Struct Chem (2008) 19:109–114
DOI 10.1007/s11224-007-9259-6

ORIGINAL PAPER

Water and time dependent interaction of iron(III) with indole-3-acetic acid

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Received: 24 July 2007 / Accepted: 8 October 2007 / Published online: 8 November 2007

Abstract The influence of water on the interaction between iron(III) and indole-3-acetic acid (IAA) was studied in different organic solutions using rapid-scan stopped-flow spectrophotometry and rapid-freeze/quench Mössbauer spectroscopy. Measurements were also performed in ethanol–water and acetone–water mixtures. The results showed that the interaction between Fe^{III} and IAA resulted in dimeric Fe^{III}–IAA complex within 1 s, followed by a slow second step to give Fe^{2+} and IAA(oxidized). No such products were formed in the absence of water. The visible and Mössbauer spectra reflect the nature of the organic solvent and that of the anion of iron(III) salts.

Keywords Indole-3-acetic acid · Iron(III) complexes · Mössbauer spectroscopy · Stopped-flow spectrophotometry

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Introduction

Indole-3-acetic acid (IAA) is one of the most important plant-growth-regulating phytohormones, which is also active in animal and yeast cells [1]. IAA stimulates cell division and promotes cell elongation [2, 3]. In the rhizosphere, IAA plays an important role in plant–microbe interactions [4, 5]. It has been suggested that a combination of IAA and horseradish peroxidase (HRP) kills human cancer cells and may thus be a new form of anticancer treatment [6, 7]. Importantly, IAA becomes active only after decarboxylation by HRP since this alone has no cytotoxic effect. The mechanism of such oxidative degradation of IAA by HRP may be understood by knowing the nature of coordination of IAA with Fe^{III} of HRP. It has been proposed that an interaction between HRP and IAA results in one electron transfer from the IAA molecule to the ferric moiety of the peroxidase hem through a triple complex (peroxidase–IAA–oxygen) [8–10]. The Fe^{III}–IAA system is therefore a good model for the peoxidase–IAA complex.

The structure of solid complexes between Fe^{III} and indole-3-alkanoic acids has been investigated using different spectroscopic methods [11, 12]. Recently, we have studied the interaction between Fe^{3+} and IAA in aqueous media using Mössbauer spectroscopy for frozen solution, vibrational spectroscopy, and solution X-ray diffraction techniques [13, 14]. The experimental results suggested the occurrence of two parallel reactions; some of the iron(III) were complexed by the IAA ligand and others were reduced to iron(II). Although these measurements were made on a time scale ranging from 20 min to several days, no insight could be obtained which of the two parallel reactions dominated. Herein, we have used a rapid scan stopped-flow spectrophotometry in conjunction with the

rapid-freeze/quench Mössbauer spectroscopy technique in order to demonstrate the water dependent interaction between Fe^{III} and IAA. It may be proposed that a dimer of Fe^{III}–IAA complex is formed by the interaction of Fe^{III} and IAA before the formation of Fe^{II} and the oxidized product of IAA. The effect of the solvent donicity was also examined using ethanol–water and acetone–water mixtures.

Experimental

Chemicals including iron(III) salts and IAA of reagent grade, purchased from either Sigma or Aldrich, were used without further purification. Two different iron salts of $FeCl_3$ and $Fe(NO_3)_3{\cdot}9H_2O$ were used. Solid Fe–IAA complex was prepared according to the method described earlier [14]. Solutions were prepared with distilled water with electric resistivity higher than 18 $M\Omega{\cdot}cm^{-1}$ using Milli-Q water purification system.

