STRUCTURE AND REDOX TRANSFORMATIONS OF IRON(III) COMPLEXES WITH SOME BIOLOGICALLY IMPORTANT INDOLE-3-ALKANOIC ACIDS IN AQUEOUS SOLUTIONS§

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Abstract: Interactions of a series of indole-3-alkanoic acids (with *n*-alkanoic acid side-chains from C_1 to C_4) with iron(III) in acidic aqueous solutions have been shown to comprise two parallel processes including complexation and redox transformations giving iron(II) hexaaquo complexes. The structure and composition of the reaction products are discussed, as analysed using a combination of instrumental techniques including ^{57}Fe Mössbauer, vibrational and ^{1}H NMR spectroscopies.

Keywords: indole-3-alkanoic acids, auxin phytohormones, iron(III) complexes, coordination structure, redox transformations.

INTRODUCTION

Earlier studies have shown [12–16] that both IAA and some other chemically and metabolically related organic substances of biological origin are capable of gradually reducing iron(III) in weakly acidic nitrate-containing aqueous media even under aerobic conditions. This could be of ecological significance since iron(III) has a poor biological availability, which is due to its full hydrolysis and extremely low solubility of ferric hydroxides in a wide pH range, whereas iron(II) species are more soluble and, therefore, more biologically available both for plants and for soil microorganisms. Note also that acidic soils are rather widely spread, comprising about 30% of only arable territories [17]. On the other hand, these processes can result in oxidative degradation of the organic biomolecules involved in plant-microbe interactions in soil [7]. Therefore, knowledge of the chemical processes is of interest both for basic research and in applied fields, particularly those related to agricultural and environmental biotechnology.

In the present work, chemical reactions are considered which occur between indole-3-alkanoic acids (with *n*-alkanoic acid side chains from Indole-3-acetic acid (IAA) and its close structural analogues, indole-3-carboxylic (ICA), indole-3-propionic (IPA) and indole-3-butyric (IBA) acids, are natural and synthetic phytohormones of the auxin series that regulate plant growth and development [1–3]. IAA (along with some other indolic auxins), being ubiquitous in plants, is also well documented to be synthesized by many soil microorganisms which exude it into soil [4], where it plays an essential role in plant-microbe interactions [5, 6]. Thus, within the soil and/or aquifer environment, auxin molecules can readily be subjected to chemical reactions involving different metal ions including iron [7], which is commonly ubiquitous in soil [8] and is one of the most important micronutrients for virtually all organisms [9]. In the biomedical field, prooxidant activity and cytotoxic effects of IAA and its derivatives upon peroxidase-catalysed oxidation have also been tested for potential novel applications in antitumour therapy [10, 11].

C_1 to C_4) and iron(III) in acidic aqueous solutions under aerobic conditions, and their products are analysed using a combination of physicochemical instrumental techniques.

RESULTS AND DISCUSSION

In order to follow *in-situ* redox processes involving iron species in aqueous solutions, ^{57}Fe Mössbauer spectroscopy is a very convenient and informative technique giving, in particular, direct quantitative information on the Fe(II)-to-Fe(III) ratio. As Mössbauer spectra can be obtained for solid matrices only (or, for non-solids, in a solidified state, e.g. rapidly frozen), aqueous solutions can be studied in the frozen state [18]. Note also that rapid freezing (e.g., by inserting small portions of a solution into liquid nitrogen) allows one to obtain a glassy solid that reflects the structure of the initial

