Biometals (2009) 22:679–689
DOI 10.1007/s10534-009-9233-4

The redox hypothesis in siderophore-mediated iron uptake

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Received: 31 October 2008 / Accepted: 23 March 2009 / Published online: 9 April 2009

Abstract The viability of iron(III/II) reduction as the initial step in the in vivo release of iron from its thermodynamically stable siderophore complex is explored.

Keywords Iron bioavailability · Iron transport · Redox potentials · Redox kinetics

Siderophores and the iron paradox

Almost all organisms have evolved to require iron for biological processes integral to supporting life, in part due to the abundance of iron on the planet. However, much of the time, iron is not bioavailable due to its readiness to form insoluble oxides at physiological pH. To solve this problem, many organisms have evolved the ability to produce proteins for the binding of iron, or small molecules called siderophores that are specific for sequestering iron in the presence of other metals. Siderophores have evolved to have high thermodynamic affinity for binding iron(III), which upon chelation will prevent the hydrolysis and subsequent precipitation of the metal, and allow specific recognition and uptake of the metal at the cell surface.

Another function of the siderophore is to control the redox properties of the iron center. This review will focus on the redox properties of iron-siderophore complexes and the possible importance of reduction in the iron uptake cycle of many cells. There have been a number of other reviews on the topic of iron-siderophore complex thermodynamic stability and mechanism of complex formation, as well as the uptake process in a variety of organisms (Albrecht-Gary and Crumbliss 1998; Boukhalfa and Crumbliss 2002; Crichton 2001; Crumbliss 1991; Crumbliss and Harrington 2008; Dhungana and Crumbliss 2005; Miethke and Marahiel 2007; Raymond and Dertz 2004; Winkelmann 1991). As such those topics will not be discussed at great length here. In this review, we will focus on the redox properties of iron when bound in a siderophore complex and the relationship of this process to in vivo iron release to the cell.

Redox potential shift

Redox control by chelation

The one-electron reduction reaction of aqueous iron(III) to iron(II) has a potential of +770 mV versus NHE. A normal byproduct of many biological reactions is the superoxide anion, which with an O_2/O_2^- redox potential of −160 mV versus NHE at

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pH 7.0 will reduce aqueous iron(III) (Eq. 1). The iron(II) that is produced can then interact with hydrogen peroxide present in aerobic systems to produce hydroxyl radicals (Eq. 2). This series of reactions catalyzed by free iron, called the Haber–Weiss cycle, produces reactive oxygen species which can damage cells through reactions with lipids and other biomolecules (Haber and Weiss 1934).

$$Fe^{3+} + O_2^- \leftrightarrows Fe^{2+} + O_2 \quad (1)$$

$$Fe^{2+} + H_2O_2 \leftrightarrows Fe^{3+} + OH^{\bullet} + OH^- \quad (2)$$

Redox studies of iron-siderophore complexes have shown them to have much lower redox potentials for the Fe(III)/Fe(II) redox couple, ranging from −82 mV versus NHE for rhizoferrin to −990 mV versus NHE for the complex of enterobactin with iron(III) (Fig. 1) (Carrano et al. 1996; Cooper et al. 1978; Lee et al. 1985). The chelation of iron(III) by siderophores shifts the iron redox potential to much more negative values, effectively controlling the redox behavior of the metal center and preventing damage to the organism by redox cycling and production of reactive oxygen species. This shift of redox potential is due to a strong selectivity of the siderophore donor groups for iron(III) over iron(II) (Boukhalfa and Crumbliss 2002; Dhungana and Crumbliss 2005). The hard negatively charged oxygen donors which comprise the metal binding site in most siderophores effectively stabilizes the metal center in the higher 3+ oxidation state, making it more difficult to reduce the complexed metal ion.

The deep thermodynamic well for the formation of the iron-siderophore complex and the resultant shift in redox potential, coupled with the slow kinetics of iron(III) release, pose a problem to organisms in need of iron for survival. It is necessary for the organism to obtain the metal from the complex in a time- and site-specific manner, where the metal is released at the surface of the receiving cell or following uptake of the entire complex. This must be achieved so that the metal is released to specific iron-requiring sites within the cell, and not to another location.

