Metal Ions in Biology and Medicine: vol. 10. Eds Ph. Collery, I. Maymard, T. Theophanides, L. Khassanova, T. Collery.
John Libbey Eurotext, Paris © 2008 pp. 191-196.

Iron(III) reduction by microbial autoinducer molecules: oxidative degradation of a signal as a chemical interference in remote cell–cell communication

Alexander A. Kamnev[1], Krisztina Kovács[2], Ernó Kuzmann[2,3], Leonid A. Kulikov[4], Yurii D. Perfiliev[4], Borbála Biró[5], Attila Vértes[2,3]

[1]*Laboratory of Biochemistry, Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, 410049 Saratov, Russia; e-mail: aakamnev@ibppm.sgu.ru)*
[2]*Laboratory of Nuclear Chemistry, Institute of Chemistry, Eötvös Loránd University, Budapest H-1117, Hungary*
[3]*Chemical Research Center, Hungarian Academy of Sciences, Budapest, Hungary*
[4]*Laboratory of Nuclear Chemistry Techniques, Department of Radiochemistry, Faculty of Chemistry, M.V. Lomonosov Moscow State University, 119992 Moscow, Russia*
[5]*Laboratory of Rhizobiology, Research Institute for Soil Science and Agricultural Chemistry, Hungarian Academy of Sciences, Budapest H-1025, Hungary*

ABSTRACT

A diversity of microbial metabolic processes and behavioural features are now known to be regulated via intercellular signalling which involves special low-MW diffusible molecules. The microbial producer cells respond to their own signals ("autoinducers") if the latter accumulate up to a threshold concentration, reflecting the cells' perception of the cell density ("quorum sensing"), mass transfer properties of the medium ("diffusion sensing") or a combination of these two, including also spatial cell distribution ("efficiency sensing"). Thus, it appears possible to regulate the microbial metabolism and behaviour by influencing not the whole consortium but just its signalling. On the other hand, abiotic effects of the environment on signalling molecules, including any chemical interactions, also represent direct interferences in the signalling processes. In this report, the processes of iron(III) reduction in moderately acidic aqueous media by some chemical analogues of microbial autoinducers are considered using ^{57}Fe Moessbauer spectroscopic measurements in frozen solutions. The results obtained indicate that some alkylresorcinols, chemical analogues of bacterial autoregulatory substances, can be abiotically oxidised by soil iron(III) in moderately acidic media. Such redox processes are very likely to exclude the biomolecules upon their oxidative degradation from signalling pathways, which is equal to "message undelivery", thus directly affecting cell-cell communication.

Key words: iron complexes, redox transformations, cell-cell signalling, microbial autoinducer molecules, alkylresorcinols, Moessbauer spectroscopy

INTRODUCTION

The processes of microbial cell-cell communication and signalling are now at the peak of research activities in microbiology-related fields, from bacteriology and medical microbiology to plant–microbe interactions (see, e.g. recent reviews [1-6]). This unprecedented interest is quite understandable, since a wide variety of microbial metabolic processes and behavioural features have been found to be regulated via remote intercellular signalling which involves special low-MW molecules. Such diffusible chemical signals, featured by relatively small extracellularly secreted molecules of various chemical structures, are used by microbial cells as a means of communication ("chemical language") in order to coordinate the behaviour of the microbial consortium as a'social

unit'. The producer cells respond to their own molecular signals (autoinducers; autoregulators) if the latter accumulate up to a threshold concentration, reflecting the cells' capability of sensing (*i*) their'population density' sufficient for concerted actions ("quorum sensing") [6,7], or (*ii*) the'rate of disappearance' of effector molecules owing to mass transfer in the environment (i.e., "diffusion sensing"), or (*iii*) a combination of (*i*) and (*ii*), including also spatial cell distribution, as a unifying hypothesis ("efficiency sensing") [8].

The importance of such studies consists, in particular, in the possibility of regulating microbial metabolism and behaviour by influencing not the whole microbial consortium but just its signalling pathways [9-11]. For example, the naturally occurring mechanisms of suppressing (quenching) microbial quorum sensing (i.e., "quorum quenching") have been found to play important roles in microbe – microbe and pathogen – host interactions [11-14]. In biomedicine, such studies can pave a way to formulating new generations of antimicrobial agents in a novel therapeutic approach, where the target is the quorum-sensing circuitry of pathogens (which controls pathogenicity and biofilm formation) but not their growth *per se* [11,15].

