercomplex of GdP(acac) with (*S*)-Aep gave an S-shape CD spectrum, CD chirality sensing with gadolinium(III)porphyrin was demonstrated in the cases of not only amino acids but also amino phosphoric acids.

When cystine possessing two amino acid moieties in a molecule was employed as a guest, the benzene solution after stirring gave a peak at 438 nm in the visible spectrum (Fig. 3A). The solution showed the chirality specific CD spectrum after extraction; reverse S-shape for L-cystine and S-shape for D-cystine (Fig. 3B) as observed with many α-amino acids.[4,5] The extracted amount of L-cystine from aqueous to benzene phases increased with an increase in the initial amount of L-cystine in water and reached a maximum at the half molar equivalent to GdP(acac) as shown in Figure 4A. The dependency of the CD intensity ($\Delta\theta=\theta$(at 425 nm)$-\theta$(at 436 nm)) with the initial amount of L-cystine (Fig. 4B) also indicated that a half mole of L-cystine to GdP(acac) was enough to form a CD active supercomplex in 100% yield. The observations clearly indicate that GdP(acac) formed supercomplexes with cystine having 2:1 (porphyrin–cystine) stoichiometry, and also that two porphyrin chromophores did not interact with each other in a supercomplex.

L-Alanyl-L-alanine (L-Ala-L-Ala) afforded the same reverse S-shape CD spectrum as L-Ala (see R′=CH_3/R″=H at lower part of Scheme 4), but its intensity was lower; $\Delta\theta$=14.7→5.5 mdeg. Tripeptide $(\text{L-Ala})_3$ gave an even smaller reverse S-shape CD spectrum, while no obvious CD

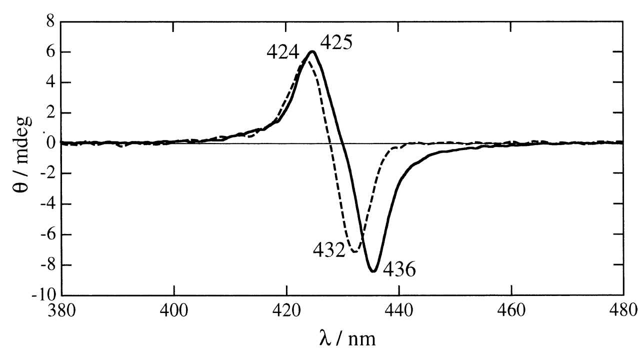


Figure 2. CD spectra of GdP(acac) in benzene (10 mL, 1.6×10^{-5} M) after stirring with an aqueous solution (0.6 mL, 2.7×10^{-4} M) of L-Ala (—) and (*R*)-Aep (- - -).

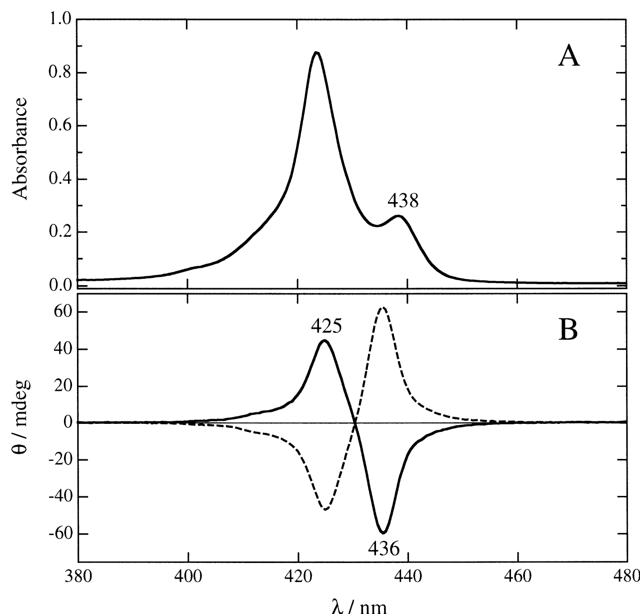


Figure 3. Visible (A) and CD spectra (B) of GdP(acac) in benzene (10 mL, 1.6×10^{-5} M) after stirring with an aqueous solution (0.6 mL, 2.7×10^{-4} M) of L-cystine (—) and D-cystine (- - -).

