Iron Transport in *Streptomyces pilosus* Mediated by Ferrichrome Siderophores, Rhodotorulic Acid, and Enantio-Rhodotorulic Acid†

GERTRAUD MÜLLER, BERTHOLD F. MATZANKE, AND KENNETH N. RAYMOND*

Department of Chemistry, University of California, Berkeley, California 94720

Received 25 May 1984/Accepted 27 July 1984

Streptomyces pilosus is one of several microbes which produce ferrioxamine siderophores. In the accompanying paper (G. Müller and K. Raymond, J. Bacteriol. 160:304–312), the mechanism of iron uptake mediated by the endogenous ferrioxamines B, D₁, D₂, and E was examined. Here we report iron transport behavior in *S. pilosus* as mediated by the exogenous siderophores ferrichrome, ferrichrysin, rhodotorulic acid (RA), and synthetic enantio-RA. In each case iron acquisition depended on metabolic energy and had uptake rates comparable to that of [⁵⁵Fe]ferrioxamine B. However, the synthetic ferric enantio-RA (which has the same preferred chirality at the metal center as ferrichrome) was twice as effective in supplying iron as was the natural ferric RA complex, suggesting that stereospecific recognition at the metal center is involved in the transport process. Iron uptake mediated by ferrichrome and ferric enantio-RA was strongly inhibited by kinetically inert chromic complexes of desferrioxamine B. These inhibition experiments indicate that iron from these exogenous siderophores is transported by the same uptake system as ferrioxamine B. Since the ligands have no structural similarity to ferrioxamine B except for the presence of three hydroxamate groups, we conclude that only the hydroxamate iron center and its direct surroundings are important for recognition and uptake. This hypothesis is supported by the fact that ferrichrome A and ferrirubin, which are both substituted at the hydroxamate carbonyl groups, were not (or were poorly) effective in supplying iron to *S. pilosus*.

As described in the previous paper (21), siderophores are iron chelating agents which are excreted by microorganisms under iron-deficient conditions to sequester iron from the environment. They are transported into the cells by specific, energy-dependent uptake systems (19, 21, 24, 27). Iron uptake by *Streptomyces pilosus* via the ferrioxamines B, D₁, D₂, and E (which are all produced by this organism) was described in the previous paper (21). Here we report iron transport by *S. pilosus* as mediated by several exogenous siderophores.

The ferrichromes are a class of structurally related siderophores (Fig. 1) which are synthesized by fungal species such as *Ustilago* (23), *Aspergillus* (18), and *Penicillium* (18). The detailed structures of ferrichrome itself (30) (after which the class of compounds is named), ferrichrome A (35), and ferrichrysin (26) have been established by single-crystal X-ray diffraction. In each case only a Δ -cis absolute configuration at the metal center was observed. By preparing the kinetically inert chromium complexes of desferriferrichrome and desferriferrichrysin, it has been established that the conformation about the metal center in solution is also Δ -cis, the same as in the crystals (20). The transport of iron by ferrichrome and ferrichrome A, their mechanism of uptake, and the regulation of their biosyntheses have been studied intensively in *Ustilago sphaerogena* (12, 13).

Although not produced by *Escherichia coli*, ferrichrome, ferricrocin, and ferrichrysin are taken up by this organism via a highly specific transport system (10, 28). An outer membrane receptor protein, specified by the *tonA* (*fhuA*) gene (9), is required for the transport of ferrichrome and its homologs. From competition experiments it is known that ferrichrome shares a receptor with bacteriophages T1, T5, and ϕ 80 and the protein colicin M, as well as with the antibiotic ferrichrome derivative albomycin (4). Ferrichrome

A, however, fails to supply iron to *E. coli* (31). In addition, it has been found that aerobactin, which is produced by *E. coli* and resembles ferrichrome only in the structure at the metal center, needs the same four gene products as ferrichrome in order to be transported: *tonA* (*fhuA*), *tonB*, *fhuB*, and *exbB* (5). In solution, aerobactin, like ferrichrome, has a preferred Λ configuration about the metal center at pH 7 (15). Evidence for stereospecific recognition has been provided by the synthetic enantiomer of ferrichrome, which as a Δ -cis configuration (22) and is taken up at a rate only 50% of that of the naturally occurring ferrichrome (33).