On the other hand, any possible abiotic effects of the environment (medium) both on the microorganism–host plant system [16-18] and on extracellular signalling molecules, including chemical interactions, have also to be considered; in the latter case, they represent direct interferences in the signalling processes [18]. For instance, the chemical reactivity of particular metal species (or their possible catalytic effects) could result in complexation (chemical binding) or redox degradation of biogenic organics involved in host–microbe interactions or microbial intercellular communication (i.e., in the aforementioned remote exchange of molecular signals, autoinducers, nutrients; quorum sensing (efficiency sensing) within microbial communities, etc.). Such processes could occur, for example, in metal-polluted environments, especially in the case of relatively acidic soils which are quite common [19].

Our earlier studies (see, e.g. [20, 21] and references therein) showed that iron(III) can be gradually reduced in moderately acidic aqueous media under aerobic conditions by some auxin phytohormones including indole-3-acetic acid (IAA). The latter, produced and excreted also by many soil microorganisms, plays an important role in plant–microbe interactions and signalling [22]. In this communication, the processes of abiotic iron(III) reduction in moderately acidic aqueous solutions by some chemical analogues of microbial autoinducers are considered, as evidenced by ^{57}Fe Moessbauer spectroscopic data. As iron(III) is ubiquitous in the environment, such redox processes could occur, e.g. in acidic soils, resulting in oxidative degradation of the biomolecules involved in cell–cell communication, thus directly affecting the latter.

EXPERIMENTAL

The following alkylresorcinols, chemical analogues of bacterial autoregulatory substances, were used: orcinol (also known as 5-methylresorcinol, or 1,3-dihydroxy-5-methylbenzene) monohydrate ($C_7H_8O_2 \cdot H_2O$; obtained from "Fluka"); 4-*n*-hexylresorcinol (1,3-dihydroxy-4-*n*--hexylbenzene, $C_{12}H_{18}O_2$; "Fluka"), as well as homoserine lactone (α-amino-γ-butyrolactone hydrobromide; $C_4H_7NO_2 \cdot HBr$; "Aldrich"). Stock solutions of the organics (1.5 ml in closed Eppendorf tubes) were prepared as 0.06 M aqueous (for orcinol and homoserine lactone) or 25%(v/v) ethanol-water (for 4-*n*-hexylresorcinol) solutions. Stock iron(III) aqueous solution contained 0.1 M $^{57}FeCl_3$ (enriched with ^{57}Fe up to 90% to increase the intensity of resonant absorption of γ-radiation). The absence of iron(II) in the $^{57}FeCl_3$ stock solution was confirmed by special tests. For Moessbauer measurements, 0.50 ml of an organic reagent were mixed with 0.10 ml of the stock iron(III) solution (initial pH was brought from ~1.5 to ~3 by adding 0.06 ml of 1 M KOH), so that the iron(III)-to-organics molar ratio was 1:3. The resulting mixture was stored at room temperature for a required period of time and then rapidly frozen in liquid nitrogen. Solid samples were prepared by drying the sample solutions in air at ambient temperature. Moessbauer spectroscopic measurements were performed at T~80 K as described earlier [21]; all isomer shift values are reported relative to α-Fe at room temperature.

RESULTS AND DISCUSSION

Alkylresorcinols (i.e., alkyl-substituted *m*-dihydroxybenzenes) comprise a group of natural phenolic substances that have a wide range of known biological functions [23]. In particular, alkylresorcinols excreted by many microorganisms into the environment have been documented to perform autoregulatory and adaptogenic functions under unfavourable conditions [24-26]. Their important role in extracellular signalling poses a question concerning their fate in the environment related to possible abiotic (e.g., chemical) effects of the latter. In this work, the aim was to test the possibility of redox transformations involving iron(III) and the aforementioned alkylresorcinols in aqueous media and upon drying, which can simulate acidic soil conditions with varying humidity. Moessbauer spectroscopy was used as a very sensitive technique for the qualitative and quantitative monitoring of chemical transformations of high-spin iron(II) and iron(III) species [21,27]. Rapid freezing (necessary for measuring Moessbauer spectra of Fe-containing solutions) allowed any ongoing chemical processes to be ceased at a certain point.