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solution. Moreover, in such a frozen solution all processes are virtually ceased, so that by rapidly freezing a successive series of solution aliquots with their subsequent low-temperature Mössbauer spectroscopic measurements, one can obtain „snapshots" of the state of processes that have been „stopped" at certain successive time points [19]. Mössbauer spectra of iron(III)-containing aqueous solutions with different indole-3-alkanoic acids, filtered and rapidly frozen 15 min and 2 days after mixing, are shown in Figure 1, *a–h*. It can be seen that in the solutions which initially contained iron(III) only, already 15 min after mixing some certain amounts of iron(II) are present, which is distinctly evidenced by the appearance of a corresponding component doublet with a large quadrupole splitting (its position is indicated in Figure 1 by a square bracket above the upper spectrum) having varying intensity for different acids (cf. Figure 1, spectra *a* to *d*). The presence of the same iron(II)-related doublet with higher intensities is detected in the spectra of the mixtures obtained after 2 days (cf. Fig. 1, spectra *e* to *h*). The Mössbauer parameters of the resulting Fe^{2+} species (i.e., isomer shifts δ = 1.39 ± 0.01 mm/s and quadrupole splittings Δ = 3.35 ± 0.03 mm/s) are typical of a hexaaquo coordination microenvironment [18].

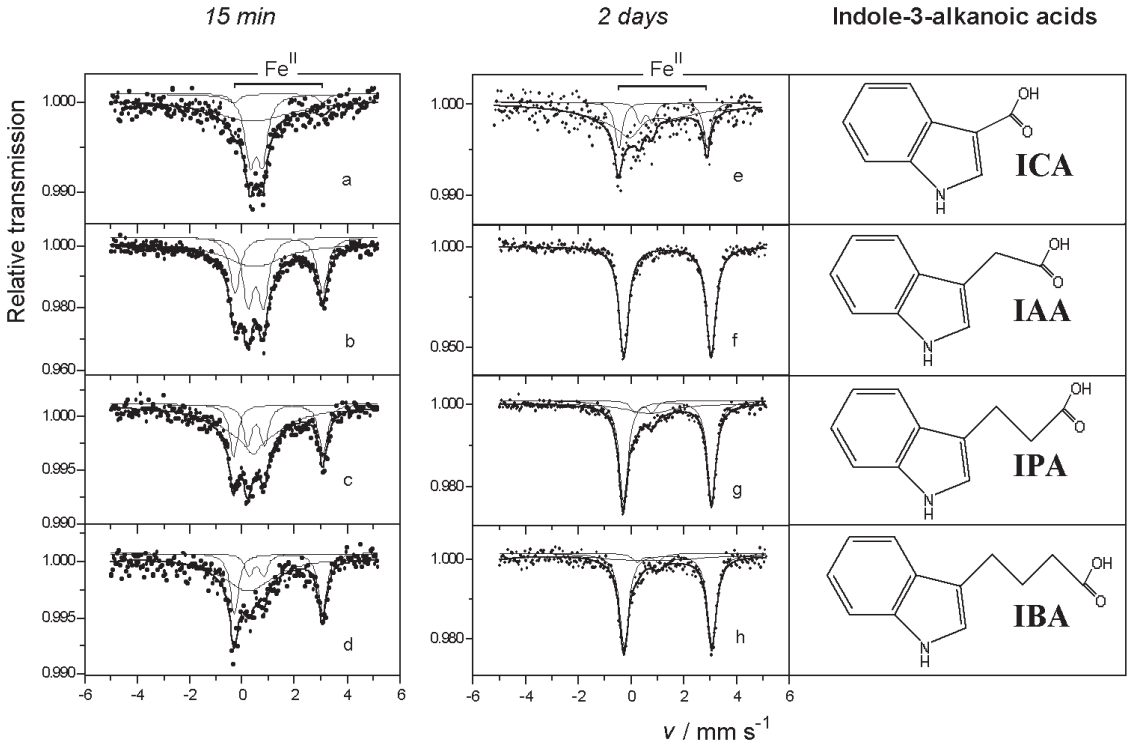

Fig. 1. Mössbauer spectra of aqueous solutions of $^{57}Fe^{III}$ nitrate and indole-3-alkanoic acids (their structures are shown in the right-hand panel) filtered and rapidly frozen (at T = 80 K) 15 min (a–d) and 2 days (e–h) after mixing the reagents (1:3 molar ratio; final pH ~ 2 to 3). Spectra (a), (e) – indole-3-carboxylic acid (ICA); (b), (f) – indole-3-acetic acid (IAA); (c), (g) – indole-3-propionic acid (IPA); (d), (h) – indole-3-butyric acid (IBA). The position of the Fe^{II}-related doublets is indicated in the upper plots by square brackets.