Rhodotorulic acid (RA) is a dihydroxamate, the dipeptide or diketopiperazine of δ -N-acetyl-L-(S)- δ -N-hydroxyornithine. It has been isolated from low-iron cultures of *Rhodotorula pilimanae* (2). Since RA is only a tetradentate ligand, it forms 2:3 six-coordinate complexes with ferric iron at pH 7 (6) (Fig. 1). Isowa et al. have synthesized protected N³-hydroxyornithine from achiral building blocks and enzymatically resolved the racemic product into its enantiomers. These served as precursors for the synthesis of DD-RA and LL-RA (16). The chromic complexes of the naturally occurring LL-RA, and by analogy the predominant configuration of the ferric complex in aqueous solution, has been shown to be Δ (7). The enantiomer DD-RA must therefore form optically active complexes of a predominantly Λ configuration around the metal center. Transport experiments with *R. pilimanae* have demonstrated that the synthetic enantio-Fe₂RA₃ complex is taken up with saturation kinetics, but at a rate reduced by 50% relative to that of LL-Fe₂RA₃ (G. Müller, Y. Isowa, and K. N. Raymond, submitted for publication).

In the preceding paper (21), we showed that the uptake of ⁵⁵Fe by *S. pilosus*, as mediated by the B, E, D₁, and D₂ ferrioxamines, could be inhibited effectively by chromic desferriferrichrome B. Hence it was concluded that only one receptor system is shared in the transport of those four ferrioxamines. This report also illustrates that ferrichrome-type siderophores and the ferric complexes of RA and its

* Corresponding author.

† Coordination Chemistry of Microbial Iron Transport Compounds, paper no. 30.

FIG. 1. (Top) Chemical structures of the ferrichromes. (Bottom) Chemical structure of RA.

enantiomer are taken up by *S. pilosus* in an energy-dependent step, although none of these siderophores is produced by *S. pilosus*.

MATERIALS AND METHODS

Materials. Reagent-grade chemicals were used in all experiments. Desferrioxamine B (Desferal) was obtained from Ciba-Geigy Corp., Summit, N.J. Desferrioxamine E, ferrichrome A, ferrichrome, ferrirubin, and enantio-RA were generous gifts from W. Keller-Schierlein, Zürich, Switzerland; D. Ecker, University of California, Berkeley; G. Winkelmann, Institut für Mikrobiologie I, Tübingen, Federal Republic of Germany; and Y. Isowa, Sagami Chemical Research Center, Kanagawa, Japan. RA and ferrichrysin were isolated as previously described (2, 17). The metal complexes were chromatographed on aluminum-backed cellulose plates or silica gel plates (Merck & Co.). ⁵⁵FeCl₃ was obtained from New England Nuclear Corp. Chromic desferrioxamine B complexes were prepared as previously described (21) by the method of Leong and Raymond (20).

Organism and growth conditions. *S. pilosus* ATCC 19797 stock cultures were kept on agar slants with 2.1% YM broth (Difco Laboratories) and 2% agar. The preparation and harvesting of spores and the cultivation of cells in iron-deficient medium were done as previously described (21).

Preparation of labeled complexes. The siderophores were labeled with ⁵⁵Fe as previously described (14), including ferrichromes, ferrioxamines B and E (as described in reference 21), RA, and enantio-RA (8). All siderophore solutions contained 20% excess ligand. The radioactive ferric complexes of nitrilotriacetic acid, citrate, and 3,4-dihydroxybenzoate were formed with a 10-fold excess of ligand. All stock solutions of the labeled metal complexes had a concentration of 0.01 mM (5 to 10 μCi/ml). The radiopurity of the solutions was tested with aluminum-backed cellulose thin-layer chromatography plates, which were cut into squares after development and counted in a liquid scintillation counter.

