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Mössbauer spectroscopic evidence for the reduction of iron(III) by anthranilic acid in aqueous solution

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Abstract—The data of Mössbauer spectroscopic measurements in rapidly frozen acidified $^{57}Fe^{III}$ nitrate solutions containing anthranilic acid are presented and discussed. The results obtained provide direct evidence that, along with the formation of an iron(III) anthranilate complex, gradual reduction of iron(III) by anthranilic acid occurs with the formation of iron(II) species even in acidic nitrate media. © 1997 Elsevier Science Ltd

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Iron(III) is known to form a weakly soluble complex with anthranilic acid in aqueous solution [1,2], its formation was reported [2] to be instantaneous and quantitative in slightly acidic (pH 4.5) media. Along with some technological applications of such complexes as additives to polymeric materials [3,4], both the metal and the acid are of significant biological value. While iron is one of the generally most essential microelements [5,6], anthranilic acid has been established as a key intermediate in the biosynthesis of L-tryptophan [7,8] and indoleacetic acid, a phytohormone (an auxin) synthesized both by plants and microorganisms [8,9]. In some bacteria anthranilic acid was identified as a metabolic product [10–12]. Moreover, iron was shown to be essential for enzymatically catalysed reactions involving anthranilic acid [13,14]. Finally, the latter was identified [15] as the only iron-solubilizing substance secreted by the nitrogen-fixing bacterium *Rhizobium leguminosarum* grown under iron deficiency. Many soil microorganisms under such conditions commonly secrete iron(III)-specific ligands (siderophores) of either the catecholate (phenolate) or hydroxamate types [5,16] that make iron available to the cells.

In order to provide more information on the chemistry of iron(III) interaction with anthranilic acid in solution, which has been scarcely represented in the literature [1,2,17], some preliminary data of our Mössbauer spectroscopic measurements performed in frozen iron(III) nitrate solutions containing anthranilic acid are reported.

EXPERIMENTAL

Iron(III) nitrate solutions were prepared by dissolving ^{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. The concentrations of iron(III) and anthranilic acid (Sigma) in aqueous solutions under study were adjusted to 0.01 and 0.03 M, respectively. After mixing, the solutions were allowed to stand at room temperature for a certain period of time and then rapidly frozen by dropwise inserting into a sample holder cooled with liquid nitrogen.

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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 spectrometer combined with a PC-operated multichannel analyser; a ^{57}Co[Rh] source was used. Standard computer statistical analysis included fitting the experimental data to a sum of Lorentzian-shaped component lines using a least squares fit, which enabled calculation of the values of isomer shift (IS, relative to alpha-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.

Addition of iron(III) nitrate to the excess of anthranilic acid in solution (up to the 1 : 3 metal-to-acid molar ratio, see above) resulted in the formation of a turbid reddish-brown solution indicating precipitation of iron(III) anthranilate. After 10 min a portion of the latter mixture was frozen and its Mössbauer spectrum at 80 K was obtained (Fig. 1(A)).

RESULTS AND DISCUSSION

It can be seen that the spectrum is a superposition of three quadrupole doublets, the parameters of which are given in Table 1. The main doublet I with IS = 0.51 and QS = 0.71 mm/s is characteristic of iron(III) in distorted octahedral coordination [18,19] and may be attributed to the iron(III) anthranilate complex which, according to Dinsel and Sweet [2],