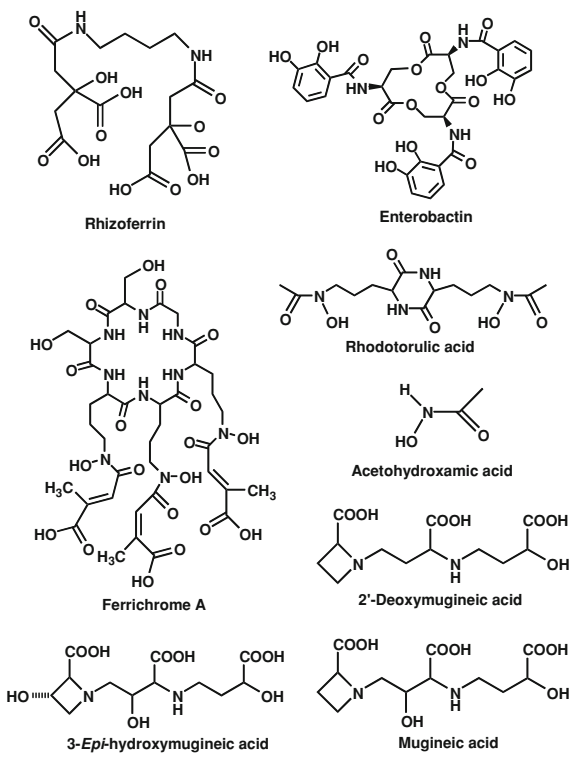

Fig. 1 Structures of selected natural siderophores, phytosiderophores, and acetohydroxamic acid

The redox hypothesis for iron release

Three main mechanisms of iron release from its thermodynamically stable siderophore complex have been proposed: hydrolysis of the siderophore, proton-assisted dissociation of the complex, and reduction of the metal center (Albrecht-Gary and Crumbliss 1998; Barchini and Cowart 1996; Dhungana and Crumbliss 2005). While an example of siderophore hydrolysis has been observed in nature, it seems problematic due to the great metabolic cost associated with the constant production of siderophores (Greenwood and Luke 1978; Raymond et al. 2003). A proton-assisted mechanism for iron release poses problems due to the extremely low pH required to promote complete dissociation of the iron(III)-siderophore complex, although it does allow recycling of the siderophores after delivery of the metal to the cell.

The redox hypothesis proposes that reduction of the metal center via in vivo reducing agents, including both small molecules and reductase enzymes, promotes the release of iron from the iron-siderophore complex. Reduction of iron(III) to iron(II) facilitates metal release by decreasing the thermodynamic stability of the siderophore complex, as well as increasing the kinetics of ligand exchange (Raymond and Dertz 2004; Richens 2005; Wilkins 1991). The reduction of the metal center from the 3+ oxidation

state to the 2+ oxidation state decreases the charge density of the metal center, converting it from a hard to a borderline hard/soft Lewis acid, which decreases its affinity for the hard negatively charged oxygen donors of the siderophores. The relationship between the stability of the iron(III) and iron(II) complexes and the redox potential can be derived from the Nernst equation as in Eq. 3,

$$E_{\text{complex}} = E^0_{\text{aq}} - 59.16 \log \frac{(\beta^{III})}{(\beta^{II})} \quad (3)$$

where E_{complex} represents the redox potential of the iron-siderophore complex, E^0_{aq} represents the redox potential of the $Fe(H_2O)_6^{3+}/Fe(H_2O)_6^{2+}$ redox couple, β^{III} is the overall thermodynamic stability constant for the iron(III)-siderophore complex and β^{II} is the stability constant of the iron(II)-siderophore complex. From Eq. 3, we can see that chelators having iron-complex redox potentials lower than +770 mV versus NHE will favor stabilization of bound iron in the 3+ oxidation state, while chelators having complex redox potentials greater than +770 mV will stabilize bound iron in the 2+ oxidation state. The reduction of iron from the 3+ to the 2+ oxidation state also increases the kinetics of iron-ligand exchange. For example, in aquated iron(III), the water exchange rate is 1.6×10^2 s^{-1}, as compared to 4.4×10^6 s^{-1} for iron(II) (Dodgen et al. 1981; Grant and Jordan 1981; Swaddle and Merbach 1981). This difference of four orders of magnitude is largely due to the decreased charge density of the metal in iron(II).