In *Figure 1* (spectra *a* and *b*), the gradual accumulation of iron(II) in aqueous orcinol–iron(III) mixtures is depicted. In *Figure 1c*, the predominance of iron(II) in the dried solid remainder is also clearly seen, representing over 3/4 of the total iron *(Table I)*. The parameters of the ferrous species formed in the solutions (isomer shift δ ~1.4 mm·s^{-1} and quadrupole splitting Δ ~3.2 mm·s^{-1} at T = 80 K) most closely correspond to hexaaquo iron(II) coordination [21].

Table I. Moessbauer parameters[a] for aqueous $^{57}Fe^{III}$-containing solutions of orcinol (see also *Figure 1*) and homoserine lactone (pH~3; 0.015 M to 0.045 M Fe-to-organics ratios), rapidly frozen after specified periods of time, and for their solid remainders obtained by drying in air at ambient temperature (measured at T = 80 K)

Organic reagent mixed with $^{57}Fe^{III}$	Time[b]	Fe oxidation state	δ,[c] mm·s^{-1}	Δ,[d] mm·s^{-1}	Γ,[e] mm·s^{-1}	S_r,[f] %
Orcinol (*solution*)	10 min	+3	0.48(1)	0.74(1)	0.52(1)	93.3
		+2	1.41(1)	3.22(1)	0.34(1)	6.7
	5.5 h	+3	0.48(1)	0.72(1)	0.50(1)	74.1
		+2	1.38(1)	3.23(1)	0.42(1)	25.9
Orcinol (*dried solid*)	–	+3	0.48(1)	0.63(2)	0.43(2)	24.5
		+2 (*form 1*)	1.31(1)	1.86(3)	0.50(3)	33.3
		+2 (*form 2*)	1.29(1)	2.65(1)	0.34(1)	42.2
Homoserine lactone (*solution*)	10 min	+3	0.483(1)	0.785(1)	0.374(1)	100
Homoserine lactone (*dried solid*)	–	+3	0.484(2)	0.766(4)	0.52(1)	97.6
		+2	1.45(1)	2.75(1)	0.52(1)	2.4

[a] Errors (in the last digits) are given in parentheses. [b] Period from mixing the reagents until rapid freezing of the solution. [c] Isomer shift (relative to α-Fe at ambient temperature). [d] Quadrupole splitting. [e] Full line width at half maximum. [f] Partial resonant absorption areas of spectral components which represent relative contents of the corresponding Fe forms assuming a common recoilless fraction for all forms (for S_r, relative error is ca. ± 4% of the given values).

However, in the dried solid in the case of orcinol, the parameters of the two forms of iron(II) species (see *Table I*) evidently represent different ferrous complexes with orcinol and/or its oxidation product(s). Thus, these Moessbauer measurements provide unambiguous evidence that iron(III) is gradually reduced by orcinol in moderately acidic (pH~3) aqueous solutions. Similar results have been obtained by us both for orcinol (5-methylresorcinol) and for 4-*n*-hexylresorcinol also at slightly lower pH~1.5 (data not shown; to be discussed in more detail elsewhere). These findings showing substantial iron(III) reduction by alkylresorcinols are still more interesting, consi-

dering the report of Pracht *et al.* [28] who found no reduction of iron(III) by resorcinol (i.e., an unalkylated *m*-dihydroxy analogue, 1,3-dihydroxybenzene) in acidic aqueous solutions (whereas both catechol and hydroquinone, its *o*- and *p*-dihydroxy isomers, respectively, substantially reduced iron(III) within the first hour). Note for comparison that two other *m*-dihydroxy isomers, 2,4- and 2,6-dihydroxybenzoic acids (at 0.01 mM), in the presence of 0.2 mM iron(III) in 20 mM sodium acetate buffer (at pH 4.5) were reported [29] to reduce ca. 1% and 4% of all iron(III) to iron(II) in 30 min, whereas catechol and hydroquinone under those conditions gave ca. 12% and 10% reduction, respectively.

