The Influence of the Chirality of Synthetic Iron Chelators Bearing *N*-Hydroxy-2(1*H*)-pyrazinones and Amino Acid Residues upon Iron Removal from Human Transferrin

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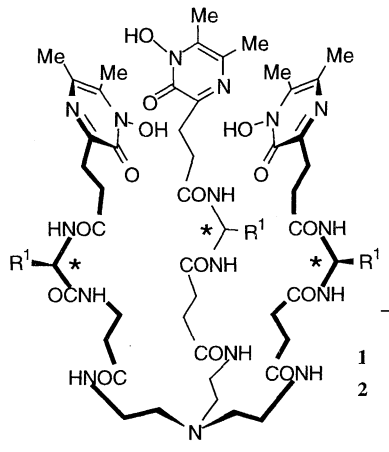
New chiral iron chelators bearing *N*-hydroxy-2(1*H*)-pyrazinone, D-amino acid residues, and tris(2-aminoethyl)amine were synthesized, and their kinetic behavior of iron removal from human transferrin was investigated. The rate of iron removal by the chelator bearing D-alanine residue was suppressed to only one fourth that of L-alanine derivative.

Recently, the application of nitrogen-containing heterocycles to iron chelators, which are used as therapeutic agents for the iron overload disease, has been one of the important issues from the view point of search for new nontoxic and oral active agents.[1] The high kinetic efficiency of iron removal from serum transferrin (iron transport protein) is requisite for acting as a nontoxic agent. Cowart and co-workers proposed that the formation of a complex between the protein and the chelator promotes a conformational change of the protein in which the metal site is exposed.[2] Raymond and co-workers also suggested that the rate of iron removal from transferrin strongly depends on the kind of the chelators.[3] However, no paper concerning the relationship between the ligand structure including the chirality and the kinetic behavior has been reported. We have focused our attention on *N*-hydroxyamide-containing pyrazines because of their potent iron-sequestering ability under physiological conditions by virtue of high acidity and high water solubility.[4] In the course of our study, it was found that chiral hexadenate chelators (**2a**, **b**) composed of 1-hydroxy-5,6-dimethyl-2(1*H*)-pyrazinone, L-amino acid residues, and tris(2-aminoethyl)amine effectively removed iron from transferrin.[4b] In order to explore the relationship between ligand structure and the iron removal efficiency, their enantiomers **1a**, **b** were synthesized. We describe here the apparent difference in the kinetic efficiency between the enantiomer on iron removal from transferrin.



	R1	
	a	b
1	Me	Bu^i (D-form)
2	Me	Bu^i (L-form)

Synthesis of **1** was achieved by a similar fashion[4b] for **2**: coupling of 1-benzyloxy-3-carboxyethyl-5,6-dimethyl-2(1*H*)-pyrazinone with a dipeptide of D-Ala for **1a** or D-Leu for **1b** and β-Ala, condensation with tris(2-aminoethyl)amine, and subsequent debenzylation by hydrogenation.[5] Uv-vis spectra of the 1:1 molar mixture of **1** and ferric ion in aqueous solutions showed characteristic LMCT band around at 450 nm (ε=3580 at 450 nm for **1a**; ε=3010 at 455 nm for **1b**), indicating the formation of intramolecular 1:1 iron complexes.[6]

Iron removal ability of **1a** from human diferric transferrin (**Tf**$_{Fe2.0}$) was evaluated at pH 7.4 by mixing a buffered solution of **Tf**$_{Fe2.0}$ with 5 times excess of **1a**, followed by monitoring the change in absorbance at 460 nm.[7] The plots of log[(A_∞-Abs)/(A_∞-A_0)] as a function of time gave a linear relationship as shown in Figure 1. It indicates that the iron removal from