In this context, reduction can be seen as a molecular switch, where reduction of the iron center facilitates a number of important processes in the iron acquisition cycle. Reduction of iron can serve to facilitate the solubilization of the metal in the environment, as iron(II) has higher solubility and lower Brønsted acidity than iron(III), as well as more rapid ligand exchange rates (Dhungana et al. 2007). Siderophores also have a reasonable affinity for iron(II), and stabilization of the iron in the 3+ oxidation state due to binding by the siderophore will facilitate re-oxidation of the metal to iron(III). Reduction also serves as a switch for removing iron from the siderophore complex, where thermodynamic stability of the siderophore complex decreases and kinetic lability of the siderophore complex increases to allow time- and site-specific delivery of the metal to the cell.

Shifting iron-siderophore complex redox potentials

In almost all iron-siderophore complexes for which data are available, there is an observed variation of the redox potential with pH. Most siderophores contain acidic moieties as binding groups, such as hydroxamic acids and catechol donor groups, and iron must compete with protons for binding to these acidic donors. As the solution pH is lowered, the proton concentration increases, and becomes a more effective competitor with Fe(III) for the acidic binding moieties (Boukhalfa and Crumbliss 2002; Dhungana and Crumbliss 2005; Dhungana et al. 2003). This results in a decrease in effective complex stability as more siderophore donor groups dissociate from the iron due to protonation. Reduction of the metal center amplifies this by reducing the effectiveness of the metal center to compete with protons. The reduced charge density of iron(II) compared to iron(III) makes it less able to compete with protons for binding to the donor moieties, resulting in increased protonation constants for the iron(II) complex compared to the iron(III) complex (Fig. 2). As the primary coordination sphere is the main determinant of the redox potential of the complex, dissociation of binding groups from iron through aquation will increase the redox potential of the metal center. This results in a gradual shift of redox potential to more positive values with variation of solution pH. The redox potential can be related quantitatively to

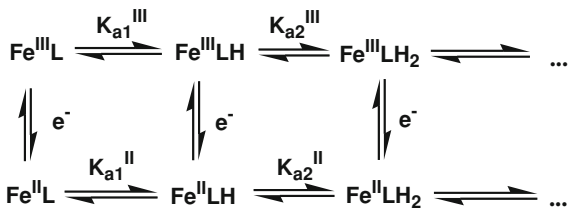

Fig. 2 Diagram illustrating the coupled protonation and reduction of the metal center in siderophore complexes. Upon reduction, the affinity of iron for the siderophore decreases, resulting in more facile protonation of the complex. This results in a shift of observed complex redox potential with pH. Here, K_a is defined as the proton association constant, where $K^{\text{II}}_{a1} > K^{\text{III}}_{a1} > K^{\text{II}}_{a2} > K^{\text{III}}_{a2} > K^{\text{II}}_{a3}$, etc

the solution pH through the Nernst equation, shown in Eq. 4,

$$E_{\mathrm{rxn}} = E_{\mathrm{complex}} + 59.16\log\left(1 + K_{a1}^{\mathrm{II}}[\mathrm{H}^+] + \beta_{a2}^{\mathrm{II}}[\mathrm{H}^+]^2 + \cdots\right) - 59.16\log\left(1 + K_{a1}^{\mathrm{III}}[H^+] + \cdots\right) \quad (4)$$

where E_{rxn} is the potential at which the reduction takes place, E_{complex} is the redox potential of the fully coordinated iron(III)-siderophore complex, K_a^{II} is the protonation constant of the iron(II) complex, K_a^{III} is the protonation constant of the iron(III) complex (Fig. 2), and $\beta_{\mathrm{an}}^{\mathrm{II}}$ are the cumulative protonation constants for the siderophores with iron(II). The variation of redox potential with pH of some iron-siderophore complexes is shown in Fig. 3.

