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# MÖSSBAUER SPECTROSCOPIC STUDY OF THE INTERACTION OF INDOLE-3-ACETIC ACID WITH IRON(III) IN AQUEOUS SOLUTION

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**Summary.** – The data of Mössbauer spectroscopic measurements in rapidly frozen $^{57}Fe^{III}$ nitrate solutions containing indole-3-acetic acid (IAA) are presented. The results obtained provide direct evidence that iron(III) is gradually reduced by IAA with the formation of soluble iron(II) complex. It has been found that further drying of the solution in air results in complete re-oxidation of iron(II) with the formation of iron(III) complex. The structure of the complexes obtained and their possible role in solubilization and transformation of iron species in soil in the presence of IAA secreted by soil microorganisms into the environment are also discussed.

**Key Words:** iron(III), iron(II), indole-3-acetic acid, complexes, Mössbauer spectroscopy

## INTRODUCTION

Indole-3-acetic acid (IAA), a phytohormone (auxin) widely distributed in higher plants [1], is well documented to be synthesized not only by the latter but also by a number of microorganisms [2,3]. In particular, IAA production is widespread among associative soil bacteria that inhabit the rhizosphere of plants [2–5] promoting their growth and development. The *in vivo* level of IAA is thought to be controlled largely by its oxidative degradation involving plant peroxidases; a specific interaction between the latter and IAA has been suggested [6] with the initial reduction of iron(III) to iron(II) in the enzyme as a key step in the mechanism of IAA oxidation.

Iron was also found [7–9] to be essential for the utilization of anthranilic acid, a key intermediate in the biosynthesis of L-tryptophan which, in its turn, is another important metabolic precursor of IAA [2,3] (a tryptophan-independent pathway of IAA synthesis in bacteria was for the first time demonstrated quite recently [4]). It is interesting to note that *Rhizobium leguminosarum* GF160

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grown under iron deficiency was shown [7] to secrete anthranilic acid as the only iron(III)-reducing and solubilizing substance instead of siderophores (ferric-specific ligands of either the catecholate (phenolate) or hydroxamate types [10,11], commonly secreted by many soil microorganisms, that make insoluble soil iron available to the cells).

Secretion of IAA by soil bacteria into the environment [2–4] might as well be accompanied by the formation of complexes and other chemical transformations involving metal cations, considering the relatively high ligating power of the acid. In this communication, we concentrate on the interaction of IAA in aqueous solution with iron as one of the generally most biologically important microelements which is abundant in soil yet requiring specific complexing agents for sequestering it from this environment [11].

Mössbauer spectroscopy used as the basic technique in the present study has proven to be a highly sensitive and convenient tool giving valuable and often unique information about the chemical environment, coordination symmetry and oxidation states of Mössbauer-active nuclides (the stable $^{57}$Fe isotope having been by far the most widely used), as well as about any changes on the molecular level [12,13]. According to the principles of Mössbauer spectroscopy, a sufficient recoilless absorption (or emission) of gamma-radiation (*i.e.*, the Mössbauer effect) can be observed only if a Mössbauer-active nuclide (*e.g.*, $^{57}$Fe) is incorporated in (or firmly bonded to) a solid phase, not necessarily crystalline (or an extremely viscous medium), so that the lattice of the solid as a whole absorbs the recoil energy. Therefore solutions containing a Mössbauer-active nuclide are commonly studied rapidly frozen [14], so that the structure of the resulting glass-like phase would reflect the structure of the solution.

## EXPERIMENTAL

***Sample preparation.*** Iron(III) nitrate solutions were prepared by dissoving $^{57}$Fe metal (95.6% enriched isotope, Kurchatov Institute of Atomic Energy, Moscow) in a slight excess of *ca.* 50% nitric acid (analytical reagent grade) at elevated temperature, partially drying the resulting solution in air at *ca.* 50°C up to the formation of crystals and further redissolving the latter in doubly distilled water up to the iron(III) concentration of 0.1 M (stock solution). The absence of iron(II) in the stock solution was confirmed by special Mössbauer measurements which gave a relaxation spectrum typical for partially hydrolysed ferric ions in solutions frozen at liquid nitrogen temperature [12-14].

The concentrations of iron(III) and IAA (Eastman Kodak, chemical purity) in acidic solutions under study were adjusted to 0.01 and 0.03 M, respectively. After mixing, the solutions were allowed to stand at room temperature in contact with air (in small closed flasks to prevent evaporation) for certain periods of time, filtered and then rapidly frozen by dropwise inserting into a sample holder cooled with liquid nitrogen.

