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Development of Soft-Based Double-Stranded Peptide Chelators which Selectively Separate Europium and Lanthanum Ions Based on the Hardness Concept

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New double-stranded peptide chelators (1) conjugated Cat (2,3-dihydroxybenzoic acid) were synthesized and formed a molecular complex 1-Eu^{3+} (or 1-Lu^{3+}) with Eu^{3+} and Lu^{3+} but not La^{3+}. The double-stranded peptide chelator may prove to be useful tools for studying the selective separation of lanthanide ions.

Key words double-stranded peptide; soft-based chelator; hardness concept; lanthanide ion; binding constant

Many studies have described the determination of luminescence, radiotherapeutic applications, and development of chelators for lanthanide ions (Ln^{3+}) in the past several years.[1—3] In the application of lanthanide ions for photometry, fluorometry and bioassay,[4,5] the development of selective chelators and probes for lanthanide ions is important. The electronic configurations of lanthanide ions have 4f electrons, for instance Eu^{3+} and Lu^{3+} have 7 and 14 4f electrons, respectively, except for Y^{3+} (4f=0) and La^{3+} (4f=0). However, information about the development of the chelators which selectively form the complexes with Y^{3+}, La^{3+}, Eu^{3+}, and Lu^{3+} ions is lacking since lanthanide ions have extremely similar chemical properties. Here, we used La (4f=0), Eu (4f=7), and Lu (4f=14) to study in order to show clearly whether the ability of complexation changes with the number of 4f electrons. Although 18-crown-6 ether (**1**),[6,7] ethylenediaminetetraacetic acid (EDTA) (**2**),[8] and calix[6]arene (**3**)[9—11] have often been used as chelators for the complexation of lanthanide ions, these chelators form the complexes with La^{3+}, Eu^{3+} and Lu^{3+} ions and have no selectivity for selective complexation of their ions. Our aims in this study are the molecular design and synthesis of novel chelators for the highly selective recognition of Y^{3+}, La^{3+}, Eu^{3+} and Lu^{3+} corresponding to the number of 4f electrons, 0, 0, 7, and 14, respectively.

To achieve the molecular design and the synthesis of functional chelators which selectively bind with Y^{3+}, La^{3+}, Eu^{3+} or Lu^{3+} ions, we synthesized target double-stranded amino acid-Cat(OH) (**8**) and double-stranded peptide-Cat(OH) (**9**) by conjugation with two 2,3-dihydroxybenzoic acid (Cat, **5**) at N-terminus of the double-stranded peptide (or amino acid) conjugated with two -Ile-Phe- or -Phe- residues to a spacer.[12,13] In designing the functional chelators we have designed them using absolute hardness (η) and absolute electronegativity (χ) based on the hardness concept.[14—17] The values of χ and η were calculated by Eqs. 1 and 2.[14,15]

$$\chi = -\mu = -(\partial E/\partial N)_{\upsilon(r)} = (I_p + E_a)/2 \qquad (1)$$

$$\eta = 1/2(\partial \mu/\partial N)_{\upsilon(r)} = 1/2(\partial^2 E/\partial N^2)_{\upsilon(r)} = (I_p - E_a)/2 \qquad (2)$$

Where E is the electronic energy of a molecule or a atom, N is the number of electrons, and $\upsilon(r)$ is the external electrostatic potential. The I_p and E_a are the ionization energy and the electron affinity (eV), respectively. To analyze the electron structure of lanthanide ions and chelators, the coordinate of the electron structure was defined as $\mathbf{r}(\chi, \eta) = \mathbf{r}(-(\partial E/\partial N)_{\upsilon(r)}, 1/2(\partial^2 E/\partial N^2)_{\upsilon(r)})$. We used χ, η, and the diagram $\mathbf{r}(\chi, \eta)$ in the development of chelators. The coordinate diagram $\mathbf{r}(\chi, \eta)$ of electronic structure showed that Ce^{3+}, Pr^{3+}, Sm^{3+}, Eu^{3+}, and Tb^{3+} groups are chemically softer than Sc^{3+}, Y^{3+}, La^{3+}, Pm^{3+}, Gd^{3+}, and Dy^{3+}–Lu^{3+} groups (Fig. 1). Absolute electronegativity and hardness values of lanthanide ions (Ln^{3+}) were calculated from the Eq. 3 using date from lit.[18]

$$\mathrm{Ln}^{3+} : \mathrm{Ln}^{3+}(E_a) \rightarrow \mathrm{Ln}^{4+}(I_p) + e^- \qquad (3)$$

For instance, the $\mathbf{r}(\chi, \eta)$ values of Eu^{3+} and La^{3+} are $\mathbf{r}(33.809, 8.893)$ and $\mathbf{r}(40.561, 20.040)$, respectively.