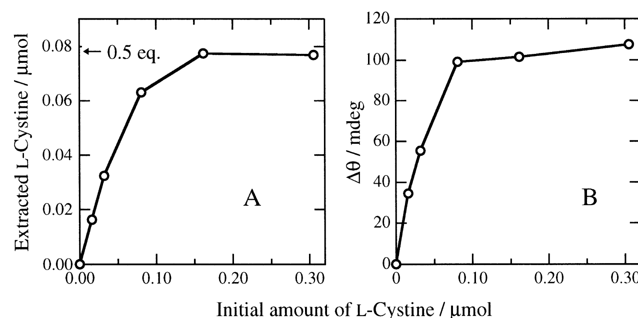


Figure 4. Dependency (A) of extracted L-cystine into benzene solution (10 mL) of GdP(acac) (0.16 μmol) upon the initial amount of L-Pgl in aqueous solution (0.6 mL). Dependency (B) of the induced CD intensity $\Delta\theta$ ($=\theta$(at 425 nm)$-\theta$(at 436 nm)) of benzene solution (GdP(acac), 0.16 μmol/10 mL) upon the initial amount of L-Pgl in aqueous solution (0.6 mL).

peaks appeared in (L-Ala)$_4$ or (L-Ala)$_5$. Therefore, the distance between amino and carboxy groups in a molecule is important in CD active supercomplexation with GdP(acac). Since its enantiomeric D-Ala-D-Ala gave an S-shape CD spectrum, the chirality of peptides as well as α-amino acids can be sensed by GdP(acac). Heterochiral dipeptides, L-Ala-D-Ala and D-Ala-L-Ala yielded S- and reverse S-shape CD spectra, respectively. D/L-Ala-L-Ala, Gly-L-Ala and L-Ala afforded reverse S-shape CD spectra (see Scheme 4), though D/L-Ala-D-Ala and D-Ala showed the mirror image S-shape. Therefore, the sign of the observed CD signal was determined by the stereochemistry of the C-terminal component in the dipeptides. The carboxy group perhaps strongly coordinate with the gadolinium center and thus the chirality of the α-position neighbor to the carboxy group largely affected the CD spectral shape. The amino group on the other terminal would be fixed around the gadolinium center to form the CD active supercomplex.[10] Considering that N^{α}-acetyl-L-ornithine (MeCONH–CH((CH_2)$_3$$NH_2$)–$CO_2$H) gave no obvious CD peaks, the amido group of the peptide guests provides weak coordination in the supercomplex.

2.4. Complexation between chiral GdP(acam) and chiral H-(Ala)$_n$-OH (n=1 or 2)

After an aqueous solution (2.0 mL) of L-Ala (1.7 μmol, 10 equiv.) was shaken with a dichloromethane solution (10 mL) of chiral GdP((+)-acam) (0.17 μmol) at room temperature for 1 h in the dark, the dichloromethane phase showed a new absorption shoulder band at 438 nm (Fig. 5A) as well as a reverse S-shape CD spectrum with a negative peak at 435 nm and a positive peak at 424 nm (solid line of Figure 5B). Since D-Ala gave the complete mirror image S-shaped spectrum (broken line of Figure 5B), no diastereomeric control operated in the present supercomplexation. GdP((−)-acam) was also examined. This

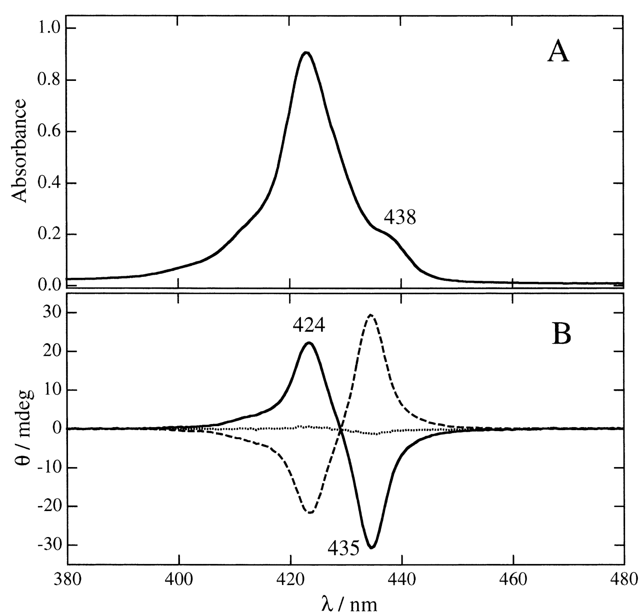


Figure 5. Visible (A) and CD spectra (B) of GdP((+)-acam) in dichloromethane (10 mL, 1.7×10^{-5} M) after stirring with an aqueous solution (2.0 mL, 8.5×10^{-4} M) of L-Ala (—) and D-Ala (- - -). The dotted line (···) was calculated by adding the solid (—) and broken lines (- - -).