***Spectra acquisition and data treatment.*** Mössbauer spectra of the frozen samples placed in a cryostat filled with liquid nitrogen (at *ca.* 80 K) were collected using a conventional constant-acceleration Mössbauer spectrometer combined with a computer-operated multichannel analyser; a $^{57}$Co[Rh] source used was calibrated relative to α-Fe at room temperature. Standard computer statistical analysis included fitting the experimental data obtained to a sum of Lorentzian-shaped

component lines using a least squares fit, which enabled calculation of the values of isomer shift (IS; all data given relative to α-Fe), quadrupole splitting (QS), linewidth (*i.e.* full width at half maximum, FWHM), as well as relative partial intensities for superimposed components composing the overall spectrum.

## RESULTS AND DISCUSSION

Addition of iron(III) nitrate to the excess of IAA in acidic solution (up to the 1:3 Fe-to-IAA molar ratio, see above) resulted in gradual development of colour in the solution from light pink (1 min after mixing) to crimson (20 min) indicating a slow formation of a complex. A portion of the solution was filtered and frozen 25 min after mixing; its Mössbauer spectrum is shown in Fig. 1,A. The central part of the spectrum evidently represents a substantial contribution (about 72% of the total spectrum area) from the broad relaxation component featuring the presence of hydrolysed non-chelated ferric ions, whereas the other component is a well-resolved quadrupole-split doublet with the parameters (Table 1) typical for high-spin iron(II). Since there was no iron(II) in the initial solution, this finding directly evidences that a gradual reduction of iron(III) by IAA occurs with the formation of a soluble iron(II) species. It is noteworthy that a similar yet much weaker partial reduction of iron(III) was observed [15] during synthesis of its complexes with tryptophan (as well as with lysine, in contrast to other amino acids studied in [15]) by precipitation and drying, as evidenced by Mössbauer spectroscopy.

In order to test the reducing capability of IAA towards iron(III), the other portion of the mixed iron(III)–IAA solution was allowed to stand for two days. Then the resulting intensively reddish-purple solution was filtered and frozen as described above. Its Mössbauer spectrum shown in Fig. 1,B consists mainly of a strong doublet of iron(II) with essentially the same parameters as for that in Fig. 1,A (see Table 1). We note that the intensity of the spectrum in Fig. 1,B (resonance effect *ca.* 17%) is significantly higher than that of the corresponding iron(II) component in Fig. 1,A (resonance effect *ca.* 2%). This finding provides evidence that long-term interaction of iron(III) with IAA in acidic nitrate solution leads to its practically complete reduction with the formation of a soluble deeply coloured iron(II) complex.

In an attempt to obtain the complex in the solid state, the latter solution was allowed to evaporate in air. Surprisingly, the Mössbauer spectrum of the resulting dark-purple crystals (80 K) showed an intensive symmetric quadrupole-split doublet (Fig. 2) with the parameters (see Table 1) typical for high-spin iron(III) in essentially distorted octahedral coordination [12–15]. Comparison of the parameters (the IS and QS values featuring, respectively, the density of *s*-electrons at the iron nucleus and the degree of asymmetry of its coordination environment [12–15]) with those for iron(III) complexes with some amino acids and other ligands with both N- and O-donor atoms [15,16] (see Table 1) shows their close similarity. Note that iron(III) complexes with only O-donor ligands, *e.g.* oxalate, formate, succinate and tartrate [17], give significantly lower IS values; their QS values are also under 0.3 mm/s [17] owing to an obviously higher coordination symmetry than in the case of