A stopped-flow spectrophotometer (SX.18 MV, Applied Photophysics, U.K.) equipped with a photomultiplier (PM) detector was used to perform rapid kinetic measurements. In the experiments, solutions were mixed at a 1:1 volume ratio. In one syringe, 0.005 M Fe^{III} solution was placed, and in other syringe IAA dissolved in either absolute ethanol or acetone. Concentrations of IAA were fixed to be 0.015 M and 0.060 M in order to achieve the Fe^{III}:[IAA] molar ratios of 1:3 and 1:12, respectively. The influence of water on the reaction was also studied by mixing the Fe^{III}–IAA solution of absolute ethanol or acetone with water. The rapid scan curves were collected in the wavelength range of 400–600 nm, and the scans at different time intervals were collected up to 50 s. The temperature of the reaction media was controlled at 25 ± 0.1 °C with a Fischer Scientific Isotemp 3016 circulating water bath.

For the Mössbauer measurements, $FeCl_3$ was prepared by dissolution of metallic ^{57}Fe (~90% enriched) in hydrochloric acid. The iron(III) salt was obtained by drying the solution on a boiling water bath. A total of 0.025 M $FeCl_3$ solution was used for the experiment. In the case of $Fe(NO_3)_3{\cdot}9H_2O$ solutions, a higher concentration of ~0.1 M was used for the experiment. Consequently, the Mössbauer spectra could be recorded for the sample prepared with natural iron. Before the Mössbauer measurements, each solution was allowed to stand for 50 s to 20 min at room temperature and then it was rapidly frozen in liquid nitrogen.

Mössbauer spectra were taken at ca. 100 K using a conventional constant acceleration type Mössbauer spectrometer with a $^{57}Co(Rh)$ source of a $\sim 10^9$ Bq. The spectrometer was calibrated with α-iron at room temperature. Isomer shift values were calculated with respect to α-iron. Spectral evaluation was performed using the Mosswinn 3.0 program [15].

Results

Rapid scan measurements

Solutions of $FeCl_3$ and IAA in absolute ethanol were mixed and rapid scans were recorded at different time intervals. Molar ratio of Fe^{III}:IAA in the mixed solution was fixed to be 1:3 (Fig. 1a). The spectra showed no significant change at time intervals between 0.05 and 50 s, suggesting no interaction between Fe^{III} and IAA. The molar ratio in the mixed solution was increased to 1:12 and the solvent of ethanol–water (50:50 v/v) was used in order to induce any possible interaction between Fe^{III} and IAA. The spectra obtained at different times are given in Fig. 1b. A broad band was observed at ca. 460–540 nm, which rapidly increased with time. A log-plot of the absorbance at 470 nm against time is shown in Fig. 1c. This clearly demonstrates that water plays a role in the formation of the complex composed of Fe^{III} and IAA.

In the second experimental set-up, solutions of $FeCl_3$ and IAA in acetone were investigated. Molar ratios of Fe^{III} to IAA were fixed to be 1:3 and 1:12, respectively. Similarly to the observation in absolute ethanol, the sspectra showed no significant change with time (Fig. 2a), indicating that no interaction occurred in pure acetone between Fe^{III} and IAA up to 50 s. However, rapid scans collected by mixing $FeCl_3$ with IAA in an acetone–water mixed solvent showed a very broad band at the same wavelength as observed before (Fig. 2b). These bands grew very rapidly within 1.0 s, which was much faster than in ethanol–water mixture (Fig. 1b), in spite that the shape of the spectra was quite similar to each other. These results indicate that nature of the organic solvent deeply influences the interaction of Fe^{III} with IAA.

Spectral scans for ferric nitrate $Fe(NO_3)_3{\cdot}9H_2O$ were conducted by mixing Fe^{III} with IAA in absolute ethanol at a molar ratio of 1:3 (Fig. 3a). As expected, a broad band appeared between 470 and 540 nm in the mixed solution, and its intensity increased with time. A log–log plot of the absorbance at 470 nm was linear, as shown in Fig. 3b. The spectral characteristics indicate the formation of chemical species composed of Fe^{III} and IAA. The spectral difference between Figs. 1a and 3a seems to be based on the difference of the crystallization water; i.e., $FeCl_3$ is anhydrous, whereas $Fe(NO_3)_3{\cdot}9H_2O$ has nine moles of crystallization water. It seems that the water coming from the ferric nitrate in the mixed solution plays an important role in the interaction between Fe^{III} and IAA.