As the soft-based chelator is favorable to bind the soft-acid metals M^{n+} ion according to the hardness concept, we synthesized and chose chelators **8** ($\mathbf{r}(3.015, 5.635)$) and **9** ($\mathbf{r}(2.810, 5.500)$)[19] as soft-based chelators which are a softer chelator than **1** ($\mathbf{r}(2.495, 8.345)$), **2** ($\mathbf{r}(3.180, 7.500)$), and **3** ($\mathbf{r}(2.370, 5.910)$). Because, the s (0.177) of **8** is smaller than that of **1** (0.120), **2** (0.133), and **3** (0.169) since the reciprocal of the η is the global softness (s); $s=1/\eta$ (eV). The s is a measure of chemical softness.

The target chelators were synthesized as shown in Fig. 2. Removal of the Boc groups of Boc-protected bis(L-Phe)-1,4-diaminodimethylbenzene (**4**) by trifluoroacetic acid (TFA) afforded the free compound (**6**) in good yield.[13] Conjugation of 2,3-bis(benzyloxy)benzoic acid, was prepared from 2,3-dihydroxybenzoic acid (**5**), with N-terminal of **6** afforded by treating CDI in dry CHCl$_3$. Deprotection of Bn group in **7** using 5% Pd/C–H$_2$ gave new functional double-stranded peptide-Cat(OH) chelators (**8**) (and (**9**)), in 60—65% yield. All compounds **6**, **7**, **8** and **9**, provided satisfactory analysis by

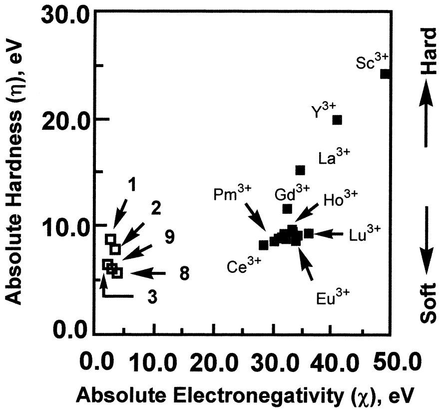

Fig. 1. Plot of a Coordinate Diagram $\mathbf{r}(\chi, \eta)$ of the Electronic Structure for Lanthanide Ions (Ln^{3+})[a] and Chelators[b]

a) The data of lanthanide ions was taken from lit.[18] *b*) At the HF/6-31G* level. *c*) Open and closed squares show the coordinate of electronic structure of chelators and lanthanide ions, respectively.

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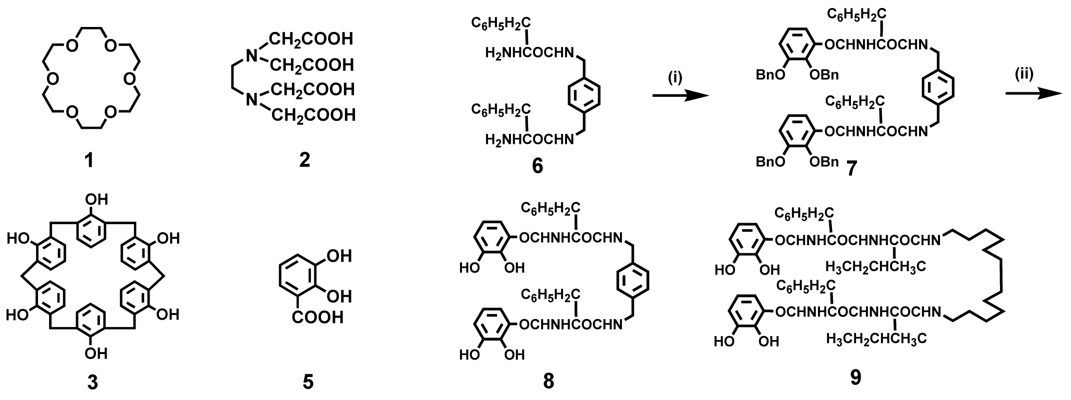

Fig. 2. Synthesis of Double-Stranded Peptide Chelators

Reagents: (i) Cat (**5**), CDI, in dry $CHCl_3$; (ii) 5%Pd–C/H_2, in MeOH.