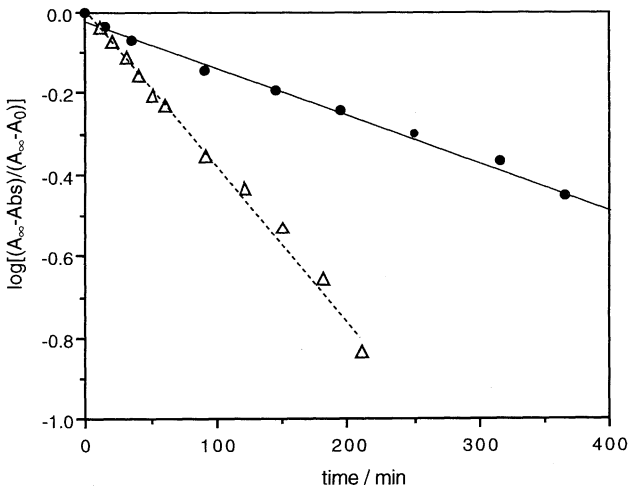


Figure 1. The plots of log [(A_∞ -Abs)]/(A_∞ -A_0)] vs. time on iron removal of **1a** (•) and **2a** (Δ) from **Tf**$_{Fe2.0}$.

transferrin by **1a** proceeds in the pseudo-first-order kinetics. The k_{obs} was calculated from the slope of the straight line. The kinetic results are summarized in Table 1 together with the data of **2a**, **b**. The rates of iron removal by **1** were greater than that by desferrioxamine B (k_{obs}=0.66 x 10^{-3} min^{-1}; [L]/[**Tf**$_{Fe2.0}$]=100),[4c] which is a currently used drug for the iron overload disease, even at a less concentration of the ligand. It is noteworthy that the rates of iron removal by alanine residue-containing ligands were significantly affected by the chirality of free ligands. The rate of iron removal by **1a** was suppressed to

Table 1. Iron Removal from Transferrin at pH 7.4

Ligand	[L]/[$\mathbf{Tf}_{Fe2.0}$][a]	k_{obs} ($\times 10^{-3}$ min^{-1})	% Fe removed[b]
1a	5	1.05	7
1b	6	1.29	8
2a[c]	5	3.80	23
2b[c]	6	0.90	9

[a] $[\mathbf{Tf}_{Fe2.0}]_0$=0.0368 mmol dm^{-3}. $\mathbf{Tf}_{Fe2.0}$ was prepared from commercially available human serum apotransferrin (Sigma). [b] At a point 30 min after the reaction was initiated. [c]Ref. 4b.

only one fourth of **2a** (Figure 1). On the other hand, no apparent difference was observed in the case of leucine residue-containing **1b** and **2b**. The CD spectra of Fe/**1a** and Fe/**2a** complexes are shown in Figure 2. The spectrum of Fe/**1a** showed a positive band at 363 nm and a negative band at 480 nm, indicating that Fe/**1a** predominantly exists in Δ configuration. On the other hand, Fe/**2a** predominantly exists in Λ-configuration.[4b]

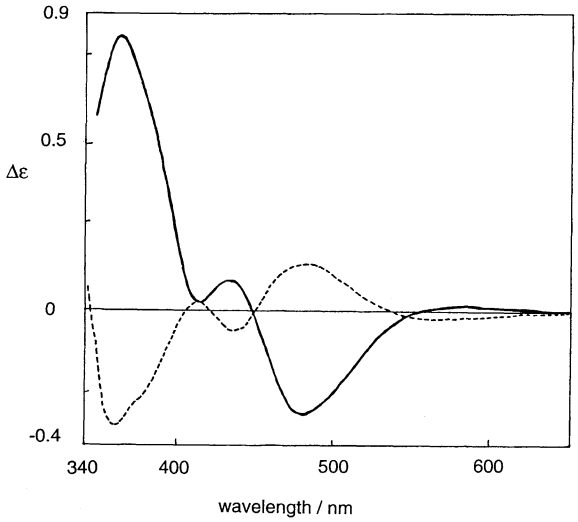


Figure 2. CD spectra of iron complexes of **1a** and **2a** in water at pH 4: [Fe/**1a**]=1.2 mmol dm^{-3} (—); [Fe/**2a**]=0.3 mmol dm^{-3} (---).