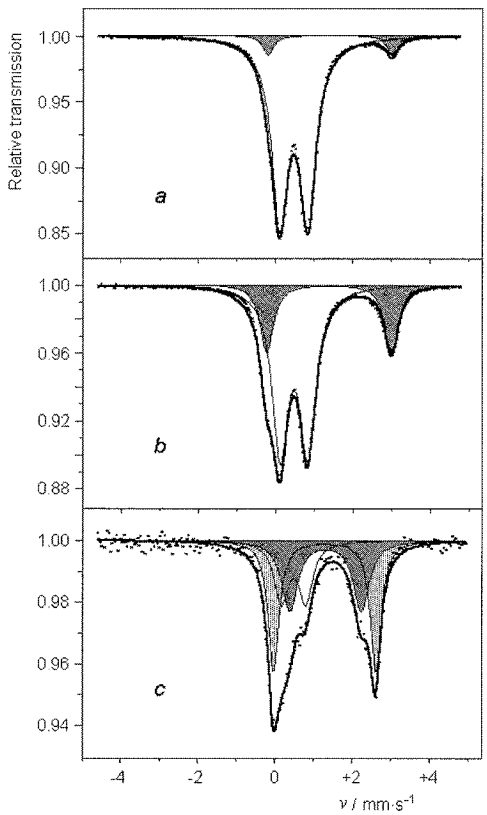

Figure 1. Moessbauer spectra of ^{57}Fe(III)-containing aqueous solutions of orcinol (1,3-dihydroxy-5-methylbenzene) rapidly frozen (*a*) 10 min and (*b*) 5.5 h after mixing, as well as of (*c*) solid remainder air-dried at room temperature (measured at T = 80 K). Relative transmission of γ-radiation is plotted against relative velocity (ν, in mm·s^{-1}) of the ^{57}Co[Rh] 14.4-keV γ-radiation source versus the absorber (^{57}Fe-containing sample), which corresponds to the energy scale according to the Doppler effect (i.e., with ± 1 mm·s^{-1} corresponding to ± 48.1 neV [27]), calibrated using α-Fe at room temperature. Shaded areas (doublets) represent contributions of iron(II) to the whole spectrum area (defined by the outer solid-line envelope); in spectrum (*c*), the more and less dark-shaded areas correspond to two different iron(II) species (forms 1 and 2, respectively; see *Table I*).

Under our conditions (see the Experimental section), homoserine lactone (an unacylated analogue of *N*-acylhomoserine lactones, a class of signalling molecules involved in quorum sensing in a variety of bacteria [1-8,11-15]), which also induces non-species-specific effects on the growth and development of different bacteria suggesting its regulatory functions (see [25] and references

therein), did not show any noticeable reduction of iron(III) in solution. Nevertheless, some iron(II) was detected in its dried remainder (see *Table I*).

It is noteworthy that, in contrast to indole-3-acetic acid (auxin; see above) which had earlier been reported to be able to totally reduce iron(III) in aqueous solutions [20,21] but, upon subsequent drying in air, all iron(II) had been found to get reoxidised back to iron(III) [30], in all cases of the alkylresorcinols studied in this work, the resulting iron(II) was evidently not reoxidised upon drying in air. For instance, for orcinol, the relative content of iron(II) in the dried solid (over 75% of total iron for both ferrous forms) was ca. 3 times higher than that in the solution 5.5 h after mixing (see*Table I*). This finding points to even stronger reducing power of the alkylresorcinol autoinducer (and/or its primary oxidation products) as compared to auxin.

It should be noted that the parameters of iron(III) forms, which are rather nonspecific and close for all the samples studied (see *Table I*), at this step do not allow the chemical nature of the ferric species to be revealed (e.g., to distinguish between possible complexes or dihydroxy-bridged dimeric moieties [21]), which would require additional studies.