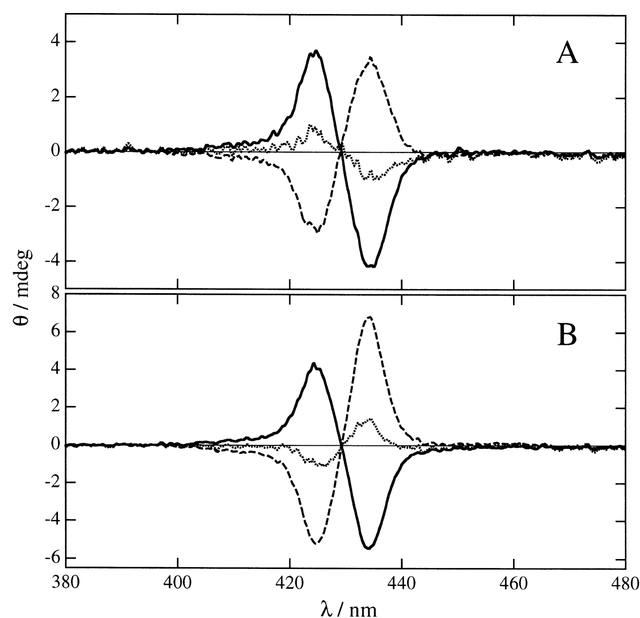


Figure 6. Corrected CD spectra of GdP((+)-acam) (A) and GdP((−)-acam) (B) in dichloromethane (10 mL, 1.7×10^{-5} M) after stirring with an aqueous solution (2.0 mL, 8.5×10^{-4} M) of L-Ala-L-Ala (—) and D-Ala-D-Ala (- - -). The dotted line (···) was calculated by adding the solid (—) and broken lines (- - -).

complex offered S- and reverse S-shaped CD spectra for D- and L-Ala, respectively and both were complete mirror images. These results were consistent with the above observation that no axial β-diketonate ligands affected the CD spectra induced by supercomplexation between achiral GdPX and L-Pgl.

Chiral gadolinium(III)porphyrins offered chiral differentiation for a series of Ala-Ala stereoisomers, though these dipeptides induced smaller CD spectra than did α-amino acids (vide supra). As shown by the solid line of Figure 6A, GdP((+)-acam) complexed L-Ala-L-Ala to give a reverse S-shape CD spectrum (θ(at 424 nm)−θ(at 435 nm)=8.3 mdeg). Its supercomplex with D-Ala-D-Ala yielded an S-shape CD spectrum (broken line of Figure 6A), but the CD intensity was lower (θ(at 424 nm)−θ(at 435 nm)=−6.6 mdeg). Addition of these two spectra gave a reverse S-shape spectrum as shown by the dotted line of Figure 6A, indicating that the supercomplex of GdP((+)-acam) with L-Ala-L-Ala had a larger CD intensity than that with D-Ala-D-Ala. GdP((−)-acam) offered a more intense CD spectrum upon supercomplexation with D-Ala-D-Ala than that with L-Ala-L-Ala (Fig. 6B). The corresponding addition spectrum (dotted line of Figure 6B) also gave an S-shape type. When D-Ala-L-Ala and L-Ala-D-Ala were compared, GdP((+)-acam) offered higher CD intensity for D-Ala-L-Ala than for L-Ala-D-Ala, and GdP((−)-acam) offered lower intensity for the former DL-form than for the latter LD-form. Therefore, diastereomeric control was realized in the supercomplexation between chiral GdP(acam) and chiral Ala-Ala.

3. Conclusion

We prepared a new series of gadolinium(III)porphyrins in

which various β-diketonate ligands were introduced. They acted as efficient CD receptors in chirality sensing of amino acids. The stereochemistry of the C-terminal amino acid residues of the dipeptides was well sensed. Since chiral gadolinium porphyrins also offered chiral differentiation of dipeptides, this type of gadolinium complexes has wide application in sensing of biological substrates.