This pH dependence of the redox potential potentially has in vivo implications. The negative redox potential of many complexes of iron(III) with natural siderophores falls out of the range of biological reductants. However, in some cases upon uptake of the iron-siderophore complex, the complexes are sequestered by intracellular compartments of lower pH (Moore et al. 2003). This decrease in pH can potentially increase the complex redox potential, shifting E_{rxn} into the range of biological reductants,

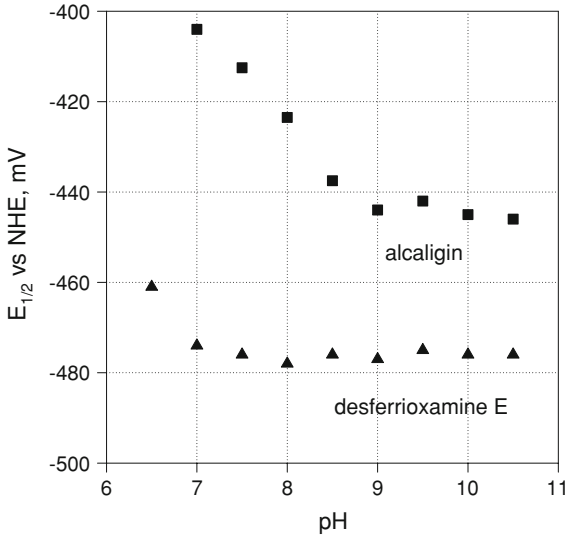

Fig. 3 The pH dependence of the redox potential ($E_{1/2}$) of the Fe complex of alcaligin and desferrioxamine E. Reprinted with permission from (Spasojevic et al. 1999). Copyright 1999 American Chemical Society

resulting in reduction of the metal center. This results in less effective competition with protons for binding by the donor moieties, protonation of binding groups, and a decrease in effective complex stability that can potentially result in exchange of iron from the complex to other binding sites within the cell.

Another factor that can potentially play a role in the exchange of iron in biological systems is the presence of competing chelators. It has been observed that competing chelators can shift the potential at which a complex is reduced due to coupled equilibria involving the competing chelator and the iron(III)-siderophore complex (Boukhalfa and Crumbliss 2002). As mentioned earlier, iron-siderophore complexes have very negative redox potentials, making the reduction of the metal center thermodynamically unfavorable. However, upon reduction, the lowered thermodynamic stability of the complex facilitates iron(II) dissociation from the siderophore, followed by complexation by a secondary chelator present. In the cases where this secondary chelator stabilizes the iron(II) oxidation state, the overall equilibrium is shifted toward the formation of iron(II) (Eqs. 5–7). This is manifested by a shift of the "effective" iron-siderophore redox potential (E_{rxn}) to more positive values, as described by Eq. 8,

$$\mathrm{Fe^{III}sid} + e^- \overset{E_{1/2}^{\mathrm{Fe(III)sid}}}{\rightleftarrows} \mathrm{Fe^{II}sid} \quad (5)$$

$$\mathrm{Fe^{II}sid} \overset{\frac{1}{K^{\mathrm{Fe(II)sid}}}}{\rightleftarrows} \mathrm{Fe^{II}} + \mathrm{sid} \quad (6)$$

$$\mathrm{Fe^{II}} + \mathrm{L} \overset{K^{\mathrm{Fe(II)L}}}{\rightleftarrows} \mathrm{Fe^{II}L} \quad (7)$$

$$E_{\mathrm{rxn}} = E_{1/2}^{\mathrm{Fe(III)sid}} + \frac{0.0592}{n}\log\left(1 + \frac{K^{\mathrm{Fe(II)L}}[\mathrm{L}]}{K^{\mathrm{Fe(II)sid}}[\mathrm{sid}]}\right) \quad (8)$$

where $K^{\mathrm{Fe(II)L}}$ is the stability constant of the complex of the competing ligand with iron(II), $K^{\mathrm{Fe(II)sid}}$ is the stability constant of the complex formed between iron(II) and the siderophore, n is the number of electrons transferred, E_{rxn} is the observed redox potential of the iron(III)-siderophore system coupled with iron(II) chelation, and $E_{1/2}^{\mathrm{Fe(III)sid}}$ is the redox potential of the iron(III)-siderophore complex. As $K^{\mathrm{Fe(II)L}}$ increases for different competing chelators, greater stabilization is offered to the 2+ oxidation state of the metal ion, resulting in a shift to more

positive redox potentials or easier reduction of the metal center.