To conclude, the results obtained unambiguously imply that alkylresorcinol autoinducers can be abiotically oxidised by soil iron(III) in moderately acidic media. Note that metal ion complexation *per se*, although changing the properties of the resulting complex as compared to the free ligand, still does not mean degradation of the latter. On the contrary, some of the signalling molecules can relatively easily be oxidised by direct action of redox-active metal species (or via their catalytic activity), similar to the aforementioned processes involving iron(III) and auxins [20,21,30] or alkylresorcinols (this study). In such cases, the resulting product is completely another substance with different properties. Thus, an oxidation process involving a particular molecular signal is very likely to exclude the latter from any signalling pathways, which is equal to "message undelivery", directly affecting cell–cell communication.

Acknowledgements: This work was supported under the NATO "Security Through Science" Programme (Grants CBP.NR.NREV 981748 and ESP.NR.NRCLG 982857), by the Hungarian Science Foundation (OTKA Projects K62691, K68135 and K68992), the Hungarian RTD Fund (Russian–Hungarian bilateral intergovernmental Project RUS-6/2004), and under the Agreement on Scientific Cooperation between the Russian and Hungarian Academies of Sciences for 2005–2007.

REFERENCES

1. Singh B.K., Millard P., Whiteley A.S., Murrell J.C. Unravelling rhizosphere-microbial interactions: opportunities and limitations. *Trends Microbiol* 2004; 12: 386-93.
2. Somers E., Vanderleyden J., Srinivasan M. Rhizosphere bacterial signalling: a love parade beneath our feet. *Crit Rev Microbiol* 2004; 30: 205-40.
3. Welch M., Mikkelsen H., Swatton J.E., Smith D., Thomas G.L., Glansdorp F.G., Spring D.R. Cell–cell communication in Gram-negative bacteria. *Mol BioSyst* 2005; 1: 196-202.
4. Barea J.M., Pozo M.J., Azcon R., Azcon-Aguilar C. Microbial co-operation in the rhizosphere. *J Exp Bot* 2005; 56: 1761-78.
5. Keller L., Surette M.G. Communication in bacteria: an ecological and evolutionary perspective. *Nature Rev Microbiol* 2006; 4: 249-58.
6. Williams P., Winzer K., Chan W.C., Camara M. Look who's talking: communication and quorum sensing in the bacterial world. *Phil Trans Royal Soc B: Biol Sci* 2007; 362: 1119-34.
7. Whitehead N.A., Barnard A.M.L., Slater H., Simpson N.J.L., Salmond G.P.C. Quorum-sensing in Gram-negative bacteria. *FEMS Microbiol Rev* 2001; 25: 365-404.
8. Hense B.A., Kuttler C., Muller J., Rothballer M., Hartmann A., Kreft J.-U. Does efficiency sensing unify diffusion and quorum sensing? *Nature Rev Microbiol* 2007; 5: 230-39.
9. Mathesius U., Mulders S., Gao M., Teplitski M., Caetano-Apolles G., Rolfe B.G., Bauer W.D. Extensive