Transport assays. The transport assays were performed as

described earlier (21). The samples were counted after equilibration in Aquasol 2 in a liquid scintillation counter.

RESULTS

⁵⁵Fe uptake mediated by ferrichromes. Figure 2 shows the time-dependent uptake characteristics of various siderophores of the ferrichrome family. Although none of the ferrichrome family of compounds are produced by *S. pilosus*, ferrichrome and ferrichrysin are accumulated at identical rates. In 10 min, 0.48 nmol of ⁵⁵Fe per mg of dried cells was incorporated, which was about 15% more than for ferrioxamine B. Uptake was linear at least from 2 to 10 min. [⁵⁵Fe]ferrichrome uptake was monitored in 10-s intervals (Fig. 2, inset). Within the initial 20 s, a fast accumulation of ⁵⁵Fe occurred; this was considered the sum of specific and nonspecific binding to the cell surface (adsorption to the filters was subtracted). Although ferrichrome and ferrichrysin differ in the amino acid sequence of the cyclic hexapeptide backbone, these differences did not significantly affect the transport rate. This is in contrast to ferrirubin, which has the same structure as ferrichrysin except that the methyl residue adjacent to the hydroxamate carbonyls is substituted by an isopentenol unit. After 10 min, 0.1 nmol of ⁵⁵Fe per mg (dry weight) of cells was taken up by the cells from ferrirubin, a decrease of 80% relative to ferrichrysin uptake. In ferrichrome A the terminal methyl groups of ferrichrysin are replaced by three Z-3-methyl-3-butenic acid residues. This siderophore fails completely in supplying iron to cells of *E. coli* (31).

Kinetics of ferrichrome uptake and effect of metabolic inhibitors. The kinetics of ferrichrome uptake were tested in a concentration range from 0.025 to 20 μM (Fig. 3). The uptake rates were determined from the linear interval between 2 and 10 min. Like ferrioxamine B, ferrichrome was taken up via a saturable process, which reached its maximum velocity at ~0.25 μM ferrichrome concentration at 60

FIG. 2. Time-dependent uptake of ⁵⁵Fe from ferrichrome siderophores and ferrioxamine B at 1 μM concentration. (Inset) Uptake of [⁵⁵Fe]ferrichrome measured at 10-s intervals.

pmol/mg (dry weight) of cells per min. This result indicates that ferrichrome, like the ferrioxamines, is transported by a carrier-mediated active process and that no diffusion takes place. This was also shown by the sensitivity of the system to metabolic inhibitors. Respiratory poisons such as sodium azide caused complete inhibition of iron transport from ferrichrome, ferrichrysin, ferric RA, and ferric enantio-RA (data not shown).

Uptake of iron from ferric RA and enantio-RA. The iron complexes of RA have a certain structural similarity to ferrichrome in the iron center and its nearest surroundings. In each case the coordination at the metal center is $\text{Fe}[\text{RN}(\text{O})\text{C}(\text{O})\text{CH}_3]_3$, so that the *cis* isomers as viewed down the trigonal face occupied by the carbonyl groups are identical. However, the predominant optical configuration around the metals of the naturally occurring R complexes is Δ (8), whereas for the ferrichromes it is Λ . Thus, the synthetic enantio-RA, like ferrichrome, forms the Λ configuration with iron in solution.

The naturally occurring Fe_2RA_3 was taken up at rates comparable to those of ferrioxamine B, whereas the complex of a siderophore analog, synthetic enantio-RA, was superior to the naturally occurring compound in its iron supply capabilities (Fig. 4). In contrast, iron transport measurements with enantio-ferrichrome in *E. coli* (33), *Neurospora crassa*, and *Aspergillus quadricinctus* (32) showed decreased uptake rates compared with those for ferrichrome. The synthetic enantiomer was found to behave similarly in a study with enterobactin and enantio-enterobactin in *E. coli* (25).