The structural feature of the ligand would give an influence on the interaction between the protein and the ligand. The bulky substituent such as Bu^{i} group may prevent access of the ligand to exposed metal site of the protein, while the iron removal process could be affected by the chirality of the ligand itself, or the absolute configuration (i.e. Λ and Δ) of the resulting iron complex of the ligand.

It is the first case suggesting the influence of the chirality of the ligand upon the rate of iron removal from transferrin, although the detailed mechanism remains obscure.

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References and Notes

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4 a) J. Ohkanda, T. Tokumitsu, K. Mitsuhashi, and A. Katoh, *Bull. Chem. Soc. Jpn.*, **66**, 841(1993). b) J. Ohkanda and A. Katoh, *J. Org. Chem.*, **60**, 1583(1995). c) J. Ohkanda and A. Katoh, *Tetrahedron*, **51**, 12995(1995).

5 The structures of all new compounds were fully characterized by the spectroscopic analyses. The data of **1a**,**b** are as follows. **1a**: pale yellow amorphous, $[\alpha]_D^{22}$ +15.1° (c=0.2 in MeOH) (**2a**: $[\alpha]_D^{25}$ -16.5° (c=0.11 in MeOH)); ^{1}H NMR (CD_3OD) δ 1.33 (d, J=7 Hz, 9H), 2.33 (s, 9H), 2.41 (s, 9H), 2.44 (t, J=7 Hz, 6H), 2.66 (t, J=7 Hz, 6H), 3.03 (t, J=7 Hz, 6H), 3.35 (m, 12H), 3.59 (m, 6H), 4.34 (q, J=7 Hz, 3H). Found: C, 49.45; H, 6.90; N, 17.83%; Calcd for $C_{51}H_{78}N_{16}O_{15}\cdot 0.5H_2O$: C, 49.19; H, 7.12; N, 18.00%. **1b**: pale yellow amorphous, $[\alpha]_D^{25}$ +9.1° (c=0.2 in MeOH) (**2b**: $[\alpha]_D^{22}$ -7.8° (c=1.0 in MeOH)); ^{1}H NMR (CD_3OD) δ 0.87 (t, J=6 Hz, 9H), 0.92 (t, J=6 Hz, 9H), 1.55 (m, 9H), 2.32 (s, 9H), 2.39 (s, 9H), 2.40 (m, 6H), 2.66 (t, J=7 Hz, 6H), 3.04 (m, 12H), 3.43 (m, 12H), 4.30 (t, J=7 Hz, 3H). Found: C, 54.05; H, 7.56; N, 17.12%; Calcd for $C_{60}H_{96}N_{16}O_{15}\cdot 3H_2O$: C, 53.95; H, 7.70; N, 16.78%.

6 Observed λ_{max} and ε values were comparable to those of 1:1 iron complexes of **2** (Ref. 4b).

7 A commercially available human serum apotransferrin (98%, Sigma) was used. $\mathbf{Tf}_{Fe2.0}$ was prepared according to the literature reported in detail by Raymond (Ref. 3). The stock solutions of **1a** (0.58 mL, 0.9 mmol dm^{-3}, pH 7.4) and $\mathbf{Tf}_{Fe2.0}$ (3 mL, 0.0368 mmol dm^{-3}) in Tris buffer were combined, and then the absorbance (Abs) of the solution was monitored at 460 nm. The pseudo-first-order rate constant (k_{obs}) was calculated from the slope of the plots of log $[(A_\infty - Abs)/(A_\infty - A_0)]$ as a function of time. A_0: the absorbance at t=0. A_∞: the absorbance after 800 min.