Comparing the spectral intensities (cf. Figure 1, *e–h*) it can be seen that after 2 days of contact of the indolic acids with iron(III), in IAA solution there is ferrous iron only, as compared to the Fe–ICA, Fe–IPA or Fe–IBA systems where some remaining ferric iron is still detectable. This indicates a stronger reducing capability of IAA towards iron(III) in the series of indole-3-alkanoic acids, evidently related to the ease of the IAA side-chain decarboxylation [20–23]. Note also that both the relative and absolute intensities of the Fe^{II} component in ICA solutions (see Figure 1, spectra *a* and *e*) are less than those for the other acids, showing the least reducing capacity of ICA in the series. The other two components of the spectra (see Figure 1, spectra *a–h*, except spectrum *f*) represent iron(III) complexes with the corresponding ligands (doublets with δ = 0.52 to 0.55 mm/s and Δ = 0.5 to 0.6 mm/s), that remain in solution after filtering out the precipitated complexes, and residual mononuclear Fe^{3+} ions (evidently partly hydrolysed at weakly acidic pH, which give a very broad single line).

The iron(III) doublets (with δ = 0.52 to 0.55 mm/s and Δ = 0.5 to 0.6 mm/s) exhibit the parameters typical for high-spin Fe^{3+} in distorted octahedral coordination. The Mössbauer spectra of the corresponding solid complexes filtered out of the aqueous solutions (for IAA, IPA and IBA) all gave an intensive symmetric quadrupole doublet with similar parameters (δ = 0.52 ± 0.01 mm/s, Δ = 0.60 ± 0.05 mm/s at T = 80 K), and Mössbauer spectra of those complexes redissolved in acetone showed the same pattern in the frozen acetone solutions [24] reflecting the possibility of their

molecular dissolution. In the frozen acetone solutions (at concentrations of each of the complexes 0.1 M and 0.01 M, using ^{57}Fe-enriched samples in the latter case to enhance the intensity of the spectra), the lack of a magnetic structure (due to fast spin-spin relaxation) provides evidence that the iron(III) species have a dimeric structure [16, 18]. This result is in good agreement with the data of elemental analyses, FTIR and FT-Raman spectroscopic results (including those for deuterated samples) for the solid complexes filtered out of the solutions, indicating a μ-$(OH)_2$-bridged structure: $[L_2Fe<(OH)_2>FeL_2]$ (where L is the deprotonated IAA, IPA or IBA moiety) [24]. It has to be noted that in the case of ICA, the data of elemental analyses pointed to the possibility of the presence of a mixture of solid products which should be studied in more detail separately.

In the case of Fe^{III}–IAA complex dissolved in methanol, a solution X-ray diffraction study was also performed [24]. Analysis of the data for Fe^{III}–IAA complex as well as, by analogy (considering the closely related Mössbauer, FTIR and FT-Raman spectroscopic results), for the corresponding IPA and IBA complexes, can be interpreted using the general structure represented in Fig. 2. Each iron atom in a complex is surrounded by six oxygen atoms in a slightly distorted octahedral symmetry as follows: four from two deprotonated IAA carboxylate ligands (in the bidentate coordination) and two from the dihydroxo bridge linking the two iron atoms. Nevertheless, it should be noted that under different conditions, a monomeric poorly water-soluble Fe(III)–IAA complex was obtained from aqueous solution which gave different spectroscopic images owing to its different structure [15].

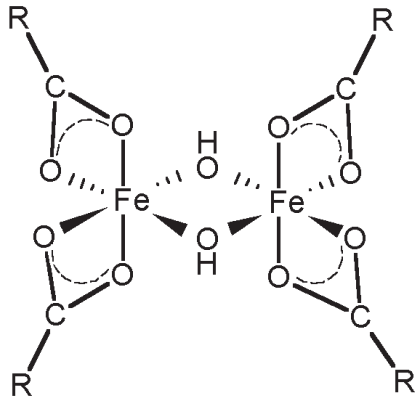

Fig. 2. Schematic representation of solid Fe(III) complexes with IAA, IPA and IBA.