This concept is demonstrated in the plots of Eq. 8 shown in Fig. 4, where the effective redox potential of the iron(III)-siderophore-L system is shown to vary with the identity of competing ligand L for four representative siderophores. In Fig. 4 the systems containing the chelator 1,10-phenanthroline are shown to have the most positive redox potential, as it is the chelator with the highest affinity for iron(II) included in the plots. However, citrate, a biological iron(III) chelator, does not stabilize iron(II) as effectively, resulting in a minimal shift in redox potential. A change in the concentrations of secondary chelator or siderophore will also result in a corresponding shift in system redox potential (E_{rxn}) due to the shift of the competing equilibria toward the chelator with higher concentrations.

Another factor that controls the redox potential of iron-siderophore complexes is the denticity of the siderophore (Albrecht-Gary and Crumbliss 1998; Crumbliss and Harrington 2008). This can be observed by comparing the pH-limited redox potentials of the iron complexes of hexadentate siderophores to those of complexes with siderophores featuring homologous

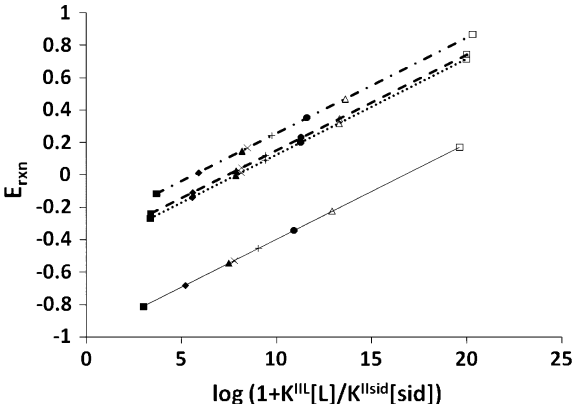

Fig. 4 Graph demonstrating the effect of coupled equilibria involving competing chelators on the observed reaction redox potential (E_{rxn}) for iron-siderophore complex reductions. Plot of E_{rxn} versus log $(1 + K^{IIL}[L]/K^{IIsid}[sid])$ according to Eqs. 5–8. Conditions: $[sid] = [L] = 10^{-5}$ M. Siderophores (sid): enterobactin (————), desferrioxamine B (·····), ferrichrome A (——) and aerobactin (-·-·-·-). Secondary ligands (L): citric acid (■), cystine (◆), glycine (▲),ethylene diammine (x), histidine (+), 2-pyridine carboxylic acid (●), EDTA (Δ), and 1,10-phenanthroline (□). Data taken from various literature sources (Crumbliss and Harrington 2008; Martell and Smith 1989)

binding groups, but lower denticity. One such example using the hydroxamic acid donor group can be seen by comparing the redox potentials of the hexacoordinate iron(III) complexes of ferrichrome A (hexadentate; −440 mV vs. NHE), rhodotorulic acid (tetradentate; −359 mV vs. NHE), and acetohydroxamic acid (bidentate; −293 mV vs. NHE), shown in Fig. 1 (Carrano et al. 1979; Spasojevic et al. 1999; Wawrousek and McArdle 1982; Wirgau et al. 2002). It is also worth noting that the donor groups incorporated in siderophores will influence the redox potential of the complexes, with catechol complexes having the most negative redox potentials, followed by hydroxamic acid complexes and α-hydroxycarboxylic acid complexes featuring the least negative redox potentials (see the above redox potentials of rhizoferrin, an α-hydroxycarboxylic acid and enterobactin, a catechol siderophore) (Crumbliss and Harrington 2008).

Redox potential shift due to ternary complex formation

As was stated earlier, the redox potentials of iron-siderophore complexes are often too negative to allow the reduction of the metal center by biological reductants. While it is possible that a decrease in pH would shift the complex redox potential into an accessible range, there are many cases where such a shift would require a pH shift out of reach to organisms. An alternative mechanism of shifting the redox potential of iron-siderophore complexes into the range of biological reductants was proposed by Mies et al., involving the formation of ternary complexes between the iron-siderophore complex and iron(II) chelators (Mies et al. 2006). In their experiments, the removal of iron(III) from the desferrioxamine B complex catalyzed by glutathione and ascorbic acid was monitored both in the presence and absence of an iron(II) chelator, here sulfonated bathophenanthroline (BPDS). In the absence of BPDS, no reduction and removal of iron from the siderophore was observed, while the addition of bathophenanthroline facilitated the removal of iron from the complex and the formation of the iron(II)-BPDS complex. It was proposed based on spectrophotometric evidence that a ternary complex was formed between iron(III), desferrioxamine B, and BPDS (Fig. 5). In the case of reductive release of iron by reaction with NADH, parallel paths were

