Design and Synthesis of Artificial Siderophores: Lysine-based Triscatecholate Ligands as a Model for Enterobactin

Masayasu Akiyama* and Tsuyoshi Ikeda
Department of Applied Chemistry, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184

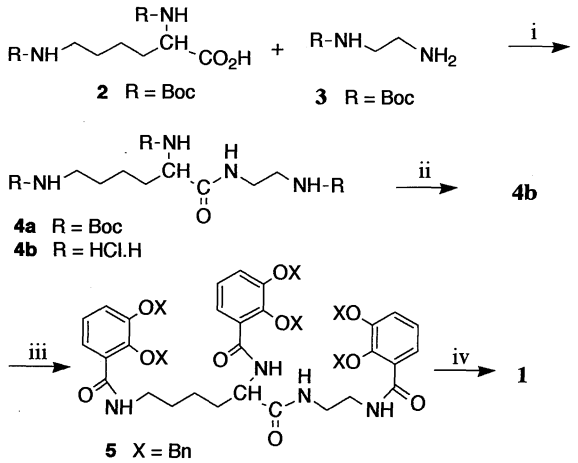
(Received June 13, 1995)

An enantiomeric pair of triscatecholate ligands have been prepared as a model for siderophore enterobactin, using protected derivatives of L- or D-lysine, 1,2-diaminoethane and 2,3-dihydroxybenzoic acid. The ligands have greater chemical stability and higher water solubility than enterobactin, and form chiral complexes with iron(III), as enterobactin does.

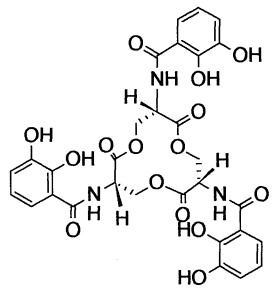
In microbial iron assimilation, powerful chelating agents called siderophores sequester iron(III) in the form of their complexes, and the complexes are subsequently transported into the cells after recognition by cell membrane receptors.[1-3] It is known that there are several factors involved in the recognition processes, including the chirality, shape, and size of siderophore iron(III) complexes. In the treatment of iron-overload diseases, there is a critical need for effective chelating agents that do not promote microbial growth.[4] Thus, considerable attention has been paid to the design and synthesis of artificial siderophores that serve as growth factors for, or have bacteriostatic effects[5] upon microorganisms.

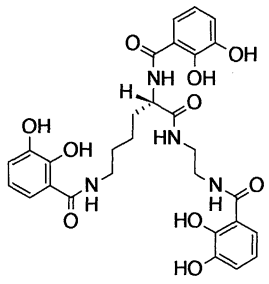
Enterobactin is a representative triscatecholate siderophore produced from *Esherichia coli* and its related enteric bacteria under iron deficient conditions.[6] It has three 2,3-dihydroxybenzoyl units appended to a cyclic L-serine triester platform, so as to form a hexadentate octahedral complex with iron(III) in the Δ-*cis* configuration. A striking feature of enterobactin is that it can compete with transferrin for iron(III) in the enteric environment by virture of its high affinity for the metal ion;[1-3] its stability constant reaching 10^{49}.[7]

To date, a number of triscatecholate derivatives have been synthesized as enterobactin models, which include analogs of a



Scheme 1. *Reagents and conditions*: i, EDC.HCl, HOBt, CH_2Cl_2, room temp, 12 h, 77%; ii, HCl in dioxane - HCO_2H, room temp, 1 h; iii, *O,O* -Dibenzyl-2,3-dihydroxybenzoic acid, EDC.HCl, HOBt, Et_3N, CH_2Cl_2 -DMF, room temp, 12 h, 95%; iv, H_2, Pd-C, MeOH, room temp, 1 h, 95%.


Enterobactin


1L

carbocycle[8] and of tris(aminomethyl)benzene (mecam)[1,2], a series of cam-derivatives such as cycam, licam, trencam and trimcam,[2] and analogs possessing chiral centers in their frameworks.[9] In particular, chiral analogs are interesting in terms of molecular recognition, since microbial growth promotion tests have shown a chiral discrimination between the natural Δ-*cis* Fe(III)-enterobactin and its synthetic counterpart; the D-serine-based enantiomeric Λ-*cis* complex showed a mismatching with the outer membrane receptor of a mutant of *E. Coli*.[10]

The synthesis of these chiral analogs required a long elaborate sequence of reactions, rendering it difficult to provide them in quantity, and yet, most of them were not close analogs in size and shape. We report here a simple synthesis of an enantiomeric pair of lysine-based triscatecholate derivatives as a model for enterobactin. As shown above, our model (**1L**) has a linear structure with a 9-atom spacing between the catecholate units, so that it can be an intimate analog of the linear enterobactin.[6a]