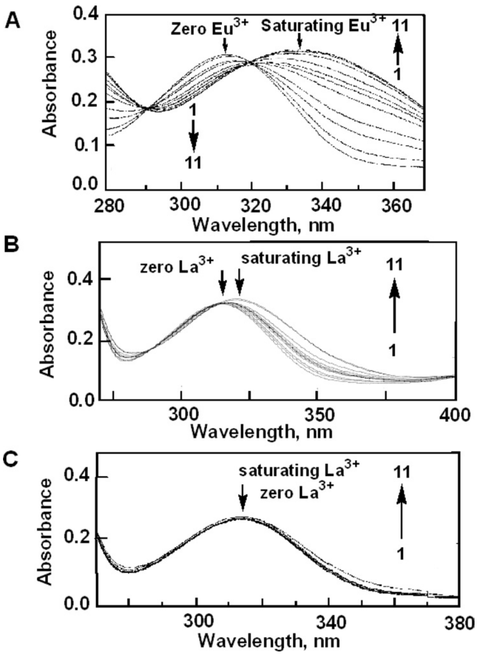

Fig. 3. UV Spectra for Double-Stranded Peptide Chelator as a Function of Eu^{3+} (A) and La^{3+} (B, C) Ions

(A) [**8**]=7.26×10^{-4} mol/l; [Eu^{3+}]=0.0, 1.0×10^{-5}, 2.0×10^{-5}, 3.0×10^{-5}, 5.0×10^{-5}, 6.0×10^{-5}, 8.0×10^{-5}, 10.0×10^{-5}, 20.0×10^{-5}, 40.0×10^{-5}, and 50.0×10^{-5} mol/l, (B) [**8**]=6.48×10^{-4} mol/l; [La^{3+}]=0.0, 1.0×10^{-5}, 2.0×10^{-5}, 3.0×10^{-5}, 5.0×10^{-5}, 6.0×10^{-5}, 8.0×10^{-5}, 10.0×10^{-5}, 20.0×10^{-5}, 40.0×10^{-5}, and 50.0×10^{-5} mol/l, and (C) [**9**]=5.74×10^{-4} mol/l; [La^{3+}]=0.0, 1.0×10^{-5}, 2.0×10^{-5}, 3.0×10^{-5}, 5.0×10^{-5}, 6.0×10^{-5}, 8.0×10^{-5}, 10.0×10^{-5}, 20.0×10^{-5}, 40.0×10^{-5}, and 50.0×10^{-5} mol/l 10 mM HEPES buffer (85% MeCN, pH=5.8).

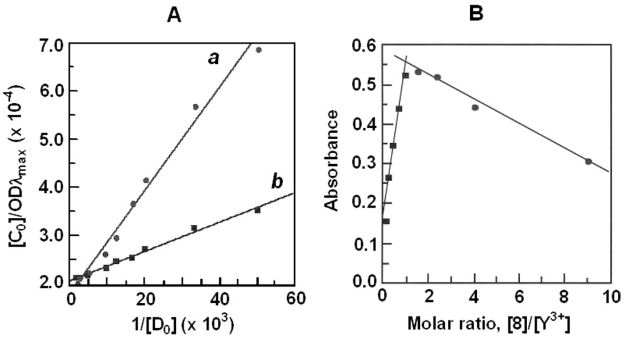

Fig. 4. Benesi–Hildebrand Plots (A) for Chelation of Double-Stranded Peptide Chelator **8** with Lanthanide Ion and Determination of 1 : 1 Complex Formation (B)

(A) *a*, [**8**]=6.13×10^{-4} mol/l. [Y^{3+}]=2.0×10^{-5}, 3.0×10^{-5}, 5.0×10^{-5}, 6.0×10^{-5}, 8.0×10^{-5}, 10.0×10^{-5}, 20.0×10^{-5}, and 40.0×10^{-5} mol/l in 10 mM HEPES 85% MeCN buffer (pH=5.8). *b*, [**8**]=7.26×10^{-4} mol/l. [Eu^{3+}]=2.0×10^{-5}, 3.0×10^{-5}, 5.0×10^{-5}, 6.0×10^{-5}, 8.0×10^{-5}, 10.0×10^{-5}, 20.0×10^{-5}, and 40.0×10^{-5} mol/l; in 10 mM HEPES 85% MeCN buffer (pH=5.8), (B) [**8**]/[Y^{3+}]=0.11, 0.25, 0.43, 0.67, 1.00, 1.50, 2.33, 4.0, and 9.0 (from left); at λ_{max}=348.0 nm; in 10 mM HEPES 85% MeCN buffer (pH=5.8).