Inhibition of ^{55}Fe uptake by chromic desferrioxamine B. Since neither RA nor any of the ferrichrome siderophores is synthesized by *S. pilosus*, the question arises of which transport system is used by those substances. Are there individual, specific receptors for each ferric siderophore, or do these complexes slip in by systems which are provided for other substances, such as peptides, which are structurally similar?

Inhibition experiments provide evidence about whether different substrates interact with one uptake system. The interference of chromic desferrioxamine B with ^{55}Fe uptake by ferrichrome, ferric enantio-RA, and ferrioxamine E was

FIG. 3. Relative rate of ^{55}Fe uptake as a function of the $[^{55}\text{Fe}]$ -ferrichrome concentration.

FIG. 4. ^{55}Fe uptake of iron complexes of naturally occurring RA (●), synthetic RA enantiomer (▲), and ferrioxamine B (■) (in minutes). The concentration in the medium was $1 \mu\text{M}$ for all three siderophores.

tested. The kinetically inert chromic complex of desferrioxamine B was used as the inhibitor instead of unlabeled ferrioxamine B complexes to avoid ligand exchange (and thus dilution of the ^{55}Fe label of the transported siderophores, which could be mistaken for inhibition).

At an inhibitor concentration of $5 \mu\text{M}$, ^{55}Fe uptake by ferrioxamine E was reduced to 67% of that without inhibitor, and ^{55}Fe uptake by ferrichrome and ferric enantio-RA was only 30 and 40%, respectively (Fig. 5). The ^{55}Fe complexes were added at $1 \mu\text{M}$ concentrations; 100% uptake was calculated from the transport rates without inhibitor. The results suggest that ferrioxamine B interacts strongly with the transport systems of the siderophores tested.

Transport mediated by other ferric chelators. Additional exogenous ligands with a range of coordination properties were tested for their ability to serve as iron donors to *S. pilosus*. Since Dyer et al. (11) described the occurrence of dihydroxybenzoic acid (DHB) in the culture filtrate of an iron-deficient *Streptomyces* strain, we tested the uptake of $^{55}\text{Fe}(\text{DHB})_3$. However, this negatively charged molecule was not taken up. Similarly, nitrilotriacetic acid was found not to be an iron transport ligand. This biological inactivity of nitrilotriacetic acid has also been reported for *E. coli* (28). In contrast, citrate appeared to be very effective as an iron transport agent (Fig. 6), although there were remarkable differences between it and ferrioxamine B in uptake. The citrate uptake system clearly consists of two superimposed processes: (i) an active transport system, dependent on metabolic energy, which becomes inactive when sodium azide, a respiratory poison, is added, and (ii) a passive process which constitutes what is left after the inhibition. By subtracting the passive part from total transport (the upper curve), the resultant curve shows that active transport reached maximum velocity at about $10 \mu\text{M}$ $[^{55}\text{Fe}]$ -citrate.

Citrate is known to have a much lower complex formation constant ($\log \beta = 11.8$) (27) than ferrioxamine B ($\log \beta = 30.5$) (1); correspondingly, it seems to be very inefficient in supplying iron at the concentrations that are optimal for ferrioxamine B, i.e., at substrate concentrations lower than $1 \mu\text{M}$, when $[^{55}\text{Fe}]$ -citrate uptake was only $0.0014 \text{ nmol/mg per}$

FIG. 5. Inhibition of ^{55}Fe uptake via ferrioxamine E, enantio-RA, and ferrichrome at $1\ \mu\text{M}$ concentrations by increasing concentrations of chromic desferriferrioxamine B.

min. However, at higher substrate concentrations, citrate became superior to ferrioxamine B in supplying iron: at $10\ \mu\text{M}$ the uptake rate was about three times higher than that of ferrioxamine B. This relative behavior of the ^{55}Fe uptake rates was not consistent with the relatively slow iron exchange reactions between citrate and the endogenous desferriferrioxamines; thus, simple iron exchange between nonspecifically recognized siderophores and a specifically recognized siderophore (such as ferrioxamine) is not an acceptable model.