The synthetic procedure is shown in Scheme 1.[11] Starting with N^2,N^6-(Boc)$_2$-lysine (**2L** or **2D**), amide bond formation with Boc-NHCH$_2$CH$_2$NH$_2$ (**3**) gave the protected triamine derivative (**4**). Compound **4**, after careful removal of the Boc groups, was condensed with *O,O*-dibenzyl-2,3-dihydroxybenzoic acid by use of a carbodiimide, yielding the protected derivative (**5L** or **5D**). Hydrogenation removed the protective groups to afford the desired product (**1L** or **1D**), which was characterized by HPLC, ^{1}H NMR, IR, and elemental analysis.[12]

The ligand (**1L** or **1D**) produced an iron(III) complex in aq solution containg MeOH (1% v/v) when its MeOH solution was mixed with an equimolar amount of an aq Fe(NO$_3$)$_3$ solution and then neutralized with 0.1 M NaOH. The UV-Vis spectrum exhibited λ_{max} 495 nm and ε 5200 mol^{-1} dm^3 cm^{-1} (Figure 1), close to the values (λ_{max} 495 nm and ε 5600) of the enterobactin iron(III) complex.[2] The **1L**-iron(III) complex is present over a pH range of 7 - 11 (data not shown), showing its chemical stability and water solubility. As the pH of the solution was lowered, the spectra showed a decrease in molar absorptivity with an isosbestic point at 553 nm, representing the following protonation equilibrium (equation 1).

$$\text{Fe(III)-L}^{3-} + \text{H}^+ = \text{Fe(III)-HL}^{2-} \quad (1)$$

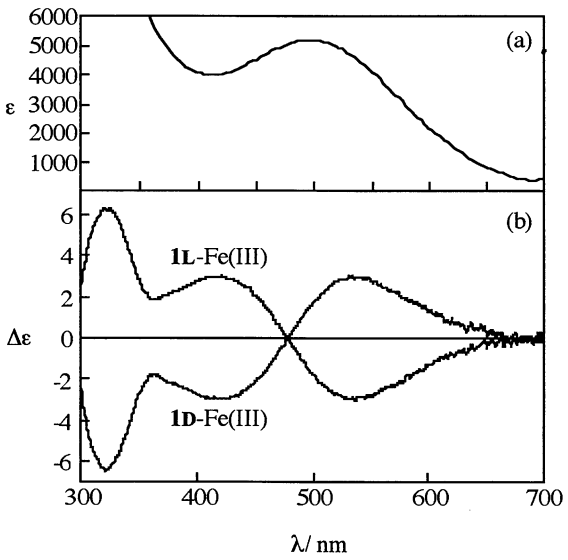


Figure 1. (a) UV-Vis spectrum of **1L**-Fe(III) complex at pH 7.8; (b) CD spectra of **1L**-Fe(III) and **1D**-Fe(III) complexes at pH 8.3. Both in water containing 1% (V/V) MeOH at 25 °C.

A Schwarzenbach plot[13] for the pH region of 7.2 - 6.0 gave a straight line with a value of $K_{Fe.HL}$=1.9 x 10^6 {$K_{Fe.HL}$ = [Fe(III)-HL]/[Fe(III)-L][H^+]}, when the data was treated as a single protonation equilibrium (data not shown). This is another piece of evidence for the formation of a 1:3 iron(III) complex, that is, one iron(III) ion to three catecholate groups.

The CD spectra of an enantiomeric pair of **1L**- and **1D**-iron(III) complexes are shown in Figure 1, which demonstrates that these complexes produce a pair of opposite configurations (Δ ε = ± 3.0 at 535 nm) around the metal ion. Examination of CPK molecular models, with reference to the literature determination,[14] allows us to assign **1L**-iron(III) and **1D**-iron(III) complexes to the Δ-*cis* and Λ-*cis* configurations, respectively. It is worthy of note that the ligand contains only one chiral center, yet this center effectively controls the three catecholate groups in their chiral coordination to the metal ion. A schematic representation for the **1L**-iron(III) complex is given in Figure 2.