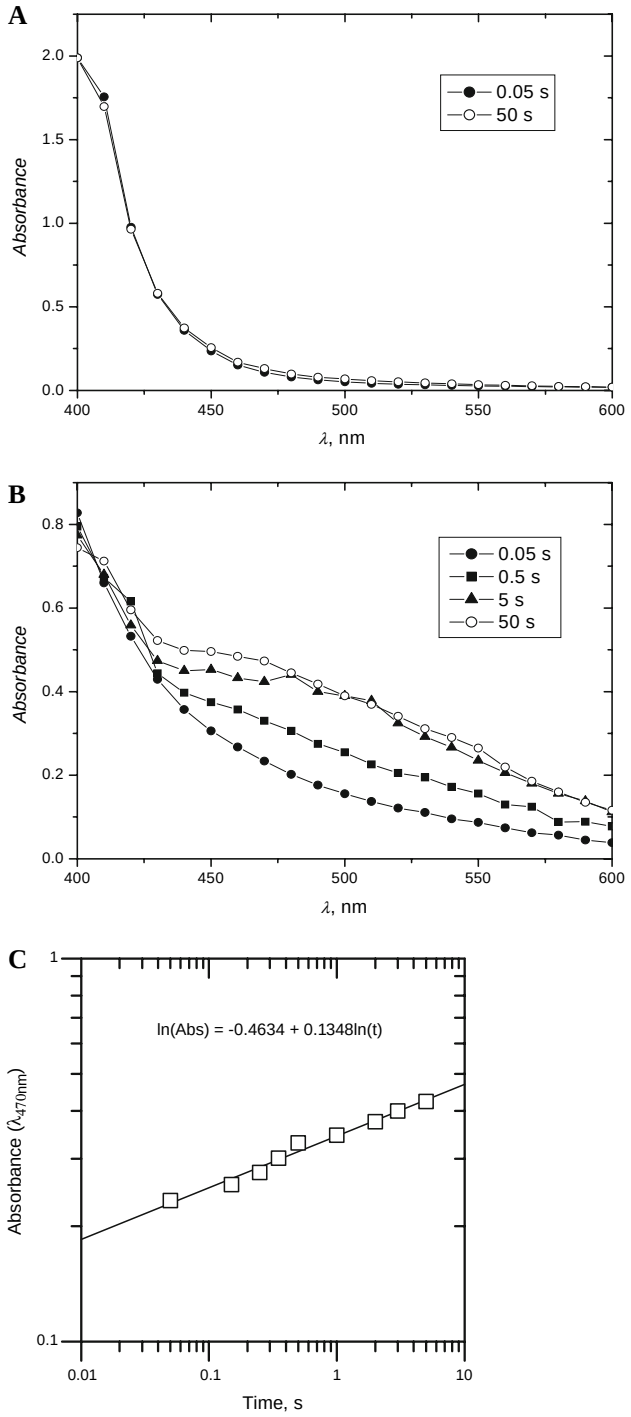

Fig. 1 Rapid scan time course of the reaction of $FeCl_3$ with IAA at 25°C. (**a**) molar ratio of 1:3 (Fe^{III}:IAA) in absolute ethanol. (**b**) molar ratio of 1:12 (Fe^{III}:IAA) in ethanol–water solvent (50:50v/v), (**c**) increase of the absorbance at $\lambda = 470$ nm in the ethanol–water mixture (50:50v/v)

Finally, solid Fe–IAA complex was dissolved in absolute ethanol, and water was added to the solution. Rapid scan data are shown in Fig. 4. As can be seen, the spectra have a broad absorption in the same region as mentioned above, and no significant change was observed caused by the addition of water.