DISCUSSION

This study shows that the iron of ferrichrome siderophores, such as ferrichrome and ferrichrysin, is taken up by gram-positive *S. pilosus* in an energy-dependent active transport process (Fig. 2). Furthermore, the ferrichrome iron accumulation is strongly inhibited by chromic desferriferrioxamine B (Fig. 5). Thus, ferrichrome and ferrioxamine compete for the same uptake system. In a parallel investigation, the same inhibition effect by chromic desferriferrioxamine B was demonstrated in the incorporation of the ferrioxamines E, D₂, and D₁ (21). From these results it can be inferred that ferrichrome and the ferrioxamines use the same carrier system. Striking confirmation of this conclusion can be found in the early results of Zähler et al. (34) and Bickel et al. (3), who in 1960 investigated antagonism between sideromycins and other siderophores.

The sideromycins albomycin and ferrimycin are homologs of ferrichrome and ferrioxamine B, respectively. Substitutions in the ligand structures which are well separated from the metal coordination sites convert what would be growth factors into powerful antibiotics. A very important fact is the cross-reactivity between the two classes of hydroxamates in gram-positive bacteria. In these strains ferrioxamines antagonize the antibiotic action not only of ferrimycin but also of albomycin, which is a ferrichrome analog. Likewise, ferri-

chrome shows antagonism with both albomycin and ferrimycin. Ferrichrome A was found to be inactive. The clue to this cross-reactivity is the existence of a common transport system for ferrichrome and the ferrioxamines, as shown by our study.

The characteristic structural feature of the ferrichrome family is a cyclic hexapeptide which contains a tripeptide sequence of δ -N-acyl-L- δ -N-hydroxyornithine, common for all ferrichromes, and a variable tripeptide building block of various neutral amino acids. Ferrichrome contains three glycine units, whereas the sequence seryl-seryl-glycine is common to ferrichrysin, ferrirubin, and ferrichrome A. Since ferrichrysin and ferrichrome are taken up at the same rate, it is apparent that the serine-for-glycine substitution has little effect on iron transport. Moreover, despite pronounced differences in their molecular architecture, ferrichrome and ferrioxamine B are taken up by the same transport system. These findings indicate that changes in the ligand skeleton structure per se are of minor importance for recognition and transport in *S. pilosus*.

For two siderophore ligands with the same architecture, ferrioxamine B and ferrioxamine D₁, it has been shown that the lack of a positive charge in ferrioxamine D₁ results in an uptake rate which is 40% lower than that of ferrioxamine B (21). Although no positive charge exists in the ferrichromes, their accumulation was comparable to that by ferrioxamine B. Whereas complexes of the linear ferrioxamine B in solution are a mixture of *cis* and *trans* isomers (20), the cyclic ferrichromes are exclusively *cis*. This configuration may compensate to some extent for the lack of a positive charge.

The ferrichrysin derivatives ferrichrome A and ferrirubin were not or were only slightly taken up, respectively (Fig. 2). In ferrichrome A, the methyl group adjacent to the hydroxamate carbonyl is substituted by Z-3-methyl-3-buteonic acid, and in ferrirubin it is substituted by isopentenol residues. The tri-negative charge of ferrichrome A totally prevented

FIG. 6. ^{55}Fe citrate uptake in *S. pilosus* as a function of the ferric citrate concentration with (■) and without (●) incubation with $10\ \text{mM}\ \text{NaN}_3$ for 5 min.

uptake. This repulsing effect is not surprising since the positive charge of ferrioxamine B plays an important role in the recognition and uptake process. Ferrirubin was taken up only very slowly.