4. Experimental

4.1. Apparatus

Visible absorption spectra were measured with a Hitachi U-3500 spectrophotometer. CD spectra were recorded with a Jasco J-720W spectropolarimeter. ^{1}H and ^{13}C NMR spectra including H/H-COSY, H/H-NOESY, H/C-COSY and long-range H/C-COSY were measured with a Bruker AC-300 or JEOL JNM-A400 spectrometer; as an internal reference, $CHCl_3$ was used for ^{1}H NMR (δ_H=7.26 ppm) and $CDCl_3$ was used for ^{13}C NMR (δ_C=77.0 ppm). FAB-MS spectra were measured with a JEOL Gcmate II or JEOL HX-100 spectrometer; FAB-MS samples were dissolved in CH_2Cl_2 or $CHCl_3$ and *m*-nitrobenzyl alcohol or dithranol was used as the matrix. FT-IR spectra were measured with a Shimadzu FTIR-8600. HPLC analysis was performed with a Shimadzu LC-10AD liquid chromatograph and SPD-M10A diode-array detector.

4.2. Materials

Organic solvents for visible and CD spectroscopic studies were purchased from Nacalai Tesque (Grade for UV-spectroscopy). $Gd(acac)_3$ and $Gd(dpm)_3$ were purchased from Strem Chemicals. The other GdX_3 were prepared by slight modification of reported procedures[11] as described below. All the GdPX were prepared according to reported procedures[5,12] and the details are described below. α-Amino acids, protected amino acids and oligoalanines were purchased from Nacalai Tesque, Watanabe Chemical Ind. and Bachem, respectively, and used without further purification.

4.2.1. Synthesis of 3-acetylcamphor (H-acam). A 1,2-dimethoxyethane (DME) solution (30 mL) of (+)-camphor (4.80 g; 31.5 mmol, Nacalai Tesque) and NaH (3.0 g; 63 mmol, 50% in paraffin) was refluxed for 2 h under N_2 and a DME solution (20 mL) of freshly distilled acetyl chloride (9.36 mL; 132 mmol) was added over a period of 2 h. Hydrogen gas evolved during addition of acetyl chloride. After refluxing for 2 h, the reaction mixture was cooled and an excess amount of ethanol was added to consume the remaining NaH. The reaction mixture was poured into 2% aqueous HCl and extracted with diethyl ether several times. The combined ether extracts were washed with 4% aqueous $NaHCO_3$ and water, dried over Na_2SO_4, and concentrated in vacuo to give a yellow oily residue. The crude products were used in the following hydrolysis. The residue was purified with flash column chromatography over silica gel (50% diethyl ether/hexane) to give pure 3^1-acetate ester of H-(+)-acam-enol(I); yellowish oil; ^{1}H NMR ($CDCl_3$) δ 2.58 (1H, d, J=4 Hz, 4-H), 2.17 (3H, s, 3^1-$OCOCH_3$), 2.01–1.94 (1H, m, 5-H-exo), 1.90 (3H, s, 3^1-CH_3), 1.65–1.58 (1H, m, 6-H-exo), 1.46–1.40 (1H, m, 5-H-endo), 1.40–1.33 (1H, m, 6-H-endo), 0.89 (3H, s, 7-CH_3-b), 0.87 (3H, s, 1-CH_3), 0.81 (3H, s, 7-CH_3-a); ^{13}C NMR ($CDCl_3$) δ 204.3 (C^2O), 168.3 (3^1-OC), 146.7 (3-CO), 129.2 (C^3), 58.3, 46.6 (C^1, C^7), 48.6 (C^4), 29.9, 26.2 (5-, 6-C), 20.8, 20.2, 18.5, 18.4, 9.1 (1-, 3^1-, 3^3-C, 7-C-a/b).