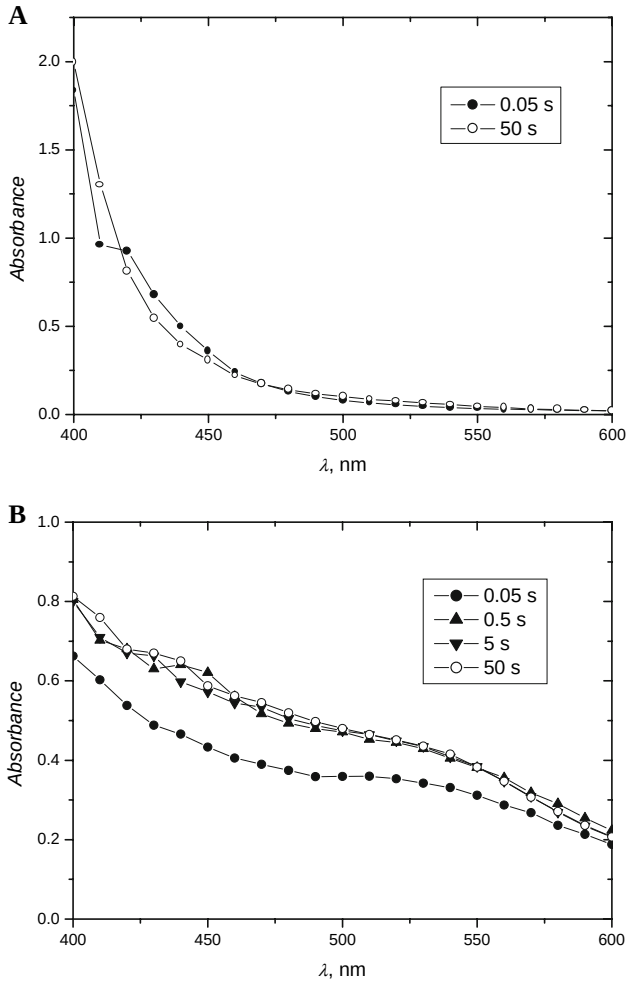

Fig. 2 Rapid scan time course of the reaction of $FeCl_3$ with IAA at 25°C. (**a**) molar ratio of 1:3 (Fe^{III}:IAA) in acetone (**b**) molar ratio of 1:12 (Fe^{III}:IAA) in acetone–water solvent (50:50v/v)

Mössbauer studies

Mössbauer spectroscopy of frozen solutions was used to elucidate further chemical information on the interaction between iron(III) and IAA, since it gives useful information about the electronic structure of iron species [16]. Mössbauer spectra of $^{57}FeCl_3$ + IAA (1:3 molar ratio) in frozen ethanol–water solution are shown in Fig. 5. From the Mössbauer parameters, different iron species were identified in the solution including that of Fe^{2+} ions that were produced as a result of the reduction of Fe^{III}.

Mössbauer parameters indicate the formation of a dimeric complex, resulting from the interaction between Fe^{III} and IAA. The lack of magnetic splitting in the spectra

