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Mössbauer Study of the Effect of pH on the Rate of Redox Interactions between Iron(III) and 4-*n*-Hexylresorcinol in Aqueous Media

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Abstract—Mössbauer spectroscopy is used for a comparative study of the rate of iron(III) reduction by 4-*n*-hexylresorcinol (4-*n*-HR, a chemical analog of microbial autoregulators excreted by cells into the environment that allow intercellular communication) in aqueous media in the pH range of 1.5–4.5 simulating acidic soil conditions. The concomitant process of 4-*n*-HR oxidation is monitored using UV spectrophotometry.

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INTRODUCTION

Interaction between soil microorganisms, their secondary metabolites excreted by cells into the environment, and its components not only plays an important role in geomicrobiology and mineralogy [1–3] but can also affect the vital activity of cells. This is true in particular of the abiotic (chemical) decomposition of microbial signaling molecules (chemical language as a means of intercellular communication) that influence the behavior of an entire microbial consortium [4–8].

In our earlier studies based on Mössbauer spectroscopy (MS), it was shown that Fe^{III} in acidic media can oxidize some of the biomolecules responsible for intercellular microbial signaling [9–12]. These abiotic processes are of interest from the viewpoint of microbial ecology for sufficiently widespread acid soils and aquifers [13, 14]. Among alkylresorcinols (1,3-dihydroxybenzenes with various alkyl substituents in positions 4 or 5), chemical analogs of microbial autoregulators of the alkylhydroxybenzene class (AHB) with adaptogenic, antioxidant, and antiradical functions [4, 5, 7, 8, 15], 4-*n*-hexylresorcinol (4-*n*-HR) was the fastest (several minutes at pH ~3) to reduce iron(III) to iron(II), according to the data in [12]. It should be noted that in the iron(III)–4-*n*-HR system, no reoxidation of the iron(II) formed in predominant amounts was observed after redox processes while drying the mixture in air [12]. This in particular is one important feature of alkylresorcinols in contrast to indole-3-acetic acid [16, 17], an important phytohormone of the auxin class that is produced by many microorganisms, participates in extracellular microbial and plant-microbial signaling [18], and can also be oxidized by iron(III) in moderately acidic media along with other auxins [9, 10, 16, 17].

EXPERIMENTAL

The aim of this work was to investigate the effect of medium acidity (pH) on the rate of redox processes via MS. We studied the reaction of 4-*n*-HR with iron(III) in the pH range of 1.5–4.5 in aqueous media (frozen solutions). The concomitant oxidation of 4-*n*-HR by iron(III) was monitored by spectrophotometry using the UV absorption spectra of soluble organic reagents.

4-*n*-HR (chemically pure, Sigma; Fig. 1) and $FeCl_3$ were used in this work (iron 90% enriched with ^{57}Fe was used in preparing samples for MS). The reaction mixtures contained 0.50 mL of initial 0.06 M solution of 4-*n*-HR (in water with 25 vol % of absolute ethanol in order to increase solubility) and 0.10 mL of 0.1 M aqueous solution of $FeCl_3$, corresponding to molar ratio Fe : AR = 1 : 3 (total concentration of Fe in the final mixture was 0.016 M). When necessary, the pH value was adjusted to the required levels by adding small amounts of 1 M KOH (or HCl when pH had to be lowered) with constant agitation; the final concentration of ethanol in all samples was 20 vol %.

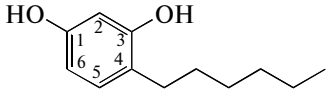

Fig. 1. Schematic representation of the molecular structure of 4-*n*-hexylresorcinol ($C_{12}H_{18}O_2$; numbers denote the arrangement of carbon atoms in the aromatic cycle).

In order to measure the Mössbauer spectra, a mixture of reagents covered with foil to avoid vaporization and exposure to light, was kept for a predetermined time at room temperature and then rapidly frozen in liquid nitrogen. The Mössbauer spectra were measured in the transmission mode by placing the frozen samples into a cryostat with liquid nitrogen (temperature of measurements, 80 K), using a WISSEL spectrometer (Germany), operating in the mode of constant acceleration with a Co(Rh) source. The obtained spectra were fitted by the least squares method using the MOSSWINN program [19]; all values of the isomer shift are given with respect to α-Fe at room temperature. Other details of the measurement procedure and instrumentation were described in [9, 11, 12].