Thus the parameters of the spectra in Figure 1 suggest the existence of two parallel reactions between Fe^{3+} and the ligands (L); namely, both a redox transformation yielding Fe_{aq}^{2+} ions and Fe^{3+}–L complex formation take place, as shown in the following scheme.

$$Fe^{3+} + L \rightarrow \begin{cases} Fe^{III} \text{ complex (precipitate and/or dissolved form)} \\ Fe_{aq}^{2+} + \text{[oxidised L product(s)]} \end{cases}$$

While enzymatic oxidation of auxin phytohormone catalysed by plant peroxidases, regarding its mechanism and products, has been under intensive investigation owing to basic interest [21–23] as well as possible biomedical applications (see, e.g. [10, 11] and references therein), chemical oxidation products of auxins are much less studied [25]. Owing to the sophisticated nature of these chemical process involving radical products and/or intermediates [26], this seems to be not an easy and straightforward task.

Some products of aerial oxidation of indole-3-acetic acid in the presence of Fe^{III} were isolated and studied using FTIR spectroscopy, ^{1}H NMR and chromatography-mass spectrometry. The formation of oxindole-3-acetic acid was shown, which formed a poorly soluble complex with Fe^{III} similar to that with indole-3-acetic acid [15] by the coordiantion mode; in particular, giving a similar FTIR spectrum [27]. From the reaction medium, using extraction with isobutanol and further chromatographic separation, two other oxidation products were isolated. One of the products gave an intensive FTIR absorption band of carbonyl (1649 cm^{-1}), a couple of bands at 2925 and 2856 cm^{-1} (aliphatic C–H stretching vibrations) and a band at 3403 cm^{-1} (N–H or O–H stretching vibrations). Its ^{1}H NMR spectrum in deuterated acetone showed a group of signals of the oxindole moiety (7.2–8.4 p.p.m.), a singlet of the >N–H proton (10.01 p.p.m.), a quadruplet (3.2–3.7 p.p.m.) of the proton in the position C3 of the oxindole moiety split at neighbouring protons of the $–CH_3$ group. Finally, the latter three magnetically equivalent protons gave a doublet (split at the vicinal C3 proton) at 1.2 p.p.m. Altogether these data provide evidence for the formation of 3-methyl-2-oxindole as an oxidation product (Fig.3). Traces of the other extracted product gave a mass spectrum which suggests that the oxidation product was formed by further oxidative splitting of the pyrrolin-2-one cycle [7].

It should be noted that both oxindole-3-acetate and 3-methyl-2-oxindole, which were found to be formed in the course of chemical oxidation of indole-3-acetic acid in the presence of Fe^{III} (see Figure 3), had earlier been reported among products of both its enzymatic [28, 29] and electrochemical oxidation [25] at physiological pH (along with 3-methylene-2-oxindole), i.e. under different conditions and involving different electron transfer modes. Nevertheless, the exact mechanism of chemical oxidation of IAA, in particular, under environmentally relevant conditions has to be elucidated in more detail, which requires further investigations.

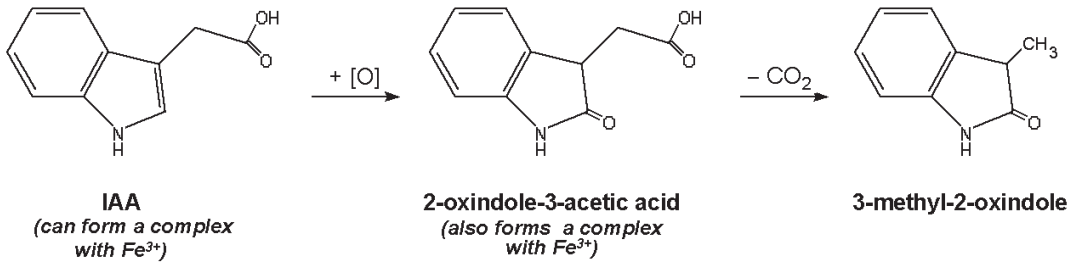

Fig. 3. Scheme and some products of chemical oxidation of indole-3-acetic acid in aqueous solution under aerobic conditions in the presence of iron(III).