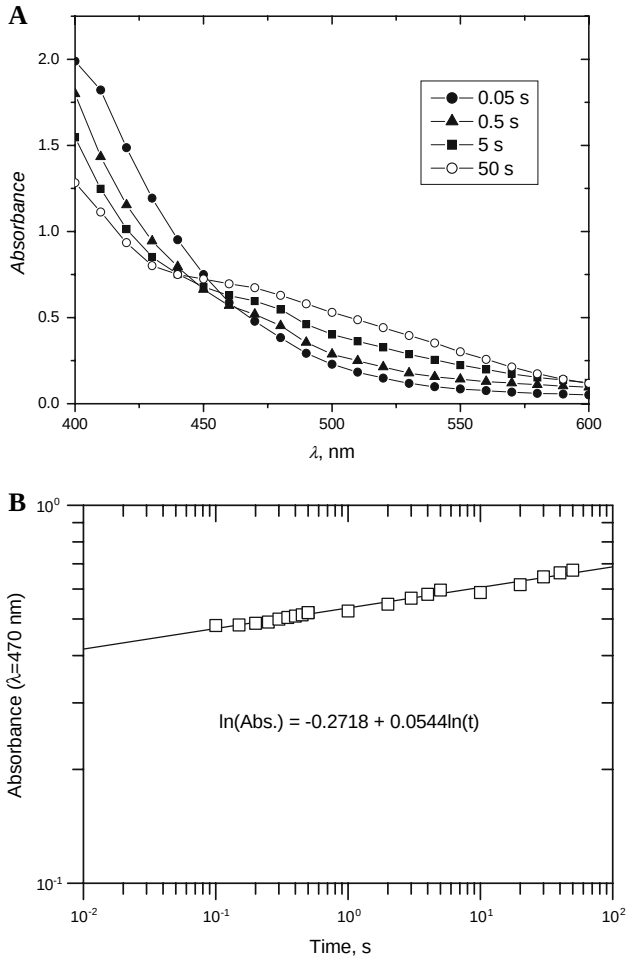

Fig. 3 (**a**) Rapid scan time course of the reaction of Fe^{III} with IAA in absolute ethanol at a molar ratio of 1:3 (Fe^{III}:IAA) using $Fe(NO_3)_3{\cdot}9H_2O$ as an iron(III) ion source. (**b**) A log–log plot of the increase of absorbance with time at λ = 470 nm

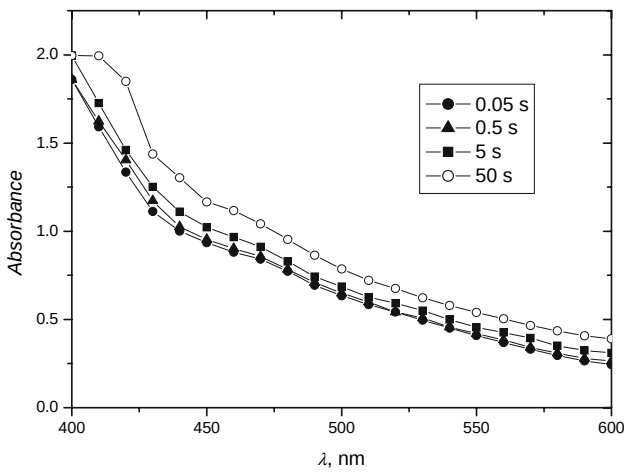

Fig. 4 Rapid scan time course of the solid Fe–IAA complex redissolved in absolute ethanol–water solvent (50:50v/v)

