

Reductive Solubilization of Fe(III) by Certain Products of Plant and Microbial Metabolism as a Possible Alternative to Siderophore Secretion

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Fe(III) oxides hydrated to various extents are the most abundant iron species in soil. Their extremely low solubility makes them almost unavailable to living organisms. To cope with iron deficiency, many microorganisms synthesize and secrete siderophores [1–5], which are low-molecular-weight compounds that specifically chelate Fe(III) to form stable complexes, into the soil (association constants of 10^{29} – 10^{32} [4]). Plants either secrete similar chelating compounds, namely, phytosiderophores, or use exogenous siderophores produced by microorganisms.

The ligand groups of siderophores are of two major types: (1) hydroxamic groups characteristic of most soil microorganisms and (2) catechol groups $\text{Ph}(\text{OH})_2$ containing hydroxyl groups in the orthoposition. However, other chelating structures, including α -hydroxyacids and 2-(2-hydroxyphenyl)-oxazolin, are also possible [4].

In some microorganisms, organic acids with a relatively low Fe(III) affinity are used to chelate iron. In microorganisms with the citric acid cycle, the mechanisms of iron transport across the cell membrane and assimilation of ferric ion, an inorganic component of the transported complex, are similar to those in microorganisms using siderophores [2, 5]. Less common pathways of iron assimilation have also been described [5].

Cells can assimilate only iron released from its complex. Several mechanisms of Fe(III) release are known: (1) an internal membrane-associated chelator substitutes for the external transporting ligand at the cell surface and further transports the iron ion into the cell (the taxicab mechanism); (2) the chelating complex reduces iron at the cell surface and releases it intracellularly (the reductive taxi mechanism); or (3) the chelating complex is transported into the cell, where iron is then reductively released with the involvement of intracellular enzymes (ferric siderophore reductases) and physiological reducing agents [2]. The organic component of

the complex can be used as a carbon source or released extracellularly in its initial form to chelate a new ferric ion (the shuttle mechanism).

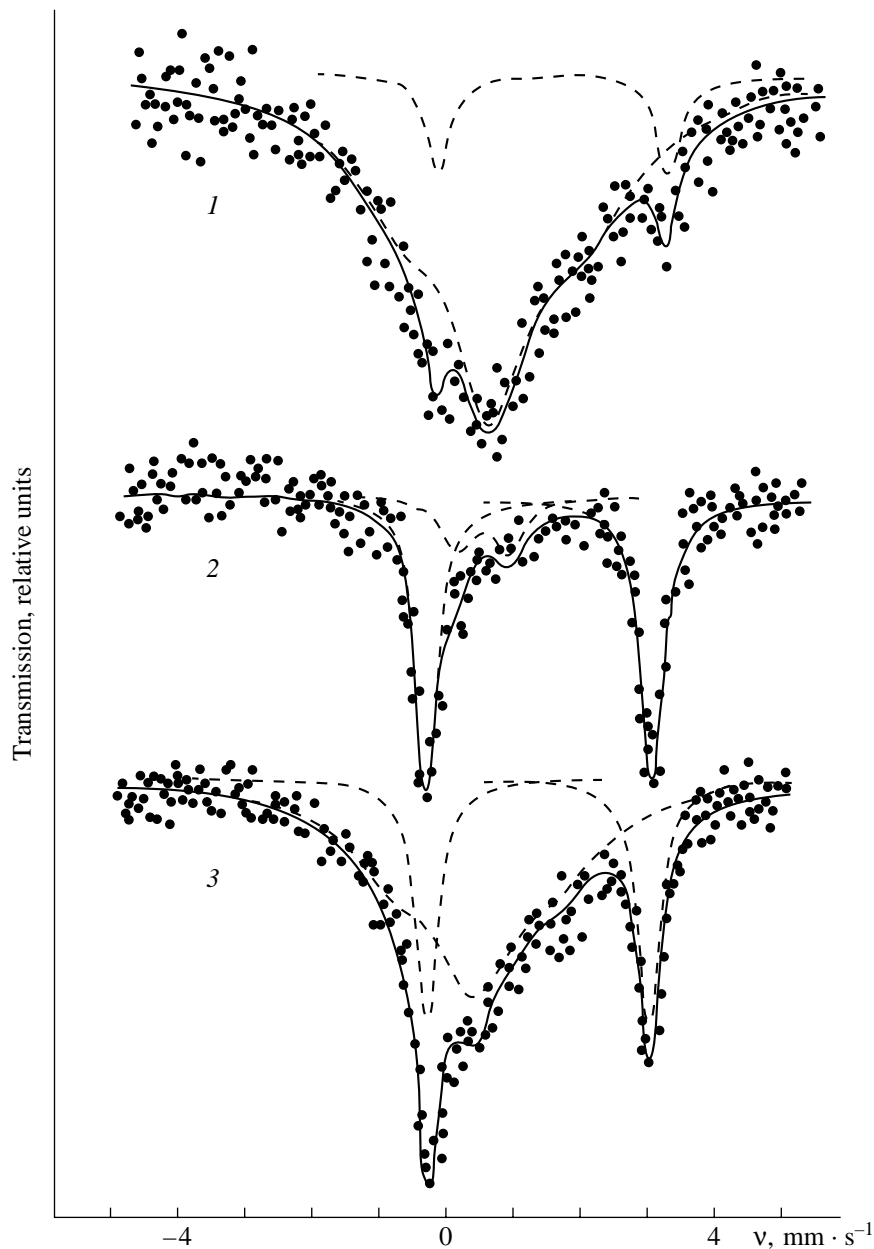
Thus, at normal physiological pH values, Fe(III) is either bound to a stable complex or hydrolyzed completely. In any case, if it is not included in magnetite or ferritin, which are intracellular iron stores [5–7], ferric iron undergoes reduction and is assimilated from its soluble complexes.