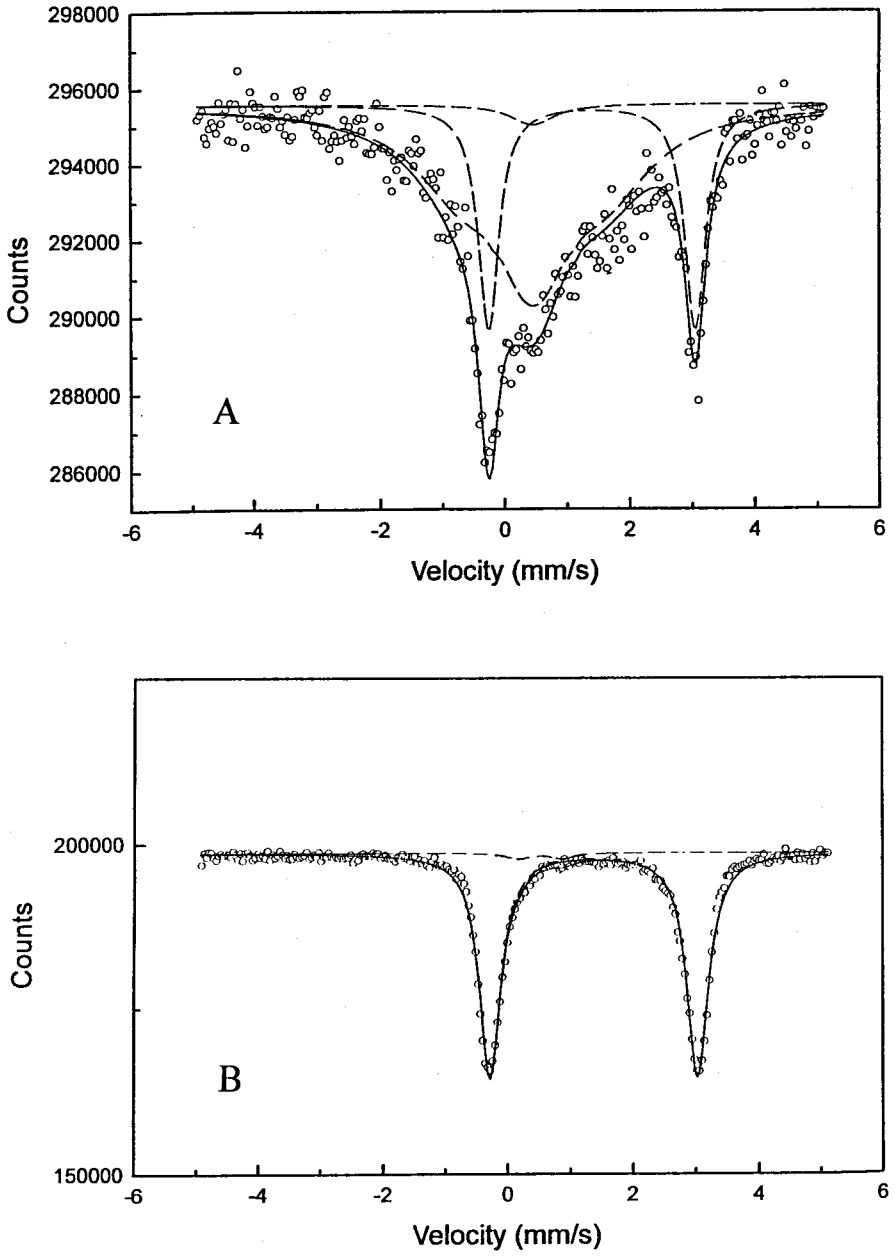


**Figure 1.** Mössbauer spectra of $^{57}Fe^{III}$ nitrate solutions containing indole-3-acetic acid (measured at T = 80 K) frozen 25 min (A) and 51 h after mixing (B). Dashed lines represent calculated components, including the relaxation spectrum of ferric ions (central part) and the Lorentzian-shaped quadrupole-split doublet of iron(II) complex (see also Table 1), which compose the resulting spectrum (solid lines) computer-fitted to the experimental data (points).

**Table 1.** Mössbauer parameters[a] for iron complexes with indole-3-acetic acid (IAA), some amino acids [15] and adriamycin [16].

| Complex | T, K | IS,[b] mm/s | QS,[c] mm/s | LWHM,[d] mm/s | $S_r$,[e] % | Reference |
|---|---|---|---|---|---|---|
| IAA–$Fe^{II}$ | 80 | 1.39(2) | 3.30(5) | 0.41(8) | 28.0 | This work (Fig. 1,A) |
| IAA–$Fe^{II}$ | 80 | 1.37(1) | 3.31(1) | 0.42(1) | 97.3 | This work (Fig. 1,B) |
| IAA–$Fe^{III}$ | 80 | 0.48(1) | 0.70(1) | 0.69(1) | 100.0 | This work (Fig. 2) |
| D-, L-tryptophan–$Fe^{III}$ | 82 | 0.53(5) | 0.70(5) | | | [15] |
| D-, L-glycine–$Fe^{III}$ | 82 | 0.45(5) | 0.65(5) | | | [15] |
| D-, L-leucine–$Fe^{III}$ | 82 | 0.52(5) | 0.61(5) | | | [15] |
| D-lysine–$Fe^{III}$ | 82 | 0.52(5) | 0.76(5) | | | [15] |
| L-lysine–$Fe^{III}$ | 82 | 0.56(5) | 0.76(5) | | | [15] |
| adriamycin–$Fe^{III}$ | 4 | 0.56(1) | 0.74(1) | | | [16] |