In order to estimate the stability constant of the complexes, the following iron(III) exchange reaction with EDTA was carried out in water at 25 °C, pH 5.7 and ionic strength 0.1.

$$\text{Fe(III)-L}^{3-} + \text{H}_2\text{EDTA}^{2-} + 4\text{H}^+ = \text{Fe(III)-EDTA}^- + \text{H}_6\text{L} \quad (2)$$

Equilibrium data for the equation, K^* = [H_6L][Fe(III)-EDTA$^-$] / {[H^+]^4[H_2EDTA^{2-}][Fe(III)-L^{-6}]}, was obtained by considering the stoichiometry of the equation and by determining the concentration of iron(III) complexes in the solution when equilibrium was reached. Potentiometric titration of the ligand (H_6L) at 25 °C and ionic strength 0.1 gave the values of pKs, pK_4, pK_5, and pK_6 being 8.50, 7.69, and 7.10, respectively. An average value of pK_1, pK_2, and pK_3 is taken to be 12.1, as has been assumed for this type of calculation.[2] Using these pK values and a value of log K = 25.1 [13] for iron(III)-EDTA, the log stability constant of the present ligand is estimated to be 46, which is comparable with those of mecam (43), linear enterobactin (43), and (Et_3)mecam (47).[7] Thus, a straightforward synthesis of lysine-based triscatecholate ligands furnishes new types of enterobactin models, which are useful for studies of iron(III) coordination and microbial growth promotion.

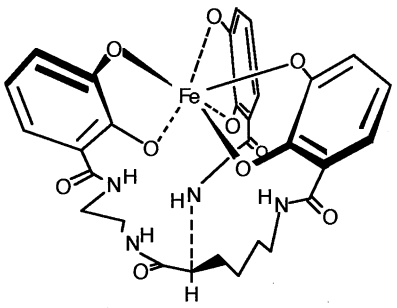


Figure 2. Schematic representation of **1L**-Fe(III) complex in the Δ-*cis* configuration.

References and Notes

1 J. B. Neilands, *Ann. Rev. Biochem.*, **50**, 715 (1981); *Struct. Bonding*, **58**, 1 (1984).

2 K. N. Raymond, G. Müller, and B. F. Matzanke, *Top. Current Chem.*, **123**, 49 (1984).

3 R. C. Hider, *Struct. Bonding*, **58**, 25 (1984).

4 "Development of Iron Chelators for Clinical Use," ed by A. E. Martel, W. F. Anderson, and D. G. Badman, Elsevier North Holland, New York (1981).

5 Microorganisms do not grow when they are unable to recognize a particular iron(III) complex into which available iron(III) is transformed.[1]

6 a) I. G. O'Brien and F. Gibson, *Biochim. Biophys Acta*, **215**, 393 (1970); b) J. R. Pollack and J. B. Neilands, *Biochem. Biophys. Res. Commun.*, **38**, 989 (1970).

7 T. D. P. Stack, Z. Hou, and K. N. Raymond, *J. Am. Chem. Soc.*, **115**, 6466 (1993).

8 E. J. Corey and S. D. Hurt, *Tetrahedron Lett.*, **1977**, 3923.

9 W. H. Rastetter, T. J. Erickson, and M. C. Venuti, *J. Org. Chem.*, **46**, 3579 (1981); Y. Hisaeda, T. Ihara, T. Ohno, and Y. Murakami, *Chem. Lett.*, **1991**, 2139; Y. Tor, J. Libman, A. Shanzer, C. Felder, and S. Lifson, *J. Am. Chem. Soc.*, **114**, 6661 (1992); B. Tse and Y. Kishi, *J. Am. Chem. Soc.*, **115**, 7892 (1993).

10 J. B. Neilands, T. J. Erickson, and W. H. Rastetter, *J. Biol. Chem.*, **256**, 3831 (1981).

11 Abbreviations: Lys, lysine; Bn, benzyl; Boc, *t*-butoxycarbonyl; EDC, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide; EDTA, ethylenediaminetetraacetic acid; HOBt, 1-hydroxybenztriazole: 1 M = 1 mol dm^{-3}.

12 ^{1}H NMR for **1L** and **1D** [270 MHz, (CD_3)$_2$SO, 30°C] δ 4.43 (α–CH, dt, J=8 and 5 Hz), 8.27 (-CHCON*H*CH$_2$-, t, J=7 Hz), 8.83 (3H, t, J=7 Hz, ArCONH-). Found: C, H, N ± 0.3% for **1L** and **1D**. Calcd as $C_{29}H_{32}N_4O_{10}\cdot H_2O$.

13 G. Anderegg, F. L'Eplattenier, and G. Schwarzenbach, *Helv. Chim. Acta*, **46**, 1409 (1963).

14 Enterobactin, Δ ε - 4.0 at 535 nm; H. J. Rogers, C. Synge, B. Kimber, and P. M. Bayley, *Biochim. Biophys. Acta*, **497**, 548 (1977): Configurations; S. S. Isied, G. Kuo, and K. N. Raymond, *J. Am. Chem. Soc.*, **98**, 1763 (1976).