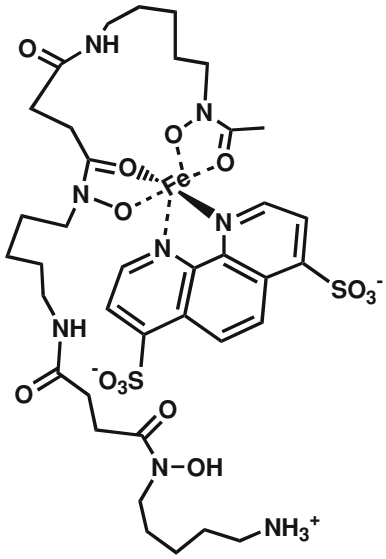

Fig. 5 Proposed structure of the ternary complex formed between iron(III), desferrioxamine B, and BPDS (Mies et al. 2006)

observed, one involving the formation of a ternary complex and a second involving direct reduction of the metal center by NADH (Mies and Crumbliss, in preparation). The formation of the ternary complex shifts the complex redox potential to a more positive value due to the increased stabilization of the 2+ oxidation state afforded by the mixed donor inner coordination sphere. The competing equilibria with the ternary ligand and the siderophore for binding iron causes the iron redox potential to shift based on the redox potential of the iron-ternary ligand complex, the concentration of the ternary ligand, and the redox potential of the siderophore complex.

Related observations were reported by Weber et al., investigating the potential role of the reductive mechanism in the siderophore mediated uptake of iron in various plants (Weber et al. 2008). Two iron uptake strategies have been observed to be operative in plants; strategy I involves reduction of the metal center by biological reductants at the plasma membrane of the root cell, followed by hydrolysis of the complex by increased secretion of protons and uptake of the iron(II); strategy II, observed primarily in grasses, involves uptake of the entire iron-siderophore complex by receptors on the root cell surface, followed by reduction within the root cell (Schmidt 2003). Graminaceous plants secrete a class of siderophores called the phytosiderophores (PS) related to the siderophore mugineic acid (Fig. 1). However, it was shown that iron could be removed from PS by nicotinamine in the presence of ascorbic acid (Weber et al. 2008). Weber et al. proposed a mechanism involving formation of a ternary complex between the iron(III)-PS complex and the biological reducing agent ascorbic acid that resulted in reduction of the metal center, followed by exchange of the ascorbate oxidation product with nicotinamine, leading to final formation of the iron(II)-nicotinamine complex. While the proposed mechanism involved the formation of a ternary complex with ascorbate, the formation of a ternary complex with iron(III), phytosiderophore, and nicotinamine, similar to the Mies et al. study, was not ruled out.

The ternary complex reduction mechanism may also be biologically relevant due to the presence of tetradentate siderophores in nature, such as rhodotorulic acid and alcaligin. These tetradentate siderophores form complexes with reduced thermodynamic stability as compared to their hexadentate analogs, but also leave two vacant sites on the metal center when binding in a 1:1 stoichiometry. These vacant (aquated) binding sites may be filled by ternary binding ligands, as described above, or by reducing agents that directly react with the metal center. Klumpp et al. recently reported on the crystal structure of a ternary complex formed between iron(III), the mixed-donor tetradentate bacterial siderophore pyochelin, and cepabactin, a bidentate hydroxypyridinone siderophore (Fig. 6) (Klumpp