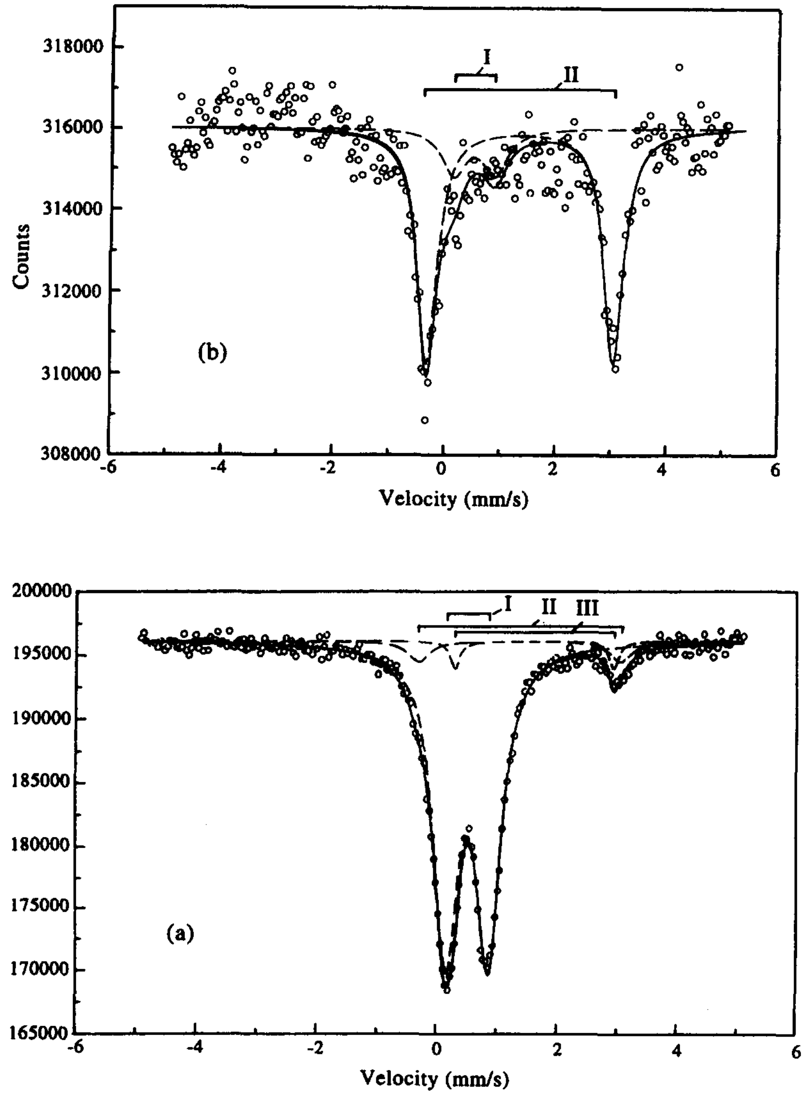

Fig. 1. Mössbauer spectra of ^{57}Fe^{III} nitrate solutions containing anthranilic acid (T = 80 K): (A) without addition of nitric acid, frozen 10 min after mixing; (B) acidified (*ca* 0.3 M HNO$_3$) solution, frozen 26 h after mixing. Dashed lines represent calculated Lorentzian-shaped components (I–III see text and Table 1) composing the resulting spectrum (solid lines) computer-fitted to the experimental data (points).

Table 1. Mössbauer parameters (see Fig. 1) for ^{57}Fe-containing anthranilic acid solutions ($T = 80$ K)

Figure	Spectral component	IS[a] (mm/s)	QS[b] (mm/s)	LWHM[c] (mm/s)	S_r[d] (%)
1(A)	Doublet I	0.51±0.01	0.71±0.01	0.49±0.02	91.5
	Doublet II	1.36±0.03	3.36±0.06	0.42±0.22	5.3
	Doublet III	1.61±0.03	2.64±0.06	0.19±0.09	3.2
1(B)	Doublet I	0.69±0.13	0.89±0.24	0.53[e]	19.1
	Doublet II	1.36±0.02	3.36±0.04	0.44±0.07	80.9

[a] Isomer shift (relative to alpha-Fe).

[b] Quadrupole splitting.

[c] Full line width at half maximum.

[d] Partial resonant absorption areas of spectral components which represent relative contents of the corresponding forms assuming a common recoilless fraction for all forms.

[e] Approximate evaluation.

consists of an iron atom coordinated by two anthranilate groups (through the oxygen of the carboxylic group and the nitrogen atoms), one hydroxyl and one water molecule. The obvious asymmetry of the coordination environment of the complex accounts for its relatively high QS value (0.71 mm/s). A distorted octahedral coordination of high-spin iron(III) through both amino and carboxylic groups in Fe^{III} complexes with some amino acids has also been assumed [20] on the basis of Mössbauer spectroscopic data.

There is also clear evidence for the presence of two forms of iron(II) species represented by doublets II and III (see Fig. 1(A) and Table 1). Since there was no iron(II) in the initial solution, this finding is direct evidence that anthranilic acid slowly reduces iron(III) with the formation of iron(II) species. This process was assumed [15] as a route for making insoluble soil iron(III) available for bacteria which had been found to secrete anthranilic acid under iron deficiency. Under such conditions, anthranilic acid may be oxidized to benzidine-3,3′-dicarboxylic acid [21] (which could similarly coordinate iron). The parameters of doublet III in Fig. 1(A) (see Table 1) obviously correspond to the iron(II) anthranilate complex which was shown [2] to conform to the formula $Fe(C_7H_6NO_2)_2$; in this case iron(II) obviously coordinates also through both the carboxylic oxygen and the nitrogen. In order to assign doublet II, part of the Fe(III)—anthranilic acid mixed solution was acidified with nitric acid (up to *ca* 0.3 M HNO_3) immediately after mixing and allowed to stand for a day. After that, a portion of the clear solution was frozen and its Mössbauer spectrum is shown in Fig. 1(B). Since under pH 2 iron is removed from acidic ligands of this type [15], it is evident that the well-resolved doublet II observed in Fig. 1(B) equal in parameters to doublet II in Fig. 1(A) (see Table 1) corresponds to hydrated Fe^{2+} ions, which is in general agreement with the Mössbauer data for frozen iron(II) salt solutions [18,19]. It is noteworthy that, according to Fig. 1(B), even in the presence of *ca* 0.3 M HNO_3 the reduced iron(II) is dominant in the anthranilic acid solution (note that the admixture of an iron(III) species featured by component I in Fig. 1(B) is difficult to assign owing to the insufficient intensity).

Thus, it has been found that anthranilic acid gradually reduces iron(III) in acidic nitrate solution. The Mössbauer parameters for iron(III) and, preliminarily, iron(II) complexes with anthranilic acid have been calculated. Further experiments on the chemistry, structure and behaviour of iron anthranilates in aqueous media including Mössbauer studies are in progress.

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