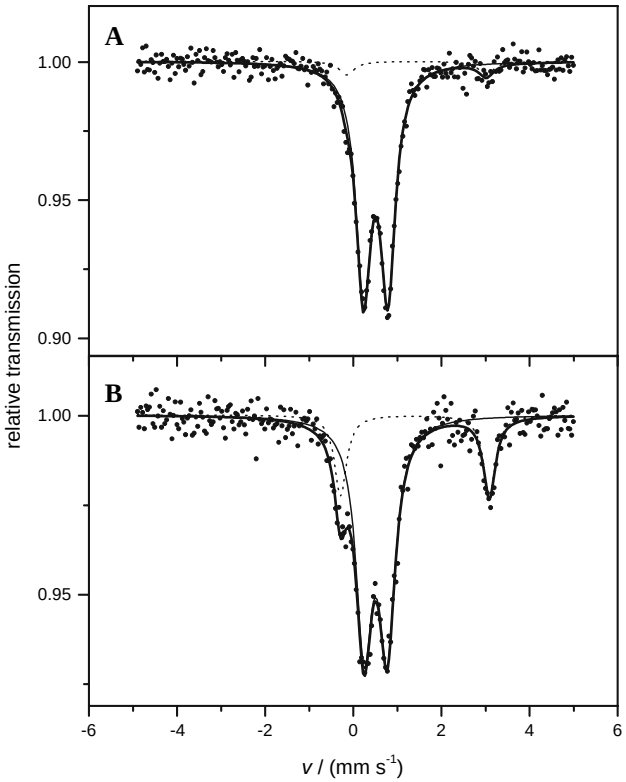

Fig. 5 Mössbauer spectra of the mixture of $^{57}FeCl_3$ and IAA at a molar ratio of 1:3 ([Fe^{3+}] = 0.025 M). Reaction mixture was prepared in ethanol–water solvent (50:50v/v) and solution was frozen at (**a**) 50 s, (**b**) 20 min. All spectra were recorded at 100 K

provides evidence that Fe^{III} does not have a monomeric structure in the solution [14, 17]. There was a significant Fe^{2+} ion formation when the same solution was frozen after 20 min. Concurrently, there was a decrease in the amount of dimeric Fe^{III}–IAA complex (Fig. 5, Table 1).

In order to understand the nature of the Fe^{III}–IAA complex, a solid Fe^{III}–IAA complex was dissolved in absolute ethanol with and without water. Each solution was aged at room temperature for 20 min and then frozen for Mössbauer measurement (Fig. 6, Table 2). Similarity of the Mössbauer parameters in ethanol–water mixed solution of this complex and those of the dimer complex of Fe^{III} and IAA [13] suggest that both complexes have essentially the same structure. One has to note that water addition causes a slight increase in the quadrupole splitting that indicates a perturbation of the ligand field of the Fe^{III} dimer complex.

Mössbauer spectra of the $Fe(NO_3)_3.9H_2O$–IAA system in absolute ethanol with and without water are shown in Fig. 7. Mössbauer parameters for the iron(III) species in pure ethanol are characteristic of the Fe^{III}–IAA complex [13, 14], which was formed within 20 min after mixing, and no Fe^{2+} could be found. In contrast, a large amount of iron(II) ($\sim$50% of the total iron content) was formed in the reaction when water (50:50 v/v) was present, and the

Table 1 Mössbauer parameters of iron(III) and IAA containing solutions ([Fe^{3+}]:[IAA] = 1:3) in different solvents

Freezing time after mixing	$FeCl_3$ + IAA Ethanol–H_2O 50:50 (v/v) 50 s	$FeCl_3$ + IAA Ethanol–H_2O 50:50 (v/v) 20 min	$Fe(NO_3)_3$ $9H_2O$ + IAA IAA 20 min	$Fe(NO_3)_3$ + IAA Ethanol–H_2O 50:50 (v/v) 20 min
Fe^{3+}, S_r%	94	79	100	50
δ/mm s^{-1}	0.508(3)	0.506(5)	0.518(7)	0.510(1)
Δ/mm s^{-1}	0.556(5)	0.528(7)	0.480(1)	0.510(2)
Γ/mm s^{-1}	0.405(7)	0.44(1)	0.38(2)	0.34(2)
Fe^{2+}, S_r%	6	21		50
δ/mm s^{-1}	1.440(3)	1.400(1)		1.483(8)
Δ/mm s^{-1}	3.160(6)	3.380(2)		3.430(2)
Γ/mm s^{-1}	0.405(7)	0.33(3)		0.32(3)

reduction of iron(III) was faster than that of $FeCl_3$–IAA in ethanol–water mixture.

Discussion

According to the earlier studies, the structure of Fe^{III}–IAA complex formed in aqueous solution was found to be dimeric, μ-$(OH)_2$-bridged, while the end product of the redox reaction was $[Fe(H_2O)_6]^{2+}$ with different oxidized indole-3-acetic acid derivatives [13]. In organic solutions, the reaction is affected by changing the type of iron salt or the water content of the organic solvent, e.g., absolute ethanol, acetone or ethanol–water, acetone–water mixtures.