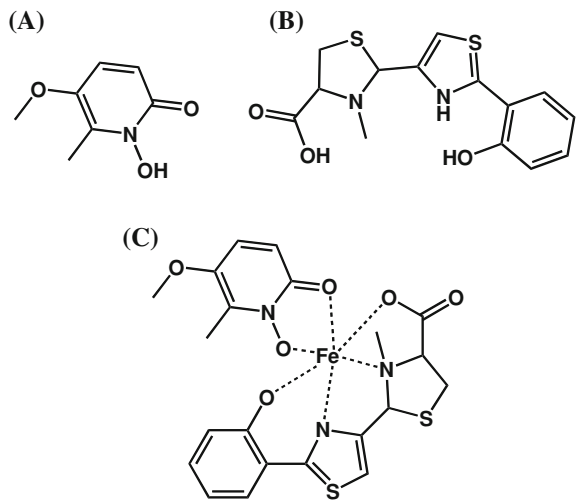

Fig. 6 **a** Cepabactin, a cyclic hydroxypyridinone siderophore, **b** pyochelin, a linear tetradentate siderophore, and **c** ternary complex formed between iron(III), pyochelin, and cepabactin

et al. 2005). The crystal structure of the ferric-pyochelin receptor protein, FptA also suggests the possible involvement of a ternary complex in the release of iron from the pyochelin complex (Cobessi et al. 2005).

Biological paths to the reductive mechanism

There have been a number of proteins identified that contribute to the iron uptake process in bacteria and fungi by catalyzing the reduction of iron (Cowart 2002; Matzanke et al. 2004. Reductases have been found to act as an in vivo reductant of iron(III) in the presence of an electron source, such as flavin mononucleotide or NADH. These reductases not only aid in removing iron from chelating agents in the environment, but also the removal of iron from minerals. At the present time, very few of the reductases have been structurally characterized while binding iron complexes, so little is known about the mechanism of redox catalysis (Chiu et al. 2001; Liger et al. 2004). A list of identified reductases is shown in Table 1.

Siderophore electron transfer reactions: kinetics and mechanism

In the study of redox reactions, the kinetics and mechanism of electron transfer are of the utmost importance. This is also of importance in understanding siderophore mediated iron bioavailability. As the siderophore varies, the mechanism and rate of reduction can vary, which can affect the viability of a redox process in vivo. There are two general mechanisms of electron transfer in metal complexes: the inner-sphere mechanism and the outer-sphere mechanism (Henderson 1994; Wilkins 1991). The inner-sphere reduction mechanism involves the transfer of electrons through direct coordination of iron by the reducing molecule, while the outer-sphere mechanism involves the transfer of electrons from the reductant to iron without disturbing the inner coordination sphere of iron. In some cases the two mechanisms can be distinguished by comparing the rate of reduction to the rate of ligand exchange. In cases where the rate of electron exchange is more rapid than the exchange of donor groups, outer-sphere electron exchange is the likely mechanism. An outer-sphere mechanism is also possible in cases where there are no suitable donor atoms for coordinating iron in the reducing molecule.

Reduction mechanisms involving the formation of a ternary complex may be classified as either inner or outer-sphere depending on the identity of the ternary ligand. If the reducing agent acts as the ternary ligand and binds in the inner sphere, then the mechanism is classified as an inner-sphere mechanism. However, if the ternary complex is formed with an iron(II) chelator that is not a reducing agent, as in the Mies et al. study, then the electron exchange mechanism is outer-sphere (Mies et al. 2006).

There are few reports of well-defined studies of the kinetics or mechanism of reduction of iron-siderophore complexes. Kazmi et al. report that the mechanism of reduction of ferrioxamine B by dithionite, V(II), and Eu(II) occurs through an outer sphere electron transfer mechanism (Kazmi et al. 1982). However, the reduction of ferrioxamine B by Cr(II) occurs through a ligand bridged mechanism. Spectral evidence suggests the formation of a bridged complex prior to electron transfer, where iron(III) is bound by two hydroxamate donor groups from ferrioxamine B and Cr(II) is bound by the third hydroxamate group. These authors argue that the inner sphere mechanism is limited by slow water exchange rates on V(II) and Eu(II), but not for Cr(II), which has rapid solvent exchange rates due to Jahn–Teller effects. Similar observations were made in the case of ferrichrome (Kazmi et al. 1984). In the case of ferrichrome A, the reduction of the iron(III) complex by Cr(II) proceeds by an outer-sphere mechanism due to the steric influence of the bulkier pendant arms. Ferrioxamine E, however, was reported to undergo an inner-sphere reduction with both Eu(II) and V(II) (Kazmi et al. 1986). It was also reported that the reduction of the iron(III)-acetohydroxamic acid complex by dithionite proceeds by an outer-sphere mechanism (Bradić and Wilkins 1984; Lambeth and Palmer 1973).