[a] Errors (in the last digit) are given in parentheses.
[b] Isomer shift (relative to α-Fe).
[c] Quadrupole splitting.
[d] Full line width at half maximum.
[e] Partial resonant absorption areas of spectral components which represent relative contents of the corresponding forms assuming a common recoilless fraction for all iron forms.

different donor atoms in the ligands (cf. Table 1). Thus it can be assumed that iron(III) in its complex with IAA is coordinated with both the carboxylic oxygen and the indole nitrogen, which can be possible considering the variable relative orientations of the side chain towards the indole plane and of the carboxylic group [18]. By analogy, a similar coordination of high-spin iron(II) in its complex with IAA may be presumed (see Table 1). Note for comparison that precise chemical analyses of iron(II) and iron(III) anthranilate complexes reported by Dinsel and Sweet [19] gave the formulae $Fe^{II}[C_7H_6NO_2]_2$ and $Fe^{III}[(OH)(C_7H_6NO_2)_2(H_2O)]$ with the obvious coordination numbers of 4 and 6, respectively, which also implies iron chelation involving both the N- and O-donor atoms of the anthranilate anion (with additional hydroxo and aqua ligands in the case of the ferric complex [19]).

The observed reduction of iron(III) to iron(II) in solution with its further re-oxidation in air upon drying (see above) is similar to the cyclic processes found for iron(III)–adriamycin chelates with particular chelation structures in aqueous solution [16] leading to molecular oxygen reduction. It may also be supposed that an increased iron(III) content in the medium under aerobic conditions could facilitate oxidative degradation of IAA secreted, *e.g.* by bacteria into the environment [2,3], similar to the processes involving plant peroxidases that occur *in vivo* [6]. On the other hand,

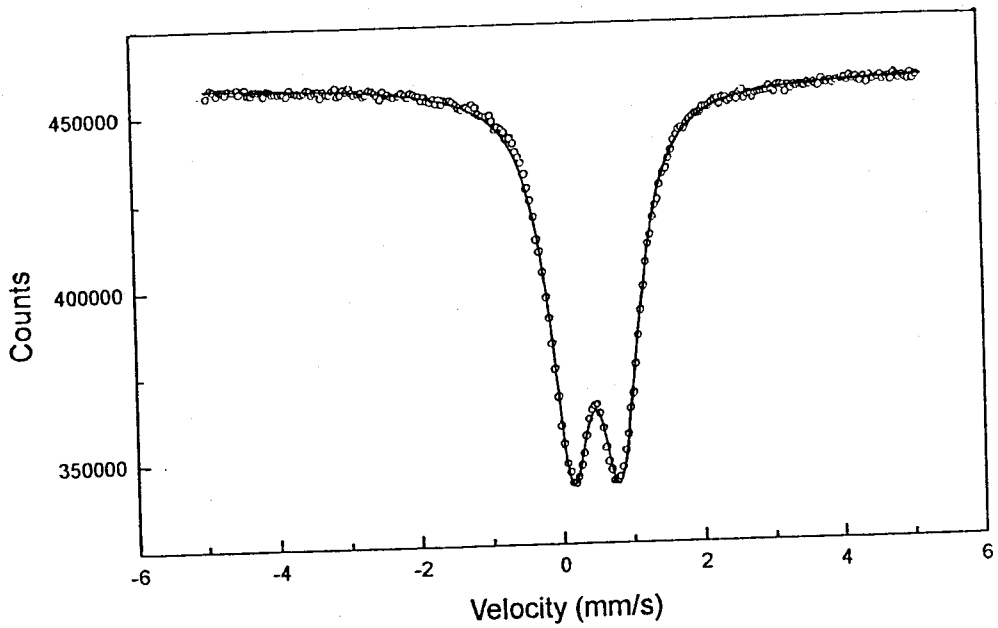


**Figure 2.** Mössbauer spectrum (measured at T = 80 K) of the crystals obtained by drying of the aged iron – indole-3-acetic acid solution (see Fig. 1,B) in air at room temperature (see also Table 1).

considering the data obtained, secretion of IAA by soil microorganisms could facilitate reductive solubilization of relatively poorly soluble iron(III) in a way similar to that well documented for siderophores [10,11] and also reported for anthranilic acid [7].

For a more detailed discussion of the chemistry, structure and behaviour of iron–IAA complexes as well as of their role in iron and IAA metabolism, further experiments are necessary including the use of Mössbauer and other spectroscopic techniques, which are currently in preparation.

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