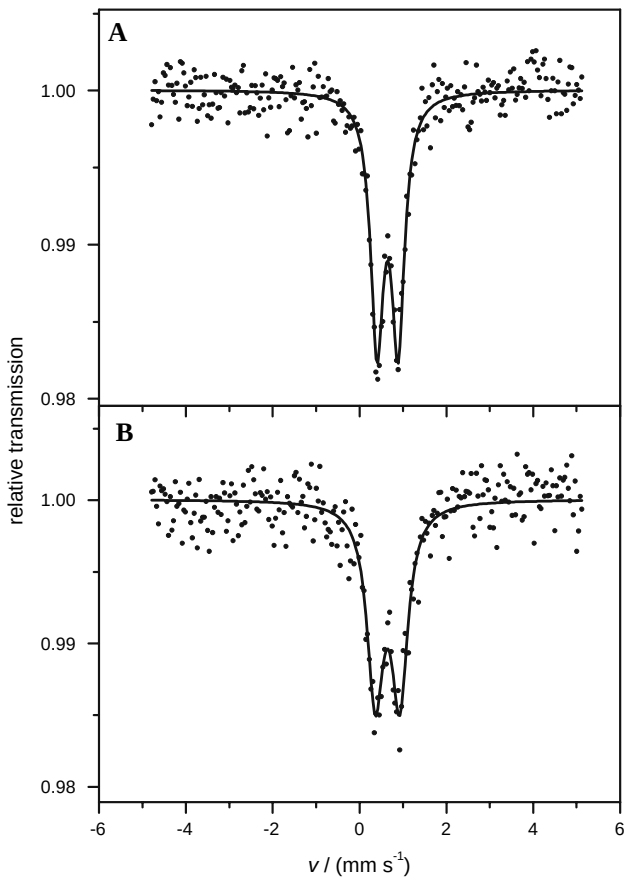

Fig. 6 Mössbauer spectra of the ^{57}Fe–IAA solid complex redissolved in absolute ethanol (**a**) and in ethanol–water solvent (50:50v/v) (**b**). Solutions were frozen after 20 min and spectra were taken at 100 K

The short-term stopped-flow scans of the $FeCl_3$–IAA system show changes in the spectra when water was present. Comparing the spectra of the freshly mixed solutions and those of the dissolved solid complex, one can see that the dimeric Fe–IAA complex gives a significant broad shoulder in the 470–540 nm region (Fig. 4). The peaks observed in the freshly mixed solutions in the presence of water are very similar to each other, suggesting the formation of essentially the same complexes (Figs. 1a, 2a and 3). It proves that water is necessary for the formation of the dimeric Fe–IAA complex. The reduction process, which results in the formation of Fe^{II} and different oxidized derivatives of IAA, will be significant only when the water content is high, as known from spectra recorded in the 50:50 v/v solutions. The increase in the fraction of Fe^{II} could be confirmed from Mössbauer spectra and UV–Vis spectra, in which a significant increase was observed in the region > 520 nm. It is speculated that the pink color in the solutions comes from some oxidized products of the IAA which can partly coordinate to Fe^{II}. This is also supported by the Mössbauer parameters obtained for the Fe^{II} species

Table 2 Mössbauer parameters of solid Fe^{III}–IAA complex after dissolving in ethanol and ethanol–water mixture and freezing the solution after 20 min

	Ethanol	Ethanol:H_2O 50:50 (v/v)
Fe^{3+}, S_r,%	100	100
δ/mm s^{-1}	0.531(5)	0.529(9)
Δ/mm s^{-1}	0.487(9)	0.570(2)
Γ/mm s^{-1}	0.36(1)	0.45(3)