IR, ^1H-, ^{13}C-NMR, C–H COSY NMR, and FAB-MS.[20,21]

The UV/Vis spectra of the chelators **8** and **9** were obtained in a 10 mM HEPES buffer in 85% MeCN (pH=5.8), and the first excited wavelength (λ_{max}) was 315 nm. The absorption intensity of **8** at 315 nm decreased by the addition of the Eu^{3+} ion increased from 0.0 to 5.00×10^{-4} mol/l under a fixed concentration of **8** (final concentration of 7.26×10^{-5} mol/l). It was found that the absorption intensity of a new λ_{max} at 338 nm increased by addition of Eu^{3+} ion. Figure 3A shows the result of the UV/Vis titration studies. However, the shift of λ_{max} at 315 nm was not observed in titration experiments of **8** and **9** with La^{3+} ion (Figs. 3B, C).

In the titration studies, the expression of a new λ_{max} confirms that the double-stranded peptide chelator **8** and **9** form the molecular complexes with Y^{3+}, Eu^{3+} and Lu^{3+} but not La^{3+}. In order to characterize the new complex, the binding constants (K_b), ratio ($n:m$) in composition of the complex, and the molar absorbance coefficient (ε) were obtained from the UV/vis titration data. The K_b values of the complexes, which were determined by a Benesi–Hildebrand plot using UV/Vis titration,[22] were about 10^4—10^5 $mol^{-1}\cdot l$ levels. It was found that the magnitude of K_b value increases in the following order: **8**-La^{3+} (K_b~0)≪**8**-Lu^{3+} ($K_b=7.11\times10^3$)<**8**-Y^{3+} ($K_b=1.751\times10^4$)<**8**-Eu^{3+} ($K_b=7.04\times10^4$). Although the K_b of **8** (and **9**-)-La^{3+} complexation was determined by a similar method, the slope of the Benesi–Hildebrand plot was parallel against the x-axis. From the slope, the binding constants for **8**-La^{3+} and **9**-La^{3+} are near 0. The K_b~0 supports in comparison with the results of calix[4]arene-La^{3+} complexation (slope=0). The stoichiometry for the ratio ($n:m$) of composition for **8**-Ln^{3+} complexation was determined by the molar ratio method[23] and was found to be 1 : 1 ratio since numbers n and m were $n=m=1$. It is found that the other chelators also form the complex of 1 : 1 ratio with Y^{3+}, Eu^{3+}, and Lu^{3+} ions.

Although the ionization potential (I_p) and electron affinity (E_a) values of lanthanide ions are almost the same value, the values of χ and η shown with horizontal and vertical lines respectively, greatly change in the $\mathbf{r}(\chi, \eta)$ diagram (Fig. 1). In the diagram, lanthanum (La^{3+})-pimeritium (Pm^{3+}) haven η values from 2.741 (eV) to 2.659 (eV) are chemically softer than Y^{3+}, Eu^{3+}, Yb^{3+}, and Lu^{3+}, *etc*. Moreover, the lanthanum (La^{3+}) ion which has a smaller value than χ value of Y^{3+}, Eu^{3+}, Yb^{3+}, and Lu^{3+} possesses powerful acidity since La without the f electron has the small first I_p (forth I_p too) in order not to receive lanthanide contraction.

Here, the double-stranded peptide chelators **8** and **9** are chemically softer than EDTA, Cat, and calixarene. It is expected that if the double-stranded peptide chelators **8** and **9** are interacted with Eu^{3+} and Y^{3+} ions, the binding of Eu^{3+} and Y^{3+} ions to **8** and **9** form stable **8** (and **9**)-Eu^{3+} and **8** (and **9**)-Y^{3+} complexes. However, the stabilization by the binding of chemically softer La^{3+} ion to **8** and **9** decreases by decreasing of the formation energy of **8** (and **9**)-Y^{3+} complex.