The UV absorption spectra of aqueous ethanol (20 vol %) solution of the initial 4-*n*-HR (at a concentration of 0.48 mM, equivalent to the one used for its mixtures with Fe^{III} at the same degree of dilution (see below); for 4-*n*-HR, the molar extinction coefficient calculated from the spectra was 2.42×10^3 M^{-1} cm^{-1}) and soluble products of its oxidation in the presence of Fe^{III} (using $FeCl_3$ not enriched with ^{57}Fe isotope) were measured on a Specord S-250 spectrophotometer (Carl Zeiss, Germany) in a quartz cell with an optical path length of 10 mm in the wavelength range of 230–300 nm relative to an aqueous solution with 20 vol % of ethanol. Measurements of the UV spectra were performed at predetermined time intervals (after centrifuging a mixture with a composition similar to the one mentioned above, in Eppendorf tubes with a volume of 2.0 mL to separate suspended solid particles on a MICROSPIN 12 microcentrifuge (United States), 13400 rpm), taking an aliquot (100 μL) of transparent supernatant after 100× dilution with water containing 20 vol % ethanol (in order to reduce absorption in the UV range to the value suitable for measurements). Immediately after sampling, the residue was again agitated, closed, and stored for the next aliquot sampling.

RESULTS AND DISCUSSION

The Mössbauer spectra of the prepared mixtures of 4-*n*-HR with iron(III) in aqueous ethanol solutions at final pH values of 1.5, 3.0 (frozen 1 min after mixing) and 3.7 (frozen 30 min after mixing), measured at 80 K, are illustrated in Fig. 2a–2c. The parameters calculated from spectroscopic data are summarized in the table.

At pH ~1.5, the Mössbauer spectrum of the sample frozen 1 min after mixing the reagents contained a broadened component with a magnetic hyperfine structure (see Fig. 2a) characteristic of monomeric hydrated ions Fe^{3+}, which under these conditions are probably partially hydrolyzed [20]. Nevertheless, the spectrum distinctly shows a Fe^{II} doublet with the parameters typical of aquo complexes $[Fe(H_2O)_6]^{2+}$ [9, 20], indicating quite rapid reduction of iron(III) under such conditions (see table; the estimate is about 8% Fe^{II}, though the accuracy of determining the relative surface area of the components is in this case not high).