Conclusions

In view of the thermodynamically stable complexes formed between iron and siderophores, an explanation is required for the ability of organisms to obtain

Table 1 Ferric chelate reductases, their source, and ferric chelate substrate

Species	Name	Fe(III) chelator	Ref
Bacteria			
A. fulgidus		EDTA	Vadas et al. (1999)
E. coli	FhuF	Hydroxamates	Matzanke et al. (2004)
E. coli	Several	Several	Vartivarian and Cowart (1999)
G. sulfurreducens		NTA	Kaufmann and Lovley (2001)
L. monocytogenes		NTA	Barchini and Cowart (1996)
L pneumophila		Citrate	Poch and Johnson (1993)
L. pneumophila	Pyomelanin	None	Chatfield and Cianciotto (2007)
M. gryphiswaldense	MSR-1	Citrate	Xia et al. (2007)
N. gonorrhoeae		Citrate, pyrophosphate	Le Faou and Morse (1991)
P. aeruginosa	Ferripyoverdine reductase	Pyoverdine	Halle and Meyer (1992)
P. aeruginosa	FPIR and FCIR	Citrate, pyochelin	Cox (1980)
P. aeruginosa	Several	Several	Vartivarian and Cowart (1999)
P. dentrificans	FerA and FerB	Citrate	Mazoch et al. (2004)
R. sphaeroides	IrA and IrB	Acetate	Moody and Dailey (1985)
Synechtocystis	DrgA	Many	Takeda et al. (2007)
S. pombe	frp1	Citrate	Roman et al. (1993)
S. cerevisiae	FRE1, FRE2, FRE3, FRE4	DFB, RA, Ent, TAFC, FC	Yun et al. (2001)
S. cerevisiae	YLR011wp	Cyanide	Liger et al. (2004)
T. scotoductus		EDTA, NTA	Moller and van Heerden (2006)
Fungus			
B. dermatitis		None	Zamowski and Woods (2005)
H. capsulatum	GSH-FR	RA, FC, dimerum acid	Timmerman and Woods (2001)
P. brasiliensis		None	Zamowski and Woods (2005)
P. chryosporin	Cellobiose oxidase	Acetate	Kremer and Wood (1992)
S. schenckii		None	Zamowski and Woods (2005)
Plant			
Arabidopsis	FRO2	EDTA	Robinson et al. (1999)
Arabidopsis	Cytochrome b561	EDTA, citrate, cyanide	Berczi et al. (2007)
A. thaliana		EDDHA	Yi and Guerinot (1996)
C. maxima	NADH:nitrate reductase	Citrate	Redinbaugh and Campbell (1983)
C. sativus	CsFRO1	EDTA	Waters et al. (2007)
P. sativum	FRO1	EDTA	Waters et al. (2002)
Z. mays	FCR	Citrate, EDTA	Sparla et al. (1999)
Mammal			
Human		NTA, cyanide	Inman et al. (1994)
Mouse	Cytochrome b561	NTA	Vargas et al. (2003)
Mouse	Dcytb	NTA	McKie et al. (2002)
Mouse	SDR2	NTA	Vargas et al. (2003)
Mouse	Steap3	NTA	Ohgami et al. (2005)
Pig	Dihydropteridine reductse	Cyanide	Lee et al. (2000)
Rat	Paraferritin	NTA	Umbreit et al. (1996)

the iron from the siderophore on demand. A redox mechanism provides a viable pathway for achieving the release of iron from the siderophore in a time- and site-specific manner at the cell surface or within the cell. The decrease in complex stability and increase in lability of first coordination shell ligand exchange due to reduction of the iron center provides a viable pathway for the uptake of iron by an organism. The ability to shift the redox potential of the siderophore complex due to changing pH or a competing chelator also allows the chemistry associated with a particular cell, or environment within a cell, to fine tune the effective redox potential, thus making iron reduction accessible to in vivo reductants.

Acknowledgments We thank the National Science Foundation (CHE 0809466) for their support of our work in this area.

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