The *in situ* chemical reduction of ferric iron is a fundamentally different way to increase its bioavailability. The only Fe(III)-solubilizing agent that the aerobic soil bacterium *Rhizobium leguminosarum* secretes under iron-deficient conditions was identified as anthranilic acid [3]. It was suggested that anthranilic acid reduces Fe(III). Anthranilic acid is the key intermediate in the synthesis of *L*-tryptophan [8] and a metabolically important precursor of indole-3-acetic acid (IAA), known as the phytohormone auxin [9], which is synthesized by plants and many microorganisms [8, 10].

Due to the high chelating activity of tryptophan, IAA, anthranilic acid, and other metabolites of soil bacteria, the release of these compounds (e.g., upon decomposition of dead cells) may result in their chemical conversion with the involvement of metal ions [11–13]. In this work, we demonstrated by Mössbauer spectroscopy that formation of Fe(III) complexes with anthranilic acid, tryptophan, and IAA in nitrate aqueous solutions (a model of aerobic conditions) was accompanied by gradual reduction of Fe(III).

To prepare $^{57}\text{Fe}(\text{NO}_3)_3$, enriched metallic iron (95.6% ^{57}Fe ; Center of Radionuclide Diagnostics, Russian Foundation for Basic Research) was dissolved by heating in a small excess of 50% nitric acid (analytical grade). Mössbauer measurements were performed on frozen samples at 80 K. The samples contained $^{57}\text{Fe}(\text{NO}_3)_3$ in aqueous *L*-tryptophan (Trp; Sigma), anthranilic acid (AA; Sigma), or IAA (Eastman Kodak) at a molar ratio of 1 : 3. The concentrations of Trp, AA, and IAA in these samples were up to 0.03 M.

After the addition of $^{57}\text{Fe}(\text{NO}_3)_3$, the samples were kept for a specified time and then rapidly frozen. The



Mössbauer spectra at 80 K of $^{57}\text{Fe}(\text{NO}_3)_3$ -containing aqueous solutions of (1) Trp, (2) AA, and (3) IAA frozen (1) 2.5, (2) 26, and (3) 0.4 h after the addition of Fe(III) (see the table; samples 1, 4, and 2, respectively).

frozen samples were placed in the holder of a Mössbauer spectrometer equipped with a specially constructed cryostat filled with liquid nitrogen. The standard computerized processing of spectra consisted of calculations of the isomer shift (IS) relative to α -Fe, quadrupole splitting (QS), and integral and partial intensities of spectral components. The Mössbauer spectroscopic methods used were described in more detail elsewhere [12, 13].

The addition of Fe(III) to Trp, AA, or IAA led to the appearance of spectral components characteristic of Fe(II) in spectra measured at 80 K. The contribution of

these components increased markedly with an increase in the incubation time of the reaction mixtures before freezing and, in certain cases, became dominant (table). Typical spectra of $^{57}\text{Fe}(\text{NO}_3)_3$ in Trp, AA, and IAA are shown in the figure. The spectral components corresponding to Fe(II) in the high-spin state are represented by doublets (the dashed line) with high values of quadrupole splitting (table). The midportions of the spectra (see spectra 1 and 3) are relaxation components (also indicated by a dashed line) that characterize Fe(III) ions in the high-spin state [15].

Table 1. Mössbauer parameters^a calculated for spectra of frozen ⁵⁷Fe(NO₃)₃-containing aqueous solutions of Trp, IAA, and AA and the corresponding solid phases (*T* = 80 K)

Sample (doublet)	Component	Phase	Time ^b , h	IS ^c , mm/s	QS ^d , mm/s	Sr ^e , %
1	Fe ^{II} , Trp	Solution	2.5	1.37(2)	3.27(4)	9.1
2	Fe ^{II} , IAA	"	0.4	1.39(2)	3.30(5)	28.0
3	Fe ^{II} , IAA	"	51	1.37(1)	3.31(1)	97.3
4	Fe ^{II} , AA	"	26	1.36(2)	3.36(4)	80.9
5(1)	Fe ^{II} , AA	Suspension	0.2	1.61(3)	2.64(6)	3.2
5(2)	Fe ^{II} , AA	"	0.2	1.36(3)	3.36(6)	5.3
5(3)	Fe ^{III} , AA	"	0.2	0.51(1)	0.71(1)	91.5
6 ^f	Fe ^{III} , AA	Solid phase	–	0.47(5)	0.64(8)	–
7 ^f	Fe ^{III} , Trp	"	–	0.53(5)	0.70(5)	–
8	Fe ^{III} , IAA	Solid phase ^g	–	0.48(1)	0.70(1)	100

