Synthesis and Characterization of Iron Complexes of Rhodotorulic Acid: A Novel Dihydroxamate Siderophore and Potential Chelating Drug

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Summary The iron complex of rhodotorulic acid (RA) at neutral pH has been found to be dimeric with the formulation $Fe_2(RA)_3$, where both iron atoms have the Δ-*cis* configuration; it is this dimeric complex that functions as an iron transport agent in yeast.

A GREAT deal of recent interest has been generated by the discovery that certain low moleculer weight chelating agents, such as ferrichrome, are excreted by various bacteria, moulds, and yeasts.[1] These compounds, called siderophores, serve as iron transporting agents for the organisms which produce them and are used as drugs to facilitate iron mobilization in man.[2] Most of the well characterized siderophores such as ferrichrome and ferrioxamine B co-ordinate one mole of ferric ion per mole of ligand and bind the iron octahedrally *via* hydroxamic acid groups. The resulting monomeric complexes, when the ligands are optically active, have been found to exist preferentially as the Λ-*cis* isomers.[3] We describe here an unusual iron chelating agent from yeast, rhodotorulic acid, which forms a dimeric complex with Fe^{III} and exists as the Δ-*cis* optical isomer.

Rhodotorulic acid (RA), a cyclic dipeptide containing two hydroxamate groups, can be isolated from low iron cultures of the yeast *Rhodotorula pilimanae*.[4] The iron and aluminium complexes were prepared by treatment of rhodotorulic acid with the respective hydroxides in water at elevated temperatures. These complexes gave analytical results expected for the formulation, $M_2(RA)_3$, M = Al, Fe, or Cr. Spectral data and molecular weights are consistent with a dimeric structure† (Figure).

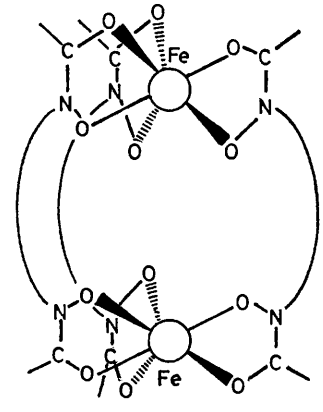

FIGURE. (A) Proposed structure of the ferric rhodotorulate dimer, showing details of the hydroxamate co-ordination sphere about each iron atom; the curved lines represent the remaining portion of the ligand (B).

† For the iron complex the calculated C, H, N, and Fe analyses agreed with those observed within $\pm 0{\cdot}3\%$. Calculated mol. wt. for the dimer 1138, found 900.

At pH 7 the orange iron complex has λ_{max} 425 nm (ϵ 2700) and at pH 2 becomes red with λ_{max} 480 nm (ϵ 1750). The c.d. spectrum at neutral pH consists of two peaks, 464 ($\Delta\epsilon$ −1·4) and 372 nm (+2·7); these peaks are reduced in intensity and shifted to longer wavelength at low pH. Based on previous work,[5] it can be concluded from the c.d. spectra that the Fe^{III} rhodotorulic acid complex exists as the Δ-*cis* isomer which is the opposite enantiomer to that found in the ferrichromes and mycobactins.

Equilibrium titration data not only confirm our formulation of the ferric complex as the dimer $Fe_2(RA)_3$ at neutral pH but also show that the red species formed at low pH is the monomer, $Fe(RA)^+$. The charge on the monomer was established by electrophoresis and ion exchange techniques while molecular weight data consistent with this formulation were found *via* gel filtration. In addition the overall stability constant (β_{320}, equation 1)‡ for the dimer was determined *via* potentiometric titration of both the ligand and the metal–ligand complex, and was found to be 2×10^{62}.

$$2\,Fe^{3+} + 3\,L^{2-} \overset{\beta_{320}}{\rightleftharpoons} Fe_2L_3 \qquad (1)$$

Since the species is a dimer, the overall stability constant cannot be directly compared with the monomeric species, ferrichrome or ferrioxamine. However, examination of the 'stability constant per iron atom' of $1{\cdot}4 \times 10^{31}$ indicates a stability comparable to that of these latter two compounds (β_3 *ca.* 10^{30}).

We have also been able to show that the iron complex of RA does indeed function as the iron transporting agent in yeast but does so in a qualitatively different fashion than does ferrichrome, in that while it facilitates iron uptake the ligand itself never enters the cell.[6]

Finally, in view of the high overall stability constant and the high synthetic capacity of the producing organism (yields averaging up to 4 g l^{-1} with 40—50% of the nitrogen fed to the yeast incorporated into RA), this natural iron chelating agent may prove to be an effective drug for iron mobilization in man.§

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‡ β_{320} refers to the overall stability constant for the species containing three ligands, two metals, and no hydroxamate hydrogens.

§ Rhodotorulic acid is presently undergoing limited clinical trials in the United States as a drug for iron chelation therapy in man, see *Chem. Eng. News*, 1977, **55**, No. 18, **24**.

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