and specific responses of a eukaryote to bacterial quorum-sensing signals. *Proc Natl Acad Sci USA* 2003; 100: 1444-49.
10. Brencic A., Winans S.C. Detection of and response to signals involved in host-microbe interactions by plant-associated bacteria. *Microbiol Mol Biol Rev* 2005; 69: 155-94.
11. Juhas M., Eberl L., Tummler B. Quorum sensing: the power of cooperation in the world of *Pseudomonas*. *Environ Microbiol* 2005; 7: 459-71.
12. Adonizio, A.L., Downum, K., Bennett, B.C., Mathee, K. Anti-quorum sensing activity of medicinal plants in southern Florida. *J Ethnopharmacol* 2006; 105: 427-35.
13. Rasmussen T.B., Givskov M. Quorum sensing inhibitors: a bargain of effects. *Microbiology* (U.K.) 2006; 152: 895-904.
14. Bjarnsholt T., Givskov M. Quorum-sensing blockade as a strategy for enhancing host defences against bacterial pathogens. *Phil Trans Royal Soc B: Biol Sci* 2007; 362: 1213-22.
15. Dong Y.-H., Wang L.-H., Zhang L.-H. Quorum-quenching microbial infections: mechanisms and implications. *Phil Trans Royal Soc B: Biol Sci* 2007; 362: 1201-11.
16. Fuzy A, Toth T, Biro B. Mycorrhizal colonisation can be altered by the direct and indirect effect of drought and salt in a split root experiment. *Cereal Res Commun* 2007; 35: 401-04.
17. Biro B., Koves-Pechy K., Voros I., Takacs T., Eggenberger P., Strasser R.J. Interrelations between *Azospirillum* and *Rhizobium* nitrogen-fixers and arbuscular mycorrhizal fungi in the rhizosphere of alfalfa in sterile, AMF-free or normal soil conditions. *Appl Soil Ecol* 2000; 15: 159-68.
18. Kamnev A.A. Metals in soil *versus* plant-microbe interactions: Biotic and chemical interferences. In E.A. Barka, C. Clement (Eds.), *Plant-Microbe Interactions*. Research Signpost, Trivandrum (Kerala, India), 2008, Chapter 13, pp. 291-318.
19. Von Uexkull H.R., Mutert E. Global extent, development and economic impact of acid soils. *Plant Soil* 1995; 171: 1-15.
20. Kamnev A.A., Kovacs K., Shchelochkov A.G., Kulikov L.A., Perfiliev Yu.D., Kuzmann E., Vertes A. Bioleaching and chemical transformations of heavy metals and radionuclides mediated by soil microorganisms. In M.C. Alpoim, P.V. Morais, M.A. Santos, A.J. Cristovao, J.A. Centeno, Ph. Collery (Eds.), *Metal Ions in Biology and Medicine*, Vol. 9. John Libbey Eurotext, Paris, 2006, pp. 220-25.
21. Kovacs K., Kamnev A.A., Mink J., Nemeth Cs., Kuzmann E., Megyes T., Grosz T., Medzihradszky-Schweiger H., Vertes A. Mossbauer, vibrational spectroscopic and solution X-ray diffraction studies of the structure of iron(III) complexes formed with indole-3-alkanoic acids in acidic aqueous solutions. *Struct Chem* 2006; 17: 105-20.
22. Spaepen S., Vanderleyden J., Remans R. Indole-3-acetic acid in microbial and microorganism-plant signaling. *FEMS Microbiol Rev* 2007; 31: 425-48.
23. Kozubek A., Tyman J.H.P. Resorcinolic lipids, the natural non-isoprenoid phenolic amphiphiles and their biological activity. *Chem Rev* 1999; 99: 1-25.
24. El'-Registan G.I., Mulyukin A.L., Nikolaev Yu.A., Suzina N.E., Gal'chenko V.F., Duda V.I. Adaptogenic functions of extracellular autoregulators of microorganisms. *Microbiology* (Moscow), 2006; 75: 380-89.
25. Mulyukin A.L., Filippova S.N., Kozlova A.N., Surgucheva N.A., Bogdanova T.I., Tsaplina I.A., El'-Registan G.I. Non-species-specific effects of unacylated homoserine lactone and hexylresorcinol, low molecular weight autoregulators, on the growth and development of bacteria. *Microbiology* (Moscow), 2006; 75: 405-14.
26. Nikolaev Yu.A., Mulyukin A.L., Stepanenko I.Yu., El'-Registan G.I. Autoregulation of stress response in microorganisms. *Microbiology* (Moscow), 2006; 75: 420-26.
27. Krebs C., Price J.C., Baldwin J., Saleh L., Green M.T., Bollinger J.M., Jr. Rapid freeze-quench ^{57}Fe Mossbauer spectroscopy: Monitoring changes of an iron-containing active site during a biochemical reaction. *Inorg Chem* 2005; 44: 742-57.
28. Pracht J., Boenigk J., Isenbeck-Schroter M., Keppler F., Scholer H.F. Abiotic Fe(III) induced mineralization of phenolic substances. *Chemosphere* 2001; 44: 613-19.
29. Aguiar A., Ferraz A. Fe^{3+}- and Cu^{2+}-reduction by phenol derivatives associated with Azure B degradation in Fenton-like reactions. *Chemosphere* 2007; 66: 947-54.
30. Kamnev A.A., Kuzmann E. Mossbauer spectroscopic study of the interaction of indole-3-acetic acid with iron(III) in aqueous solution. *Biochem Mol Biol Int* 1997; 41: 575-81.