^a Confidence intervals indicated in parentheses are of the order of the last significant figure of the corresponding mean;

^b time from Fe(III) addition to Trp, AA, or IAA solution to freezing of the solution;

^c isomer shift (relative to α -Fe);

^d quadrupole splitting;

^e relative area of a given spectral component (with respect to the total area of the spectrum), which characterizes the relative content of this iron species (if the probability of observing the Mössbauer effect is the same for each species);

^f calculated from the data in [14];

^g sample 3 dried in air at *T* = 295 ± 3 K.

The solid phases (precipitates) of Fe(III) and Fe(II) anthranilate complexes were shown to have the chemical formulas Fe(II)(C₇H₆NO₂)₂ and Fe(III)(OH)(H₂O)(C₇H₆NO₂)₂ [11], which correspond to the coordination numbers 4 and 6, respectively. It was suggested [11] that both oxygen in the carboxyl group and nitrogen in the amino group are involved in the chelation of Fe(III) and Fe(II) ions in these compounds. This suggestion is consistent with the conclusions made in [13, 14] from a comparison of Mössbauer spectra of a number of compounds. The involvement of carboxyl and amino groups in iron chelation was also suggested for Trp [14].

The carboxyl group and indole nitrogen may be involved in iron chelation by IAA [12], because the orientation of its carboxyl group and entire side chain with respect to the indole plane can vary.

Due to the low solubility of the complexes, precipitation was observed in Fe(III) mixtures with Trp, AA, or IAA. Note that filtrates of these mixtures that were obtained a certain time after Fe(III) addition contained Fe(II) (table), suggesting that solubilization of Fe(III) through its chemical reduction by AA [3], IAA, and, although somewhat slower, by Trp, is possible. This is in contrast to siderophores, which chelate and dissolve only Fe(III) [4, 5]. Similar, although less pronounced, partial reduction of Fe(III) to Fe(II) was found in Mössbauer spectra measured in the course of synthesis of Fe(III) complexes with tryptophan and lysine, which included procedures of precipitation and extraction with nonaqueous solvents [14].

Fe(III) reduction by IAA was a relatively rapid process: about 30% of the Fe(III) was reduced within

25 min. Within 2 days, the solution contained only Fe(II) in the form of dark-violet complexes (table, samples 2 and 3). Interestingly, the reduction appeared to be reversible, and after this solution had been dried in air until crystal formation, all the iron appeared to be oxidized again (table, sample 8). Under aerobic conditions, fluctuating moisture, and in the presence of excess Fe(III), this redox cycling can serve to oxidatively degrade IAA secreted by microorganisms [10].

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REFERENCES

1. *Iron Transport in Microbes, Plants and Animals*, Winkelmann, G., Van der Helm, D., and Neilands, J.B., Eds., Weinheim: VCH, 1987.
2. Riquelme, M., *Microbiologia* (Madrid), 1996, vol. 12, pp. 537–546.
3. Rioux, C.R., Jordan, D.C., and Rattray, J.B.M., *Arch. Biochem. Biophys.*, 1986, vol. 248, pp. 175–182.
4. Bossier, P., Hofte, M., and Verstraete, W., *Adv. Microb. Ecol.*, 1988, vol. 10, pp. 385–414.
5. Briat, J.-F., *J. Gen. Microbiol.*, 1992, vol. 138, pp. 2475–2483.

6. Bauminger, E.R., Cohen, S.G., Dickson, D.P.E., *et al.*, *Biochim. Biophys. Acta*, 1980, vol. 623, pp. 237–242.
7. Hawkins, C., Treffry, A., and Mackey, Z.B., *et al.*, *Nuovo Cimento*, 1996, vol. 18D, pp. 347–352.
8. Costacurta, A. and Vanderleyden, J., *Crit. Rev. Microbiol.*, 1995, vol. 21, pp. 1–18.
9. Marumo, S., *Chemistry of Plant Hormones*, Boca Raton, CRC, 1986, ch. 2, pp. 9–56.
10. Patten, C.L. and Glick, B.R., *Can. J. Microbiol.*, 1996, vol. 42, pp. 207–220.
11. Dinsel, D.L. and Sweet, T.R., *Anal. Chem.*, 1963, vol. 35, pp. 2077–2081.
12. Kamnev, A.A. and Kuzmann, E., *Biochem. Mol. Biol. Int.*, 1997, vol. 41, pp. 575–581.
13. Kamnev, A.A. and Kuzmann, E., *Polyhedron*, 1997, vol. 16, no. 19, pp. 3353–3356.
14. Raudsepp, R. and Arro, I., *Izv. Akad. Nauk Est. SSR, Fiz., Mat.*, 1972, vol. 21, no. 2, pp. 187–192.
15. *Mössbauer Spectroscopy of Frozen Solutions*, Vortes, A. and Nagy, D.L., Eds., Budapest: Akad. Kiado, 1990.