All the above enol acetate was dissolved in an aqueous 1 M KOH solution (10 mL) and methanol (30 mL). After stirring for 3 h at room temperature under N_2, hexane was added and separated from an alkaline aqueous solution containing desired β-diketonate. The aqueous alkaline phase was acidified with concentrated HCl, and extracted with diethyl ether several times. The combined ether extracts were washed with water, dried over Na_2SO_4 and concentrated. The residue was purified by vacuum distillation to give H-(+)-acam (0.91 g; 4.7 mmol) in a yield of 15%; colorless oil (turning yellow in air); bp 114–116°C/11 mm Hg. In a $CDCl_3$ solution at 293 K, H-(+)-acam was a 30:32:38 equilibrium mixture of H-(+)-acam-endo, H-(+)-acam-enol(I) and H-(+)-acam-exo. By use of (−)-camphor (Nacalai Tesque) as a starting material, H-(−)-acam was obtained in a 14% yield. H-(+)-acam-endo: ^{1}H NMR ($CDCl_3$) δ 3.33 (1H, dd, $^3J_{4\text{-H}}$=4 Hz, $^4J_{5\text{-H-exo}}$=2 Hz, 3-H), 2.43 (1H, t, $^3J_{3\text{-H},5\text{-H-exo}}$=4 Hz, 4-H), 2.23 (3H, s, 3-$COCH_3$), 1.86–1.79 (1H, m, 5-H-exo), 1.73–1.63 (1H, m, 6-H-exo), 1.53–1.26 (2H, m, 5- and 6-H-endo), 0.98 (3H, s, 7-CH_3-b), 0.89 (3H, s, 1-CH_3), 0.86 (3H, s, 7-CH_3-a); ^{13}C NMR ($CDCl_3$) δ 212.4 (C^2O), 204.3 (3-CO); IR ($CDCl_3$) 1746 (C^2=O), 1686 cm^{-1} (C=O of Ac). H-(+)-acam-enol(I): ^{1}H NMR ($CDCl_3$) δ 2.48 (1H, d, $^3J_{5\text{-H-exo}}$=4 Hz, 4-H), 2.02–1.94 (1H, m, 5-H-exo), 1.89 (3H, s, 3^1-CH_3), 1.73–1.63 (1H, m, 6-H-exo), 1.53–1.26 (2H, m, 5- and 6-H-endo), 0.94 (3H, s, 1-CH_3), 0.90 (3H, s, 7-CH_3-b), 0.80 (3H, s, 7-CH_3-a); ^{13}C NMR ($CDCl_3$) δ 211.1 (C^2O), 163.8 (3-CO), 115.6 (C^3); IR ($CDCl_3$) 1703 (C^2=O), 1620 cm^{-1} (C^3=C). H-(+)-acam-exo: ^{1}H NMR ($CDCl_3$) δ 2.87 (1H, s, 3-H), 2.69 (1H, d, $^3J_{5\text{-H-exo}}$=4 Hz, 4-H), 2.32 (3H, s, 3-$COCH_3$), 2.02–1.94 (1H, m, 5-H-exo), 1.73–1.63 (1H, m, 6-H-exo), 1.53–1.26 (2H, m, 5- and 6-H-endo), 0.94 (3H, s, 7-CH_3-b), 0.91 (3H, s, 1-CH_3), 0.59 (3H, s, 7-CH_3-a); ^{13}C NMR ($CDCl_3$) δ 212.4 (C^2O), 200.5 (3-CO); IR ($CDCl_3$) 1746 (C^2=O), 1686 cm^{-1} (C=O of Ac).

4.2.2. Synthesis of gadolinium tris(β-diketonate) (GdX_3). A 95% ethanol solution (9 mL) of β-diketone (H–X, 18 mmol) was stirred, and an aqueous 50% ethanol solution (15 mL) of NaOH (720 mg; 18 mmol) was added. The solution changed from colorless to yellow upon addition of NaOH. An aqueous 50% ethanol solution (15 mL) of gadolinium nitrate hexahydrate (2.05 g; 6.0 mmol, Strem Chemicals) was added and the mixture was stirred for 2–3 h under the reduced pressure using a vacuum pump. When the volume of the solution was reduced by 50%, the flask was opened and distilled water (100 mL) was added with vigorous stirring. The amount of white solids that began to precipitate during the vacuum removal of solvents further increased by the addition of distilled water. The resulting precipitates were separated by vacuum filtration and recrystallized from hot hexane to give desired GdX_3 as cream-colored powder (mp >300°C).

$Gd(macac)_3$ was prepared from H-macac (Tokyo Chemical Industry) in a yield of 42% and $Gd((+/-)\text{-acam})_3$ were derived from H-(+/−)-acam prepared above in yields of 31/27%, respectively. $Gd((+)\text{-acam})_3$: VIS (CH_2Cl_2) λ_{max} 314, ca. 330 nm (sh); CD (CH_2Cl_2) 288 (rel. +0.36), 305 (−0.10), 325 (+1.00), 344 nm (−0.08). $Gd((-)\text{-acam})_3$: VIS (CH_2Cl_2) λ_{max} 314, ca. 330 nm (sh); CD (CH_2Cl_2) 288 (rel. −0.36), 305 (+0.10), 325 (−1.00), 344 nm (+0.08).