In summary, we have developed new functional double-stranded peptide chelators **8** and **9**, using the coordinate diagram $\mathbf{r}(\chi, \eta)$ of electronic structure, which have the ability to recognize Y^{3+} and Eu^{3+} but not La^{3+}. In addition, chelators **8** and **9** can expect to function as the probe or luminosensor for fluorescence since maximum excitation and emission were observed at 315 and 340 nm, respectively, to **8** and **9**. This interesting selective recognition suggests that this class of double-stranded peptide chelators offer useful tools for studying the separation and the development of new peptide chelators.

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18) The date of Lanthanide ions was taken from the WWW site ⟨http://capemn.com⟩.
19) The geometries of chelators optimized structures obtained from analysis using the "conformer distribution" were optimized with Hartree-Fock *ab initio* calculations using a 6-31G* basis set.
20) Bis((2,3-bis(benzyloxy)benzoyl)-*N*,*N*-L-phenylalanyl))-4,4-dimethylbenzene (**7**): Colorless crystals, *Rf*(A)=0.51, mp 159—162 °C; FAB-MS *m/z*: 1063 (M+H)$^+$. HR-FAB-MS *m/z*: 1063.4636 (Calcd for $C_{68}H_{63}N_4O_8$: 1063.4645). Bis((2′,3′-bis(dihydroxy)benzoyl)-*N*,*N*-L-phenylalanyl))-4,4-dimethylbenzene (**8**): Colorless crystals, mp 198—200 °C, *Rf*(A)=0.18. ^{1}H-NMR ($CDCl_3$/DMSO-d_6=0.5 : 0.2): δ 2.90—2.92 (m, 1H, –CH–), 3.09—3.14 (m, 3H, –CH–, –CH_2NH–), 4.85 (m, 1H, –CαH–), 6.66, 6.93, 7.36 (m, 3H, catecholic protons), 7.15—7.80 (m, 7H, aromatic protons), 8.05 (m, 1H, –NHaCO–) and 8.49 (d, 1H, *J*=8.0 Hz, –NHbCO–); ^{13}C-NMR ($CDCl_3$/DMSO-d_6=0.5 : 0.2) ppm 37.68, 54.83, 115.00, 117.98, 118.89, 126.33, 126.39, 128.09, 128.16, 129.19, 129.22, 137.47, 146.10, 149.35, 169.58, and 170.94; IR (KBr) 3250 and 1620 cm^{-1}; FAB-MS *m/z*: 703 (M+H)$^+$. Store at 4 °C.
21) 1,12-Dodecano-bis(2′,3′-bis(dihydroxy)benzoylamido)-L-isoleucyl-L-phenylalanine (**9**): Colorless crystals, mp 157—160 °C, *Rf*(A)=0.23. ^{1}H-NMR ($CDCl_3$/DMSO-d_6): δ 0.83—0.87 (m, 6H, CH_3×2), 1.06—1.14 (m, 1H, CH), 1.23—1.25 (m, 8H, CH_2×4), 1.42—1.52 (m, 3H, CH, CH_2), 1.78—1.81 (m, 1H, CH), 3.02—3.19 (m, 4H, CH_2, CH_2-Ph), 4.21—4.25 (dd, 1H, *J*=8.3 Hz, –CH–), 4.83—4.87 (m, 1H, –CH_2-Ph), 6.61—6.67 (m, 1H, catechol protons), 6.91—6.94 (m, 1H, catechol proton), 7.20—7.23 (m, 1H, catechol proton), 7.27—7.30 (m, 5H, aromatic protons), 7.70 (m, 1H, –NHCO–), 7.95 (d, 1H, *J*=9.2 Hz, –NHCO–), and 8.70 (d, 1H, *J*=7.3 Hz, –NHCO–); ^{13}C-NMR ($CDCl_3$/DMSO-d_6) ppm 11.09, 15.38, 24.48, 26.65, 28.94, 29.18, 37.22, 37.05, 41.4 (overlap), 54.66, 57.48, 115.01, 117.59, 117.95, 118.30, 126.23, 127.99, 129.10, 137.38, 146.01, 149.12, 169.38, and 170.66; FAB-MS *m/z*: 993 (M+H)$^+$. Store at 4 °C.
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