We propose that the differences in the biological activity of ferrichrome and ferrichrysin, in contrast to those of ferrichrome A and ferrirubin, are due to the shielding or repulsing effects of groups around the hydroxamate iron center and not to changes in the amino acid skeleton. Further support for this hypothesis was supplied by the uptake experiments, in which iron complexes of naturally occurring RA and synthetic enantio-RA were used. The ferric complex of enantio-RA, which, like ferrichrome, has a Λ configuration in solution, was taken up at rates about 100% higher than those of the naturally occurring RA complex (Fig. 4). This remarkable effect cannot be due to a difference in recognition of the DD and LL optical activity of the diketopiperazine ring. Since inhibition studies with chromic desferriferrioxamine B showed strong inhibition of ferric enantio-RA uptake (Fig. 5), the same uptake system is used for ferrichrome-type and ferrioxamine-type siderophores and parts of the ligands which are distal to the metal center are not the recognized portions of the molecule.

Since enantio-RA was taken up at such an extraordinary rate, it has to be inferred that the Λ configuration is the preferred one in the uptake process. However, since the ferrioxamine complexes are racemic mixtures of Λ and Δ isomers and since separation of the chromic desferrioxamine B enantiomers from the racemic mixture has not yet been achieved, the question remains whether the Λ optical isomer of ferrioxamine B is taken up at higher rates than the Δ optical isomers.

An additional surprising result was that ^{55}Fe from synthetic enantio-RA was acquired nearly twice as fast as ferrioxamine B. At physiological pH, RA forms complexes with iron in 2:3 stoichiometry. This implies the presence of two ferric ions per molecule. Since chromic desferriferrioxamine B is accumulated as an entity, it is very likely that this is true for the RA complexes as well. Therefore, the ferric enantio-RA complex should be twice as effective in supplying iron to the cells as the monomeric ferrichrome and ferrioxamine species. This model is consistent with our observations.

What are the structural similarities between the ferrioxamines, ferrichromes, and ferric RA complexes that result in no discrimination being made by the cells? From inspection of models of these complexes it is obvious that no similarities in the ligand structures themselves exist. However, all of these molecules feature an unshielded hydroxamate ferric ion center which is $\text{Fe}[\text{R}-\text{N}(\text{O})\text{C}(\text{O})\text{CH}_3]_3$. We propose this as the molecular unit which is recognized by a receptor. Van der Helm et al. compared the geometries at the metal center obtained from the crystal structures of ferrioxamine E, ferrichrome, and ferrichrome A (30); there were no obvious differences. However, ferrichrome, ferrichrysin, and enantio-RA all exhibit a Λ configuration at the iron hydroxamate octahedron. The difference in uptake rates of enantio-RA and RA strongly suggests that the hydroxamate receptor of *S. pilosus* discriminates between Λ and Δ . A similar effect has been reported for the ferrichrome uptake system in *E. coli* (33). A second common characteristic of the complexes studied here is the rim of hydrophobic alkane functionalities (methyl and methylene groups) adjacent to the hydroxamate propellor. Changes of the substituent at the hydroxamate carbonyl, e.g., in ferrirubin, reduced the uptake rate even more drastically than the Λ coordination of ferric RA complexes. The spatial array of the methyl group

substituents changes markedly on going from the Λ to Δ configuration, and this is likely one of the determining factors of recognition and receptor binding.

ACKNOWLEDGMENTS

We thank David Ecker, Gwen Freeman, and June Smith for assistance in assembling this manuscript.

G.M. thanks the Deutsche Forschungsgemeinschaft for a fellowship. This research was supported by Public Health Service grant AI 11744 from the National Institutes of Health.