4.2.3. Synthesis of gadolinium 5,10,15,20-tetrakis(3,5-di-*tert*-butylphenyl)porphyrinate β-diketonate (GdPX). A 1,2,4-trichlorobenzene solution (10 mL) of H_2P[5] (106 mg; 0.10 mmol) and gadolinium tris(β-diketonate) (GdX_3, 0.26 mmol) was refluxed with stirring for several hours under N_2. The reaction was stopped just after disappearance of 70–80% of the visible Q-peaks of H_2P at Abs(516 nm)> Abs(560)>Abs(650)~Abs(590). The solvent was evaporated in vacuo, and the residue was purified with aluminum oxide column chromatography as follows. Unreacted H_2P and GdX_3 were removed with toluene. The remaining H_2P, a trace of GdPX, and toluene were then eluted with acetone. Finally, GdPX was eluted by dimethyl sulfoxide (DMSO). To the DMSO fraction was added freshly distilled chloroform (previously treated with a 0.1 M NaOH solution for removal of acid) and washed with water several times. After evaporation, the residue was recrystallized from dichloromethane and methanol to give pure GdPX as dark purple powder.

GdP(acac)[5] was prepared from $Gd(acac)_3$ after reflux for 2 h in 27% yield; mp >300°C; visible (C_6H_6) λ_{max} 422 (rel., 1.000), 556 (0.046), 594 nm (0.017); MS (FAB) found: *m/z* 1219. Calcd for $C_{76}H_{92}N_4^{158}Gd$: $[M-acac]^+$, 1219.

GdP(macac) was prepared from $Gd(macac)_3$ after reflux for 6 h in 11% yield; mp >300°C; visible (C_6H_6) λ_{max} 424 (rel., 1.000), 556 (0.053), 594 nm (0.021); MS (FAB) found: *m/z* 1219. Calcd for $C_{76}H_{92}N_4^{158}Gd$: $[M-macac]^+$, 1219.

GdP(dpm) was prepared from $Gd(dpm)_3$ after reflux for 3 h in 20% yield; mp 291–292°C; visible (C_6H_6) λ_{max} 428 (rel., 1.000), 557 (0.049), 596 nm (0.017); (CH_2Cl_2) λ_{max} 426 (rel., 1.000), 556 (0.043), 593 nm (0.016); MS (FAB) found: *m/z* 1219. Calcd for $C_{76}H_{92}N_4^{158}Gd$: $[M-dpm]^+$, 1219.

GdP((+)-acam) was prepared from $Gd((+)\text{-acam})_3$ after reflux for 3 h in 31% yield; mp >300°C; visible (C_6H_6) λ_{max} 423 (rel., 1.000), 558 (0.054), 596 nm (0.022); MS (FAB) found: *m/z* 1219. Calcd for $C_{76}H_{92}N_4^{158}Gd$: $[M-acam]^+$, 1219.

GdP((−)-acam) was prepared from $Gd((-)\text{-acam})_3$ after reflux for 3 h in 30% yield; mp >300°C; visible (C_6H_6) λ_{max} 423 (rel., 1.000), 558 (0.054), 596 nm (0.022); MS (FAB) found: *m/z* 1219. Calcd for $C_{76}H_{92}N_4^{158}Gd$: $[M-acam]^+$, 1219.

4.3. HPLC analysis of amino acids in an aqueous phase

L-Pgl was analyzed by HPLC (SUMICHIRAL OA-6100, 4.6ϕ×250 mm, Sumika Chemical Analysis Service) with 2 mM $CuSO_4$ in H_2O/CH_3CN=95/5 at the elution rate of 1.0 mL/min. The amount of L-cystine was determined from HPLC analysis (SUMICHIRAL OA-5000 (4.6ϕ×250 mm), 2 mM $CuSO_4$ in H_2O/CH_3CN=90/10 and 2.0 mL/min).

4.4. Correction of CD spectra induced by chiral dipeptides

Small ghost peaks which were observed in the Soret region due to a mechanical noise in the present CD measurements were relatively pronounced in the case of dipeptides and were eliminated as follows. From the CD spectra induced by complexation of GdP(acam) with chiral Ala-Ala was subtracted the CD spectrum induced by achiral GdP(acac) and a 1:1 enantiomeric mixture of L-Ala-L-Ala and D-Ala-D-Ala.

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