CONCLUSIONS

Iron(III) ions were shown to be gradually reduced by each of the indole-3-alkanoic acids with *n*-alkanoic side-chains C_1 to C_4 in acidic aqueous media under aerobic conditions using Mössbauer spectroscopic measurements in rapidly frozen solutions. The parameters of the Mössbauer spectra indicate that there are two parallel processes, *viz* iron(III) complexation and redox transformations. Within the series of the indole-3-alkanoic acids, indole-3-carboxylic acid showed the least reducing capability towards iron(III). After 2 days, indole-3-acetic acid showed virtually a complete reduction of iron(III) to iron(II), whereas iron(III) was still detectable in solutions of the other acids, along with iron(II). Mössbauer parameters of the frozen solutions provide evidence that the resulting iron(II) species is the hexaaquo complex. The solid complexes formed were found to have a dimeric μ-dihydroxo-bridged structure, that was confirmed using a combination of spectroscopic techniques for indole-3-acetic, indole-3-propionic and indole-3-butyric acids. Among the products of chemical oxidation of indole-3-acetic acid in the presence of iron(III) under aerobic conditions, oxindole-3-acetate and 3-methyl-2-oxindole were detected using vibrational and ^{1}H NMR spectroscopic measurements. The same substances had earlier been reported to be found among the products of enzymatic and electrochemical oxidation in circumneutral media, i.e. under different conditions and involving different electron transfer modes.

EXPERIMENTAL

Mössbauer measurements in aqueous solutions were performed using materials prepared from ^{57}Fe-enriched iron (ca. 90% ^{57}Fe) dissolved in nitric acid at elevated temperature. The stock solution was 0.01 M with regard to iron(III), with pH 0.9. The indole derivatives used (ICA, IAA, IPA, IBA) were dissolved in water adding KOH to the solutions up to pH 6–7. The concentration of the ligands after mixing was 0.03 M. Addition of iron(III) nitrate to an indolic acid in solution (up to the 1:3 metal-to-acid molar ratio) resulted in the colour change of the solutions and the formation of cocoa-brown precipitates indicating complexation of Fe^{3+} with the indole-3-alkanoic acids. The final pH values of the mixtures were around 2.5 (measured using an OP-211 laboratory pX/mV meter, Radelkis, Hungary).

The solutions were filtered after 15 min or 2 days, rapidly frozen in liquid nitrogen, and Mössbauer spectra of the rapidly frozen filtrates were recorded. For analysing the precipitates filtered out after 15 min, the resulting solids were dried on the filter paper at room temperature for a few days and placed in a cryostat cooled with liquid nitrogen. All Mössbauer spectra were recorded at liquid nitrogen temperature (ca. 80 K) using a conventional constant-acceleration Mössbauer spectrometer with a ^{57}Co(Rh) source using a "cold-finger" cryostat filled with liquid nitrogen. The spectrometer was calibrated using α-Fe foil at room temperature, which is the reference for all isomer shifts reported in this paper. Statistical treatment of the Mössbauer spectra was performed with the assumption of Lorentzian line shapes in order to calculate isomer shifts (δ, mm/s), quadrupole splittings (Δ, mm/s), line widths (full width at half maximum, Γ, mm/s) and partial resonant absorption areas (S_r, %) for all spectral components. ^{1}H NMR spectra were obtained on a Bruker AC-300 spectrometer (300 MHz), with $Si(CH_3)_4$ as an internal standard, in deuterated acetone solutions. All other measurements and experimental details were as described elsewhere [7, 16, 24, 27].

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