LITERATURE CITED

1. Anderegg, G., F. l'Eplattenier, and G. Schwarzenbach. 1963. Hydroxamate complexes. III. Iron(III) exchange between sideramines and complexones. *Helv. Chim. Acta* **46**:1409-1422.
2. Atkin, C. L., and J. B. Neilands. 1968. Rhodotorulic acid, a diketopiperazine dihydroxamic acid with growth-factor activity. I. Isolation and characterization. *Biochemistry* **7**:3734-3739.
3. Bickel, H., E. Gäumann, W. Keller-Schierlein, V. Prelog, E. Vischer, A. Wettstein, and H. Zährner. 1960. Sideramine und ihre Antagonisten, die eisenhaltigen Antibiotica Sideromycine. *Experientia (Basel)* **16**:129-135.
4. Braun, V., and K. Hantke. 1981. Bacterial cell surface receptors, p. 1-73. In B. K. Gosh (ed.), *Organization of procaryotic cell membranes*, vol. 2. CRC Press, Boca Raton, Fla.
5. Braun, V., R. Buckhardt, R. Schneider, and L. Zimmermann. 1982. Chromosomal genes for Col IV plasmid-determined iron-(III) aerobactin transport in *Escherichia coli*. *J. Bacteriol.* **151**:553-559.
6. Carrano, C. J., S. R. Cooper, and K. N. Raymond. 1979. Coordination chemistry of microbial iron transport compounds. 11. Solution equilibria and electrochemistry of ferric rhodotorulate complexes. *J. Am. Chem. Soc.* **101**:599-604.
7. Carrano, C. J., and K. N. Raymond. 1978. Coordination chemistry of microbial iron transport compounds. 10. Characterization of the complexes of rhodotorulic acid, a dihydroxamate siderophore. *J. Am. Chem. Soc.* **100**:5371-5374.
8. Carrano, C. J., and K. N. Raymond. 1978. Coordination chemistry of microbial iron transport compounds: rhodotorulic acid and iron uptake in *Rhodotorula pilimanae*. *J. Bacteriol.* **136**:69-74.
9. Coulton, J. W. 1982. The ferrichrome-iron receptor of *Escherichia coli* K-12. Antigenicity of the fhu A protein. *Biochim. Biophys. Acta* **717**:154-162.
10. Coulton, J. W., H.-U. Naegeli, and V. Braun. 1979. Iron supply of *Escherichia coli* with polymer-bond ferricrocin. *Eur. J. Biochem.* **99**:39-47.
11. Dyer, J. R., H. Heding, and C. P. Schaffner. 1964. Phenolic metabolites of "low iron" fermentation of *Streptomyces griseus*. Characterization of 2,3-dihydroxybenzoic acid. *J. Org. Chem.* **29**:2802.
12. Ecker, D. J., and T. Emery. 1983. Iron uptake from ferrichrome A and iron citrate in *Ustilago sphaerogena*. *J. Bacteriol.* **155**:616-622.
13. Ecker, D. J., J. R. Lancaster, Jr., and T. Emery. 1982. Siderophore iron transport followed by electron paramagnetic resonance spectroscopy. *J. Biol. Chem.* **257**:8623-8626.
14. Emery, T. 1971. Role of ferrichrome as a ferric ionophore in *Ustilago sphaerogena*. *Biochemistry* **10**:1483-1488.
15. Harris, W. R., C. J. Carrano, and K. N. Raymond. 1971. Coordination chemistry of microbial iron transport compounds. 16. Isolation, characterization and formation constants of ferric aerobactin. *J. Am. Chem. Soc.* **101**:2722-2727.
16. Isowa, Y., T. Takashima, M. Ohmori, H. Kurita, M. Sato, and K. Mori. 1972. Synthesis of rhodotorulic acid. *Bull. Chem. Soc. Jpn.* **45**:1464-1471.
17. Keller-Schierlein, W., and A. Déer. 1963. Metabolic products of microorganisms. 45. On the constitution of ferrichrysin and ferricrocin. *Helv. Chim. Acta* **46**:1907-1920.
18. Keller-Schierlein, W., V. Prelog, and H. Zährner. 1964. Siderochrome. Natural iron(III) hydroxamate complex. *Fortschr. Chem.*