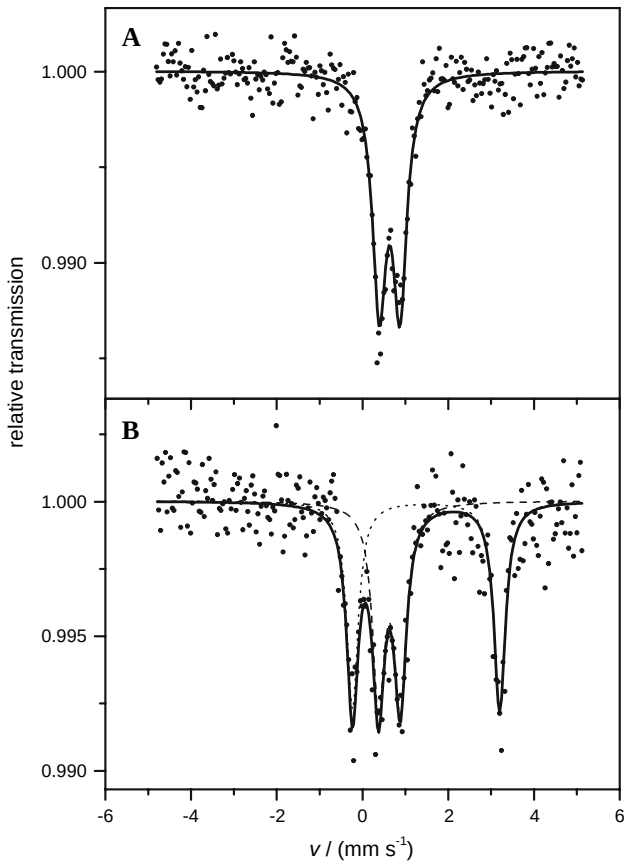

Fig. 7 Mössbauer spectra of the reaction mixture of $Fe(NO_3)_3 \cdot 9H_2O$ and IAA at a molar ratio of 1:3 ($[Fe^{3+}] = 0.1$ M). Mixtures were prepared in (**a**) absolute ethanol and (**b**) in ethanol–water solvent (50:50v/v). Solutions were frozen after 20 min and spectra were taken at 100 K

(Table 1), since the quadrupole splitting values were slightly smaller than those of characteristic of Fe^{2+} hexa-aqua complex [18].

If water is not present in the solutions, complex of Fe^{2+} could not be detected in UV–Vis and Mössbauer spectra. The quantity of the reduced iron and its complex is much higher in the solution of iron nitrate, which evidently shows the effect of anion on the reaction; namely, the probability of the coordination of Cl^- to Fe^{3+} is larger than that of NO_3^- and hence iron chloride seems to be less reactive.

It is worth mentioning that the iron complex which can be obtained in pure water solution as a precipitate [13] remains stable when redissolved in ethanol, while water addition causes only the exchange of some COO^- moiety for H_2O, as known from the increased values of quadrupole splitting (Fig. 6 and Table 2).

The spectral difference observed in different organic solvents (acetone or ethanol) can be interpreted as a consequence of different donicity to the Fe^{3+} ion. This has a great influence when dimer or monomer species are formed in the solutions, even in the case of a pure iron salt [17]. The formation of dimer moiety is more favorable in acetone than in ethanol, since the Fe^{3+} remains in the monomeric form in the latter without water.

Conclusions

The rapid scans in the visible region of UV–Vis spectrophotometry and the Mössbauer measurements of the solutions showed the importance of water during the reaction of Fe^{III} with IAA. The formation of dimeric Fe–IAA complex takes place in a relatively fast step as detected by the stopped-flow measurements, whereas the redox process goes on slowly as confirmed by the Mössbauer technique. The donicity of ethanol and acetone affects the environment around the iron atom of the dimeric Fe^{III}–IAA complex, thus changing the spectral characteristics of the complex.

Acknowledgments We acknowledge the support from the U.S.–Hungary Partnership Program provided by the National Science Foundation and the Hungarian Academy of Science, the Hungarian Science Foundation (OTKA K62691, K67835), under the Agreement on Scientific Collaboration between the Russian and Hungarian Academies of Sciences for 2005–2007, as well as by NATO (Grants CBP.NR.NREV 981748 and ESP.NR.NRCLG 982857).

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