- Org. Naturst. **22**:279–322.
19. Lankford, C. E. 1973. Bacterial assimilation of iron. Crit. Rev. Microbiol. **2**:272–331.
 20. Leong, J., and K. N. Raymond. 1974. Coordination isomers of biological iron transport compounds. II. The optical isomers of chromic desferri-ferrichrome and desferrichrysin. J. Am. Chem. Soc. **96**:6628–6630.
 21. Müller, G., and K. N. Raymond. 1984. Specificity and mechanism of ferrioxamine-mediated iron transport in *Streptomyces pilosus*. J. Bacteriol. **160**:304–312.
 22. Naegeli, H.-U., and W. Keller-Schierlein. 1978. 195. Stoffwechselprodukte von Mikroorganismen. Eine neue Synthese des Ferrichroms: enantio-Ferrichrom. Helv. Chim. Acta **61**:2088–2095.
 23. Neilands, J. B. 1952. A crystalline organo-iron pigment from a fungus, *Ustilago sphaerogena*. J. Am. Chem. Soc. **74**:4846–4847.
 24. Neilands, J. B. 1981. Iron absorption and transport in microorganisms. Annu. Rev. Nutr. **1**:27–46.
 25. Neilands, J. B., T. J. Erickson, and W. H. Rasttetter. 1981. Stereospecificity of the ferric enterobactin receptor of *Escherichia coli* K-12. J. Biol. Chem. **256**:3831–3832.
 26. Norrestam, R., B. Stensland, and C.-H. Brändén. 1975. On the conformation of cyclic iron containing hexapeptides. The crystal and molecular structure of ferrichrysin. J. Mol. Biol. **99**:501–506.
 27. Raymond, K. N., G. Müller, and B. F. Matzanke. 1984. Complexation of iron by siderophores. A review of their solution and structural chemistry and biological function. Top. Curr. Chem. **123**:49–102.
 28. Schneider, R., A. Hartmann, and V. Braun. 1981. Transport of the iron ionophore ferrichrome in *E. coli* K-12 and *S. typhimurium* LT 12. FEMS Microbiol. Lett. **11**:115–119.
 29. Sillen, I. G., and A. E. Martell. 1964. Stability constants of metal-iron complexes. Special Publication no. 17. The Chemical Society of London, London, U.K.
 30. van der Helm, D., J. R. Baker, D. L. Eng-Wilmot, M. B. Hossain, and R. A. Ioghry. 1980. Crystal structure of ferrichrome and a comparison with the structure of ferrichrome A. J. Am. Chem. Soc. **102**:4224–4231.
 31. Wayne, R., K. Frick, and J. B. Neilands. 1976. Siderophore protection against colicins M, B, V and 1a in *Escherichia coli*. J. Bacteriol. **126**:7–12.
 32. Winkelmann, G. 1979. Evidence for stereospecific uptake of iron chelates in fungi. FEBS Lett. **97**:43–46.
 33. Winkelmann, G., and V. Braun. 1981. Stereoselective recognition of ferrichrome by fungi and bacteria. FEMS Microbiol. Lett. **11**:237–238.
 34. Zähler, H., R. Hütter, and E. Bachmann. 1960. Stoffwechselprodukte von Actinomyceten. 23. Zur Kenntnis der Sideromycinwirkung. Arch. Microbiol. **36**:325–349.
 35. Zalkin, A., J. D. Forrester, and D. H. Templeton. 1966. Ferrichrome A tetrahydrate. Determination of crystal and molecular structure. J. Am. Chem. Soc. **88**:1810–1814.