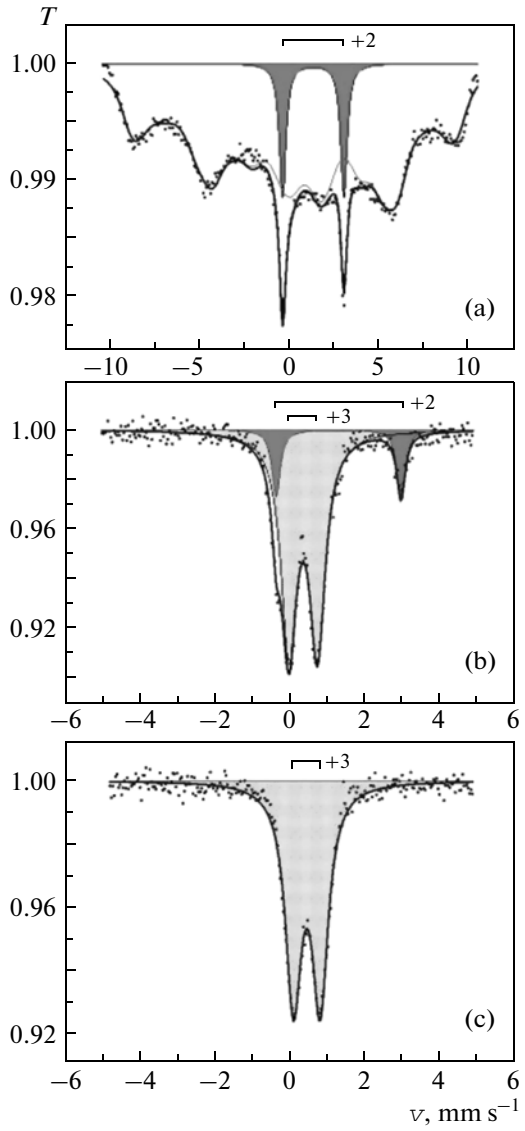

Fig. 2. Mössbauer spectra of aqueous ethanol (20 vol % ethanol) $^{57}Fe^{III}$-containing solutions of 4-*n*-hexylresorcinol (molar ratio, 1 : 3, respectively; $Fe_{total} = 0.016$ M) with final pH values of (a) 1.5, (b) 3.0, and (c) 3.7, rapidly frozen 1 min (a, b) and 30 min (c) after the mixing of reagents (measurements were performed at 80 K). The position of quadrupole doublets corresponding to different chemical forms of Fe is indicated by the brackets above the spectra (the numbers to the right of the brackets show the iron oxidation states corresponding to these forms). The broadened magnetic component (monomeric hydrolyzed forms of Fe^{III}) in spectrum (a) is shown by the thin line. Shaded regions indicate the spectral components (quadrupole doublets) corresponding to iron(III) (lightly shaded region) and iron(II) (darker shaded regions) contributing to the overall spectrum (solid line), obtained by fitting the experimental data (dots); see also table.

The rate of iron reduction remains quite high at pH ~ 3 (see Fig. 2b and table): as soon as 1 min after

Mössbauer parameters[a] of (a) aqueous ethanol (20 vol % ethanol) $^{57}Fe^{III}$-containing solutions of 4-*n*-hexylresorcinol (molar ratio 1 : 3; $Fe_{total} = 0.016$ M) with the specified pH values, rapidly frozen to 80 K at specified time intervals (the measurements were performed at 80 K; see also Figs. 2 and 3)

Conditions of preparation (pH, holding time before freezing)	Fe oxidation state	δ	Δ	Γ_{exp},	S_r, %
		mm s^{-1}			
pH 1.5 (1 min)	+3[b]	–	–	–	91.9
	+2	1.38(2)	3.44(3)	0.44(3)	8.1
pH 3.0 (1 min)	+3	0.36(1)	0.77(2)	0.51(2)	85.6
	+2	1.31(2)	3.35(4)	0.27(5)	14.4
pH 3.7 (30 min)	+3	0.46(1)	0.72(2)	0.52(2)	100
2 min at pH = 3.7 +	+3	0.47(2)	0.72(3)	0.47(4)	87.7
70 min at pH = 2.3	+2	1.44(6)	3.4(1)	0.3(1)	12.3
pH 4.5 (60 min)	+3	0.46(1)	0.71(2)	0.52(3)	100

Notes: [a] δ is the isomer shift (with respect to α-Fe at room temperature); Δ is the quadrupole splitting; Γ_{exp} is the experimental full line width at the middle of intensity (the calculated errors in the last digit are given in parentheses); S_r is the surface area of the spectral component (in % of the total surface area of a spectrum) corresponding to the Fe content in this form with respect to the total Fe content in a sample (under the condition of equal probability of the Mössbauer effect for all forms); the relative error of S_r is ±4%.
[b] Broadened component with a hyperfine magnetic structure (the approximate S_r values were estimated from the spectrum).

mixing, ~14% Fe(II) is detected. Nevertheless, the existence of Fe^{II} is not detected at pH ~ 3.7 even 30 min after mixing (see Fig. 2c and table; similar data were obtained for the mixture held at pH ~4.5 for one hour; the spectrum is not shown) (see table).

To check the possibility of resuming the redox process upon a subsequent reduction in pH, a $^{57}Fe^{III}$–4-*n*-HR mixture held for 2 min at pH ~ 3.7 and room temperature was acidified (by slowly adding small amounts of HCl with agitation) to pH ~2.3 and then held for 50 min at room temperature. An Fe^{II} quadrupole doublet appeared in the Mössbauer spectrum of this rapidly frozen mixture (with a relative content of ~12%) along with the respective Fe^{III} doublet (Fig. 3).

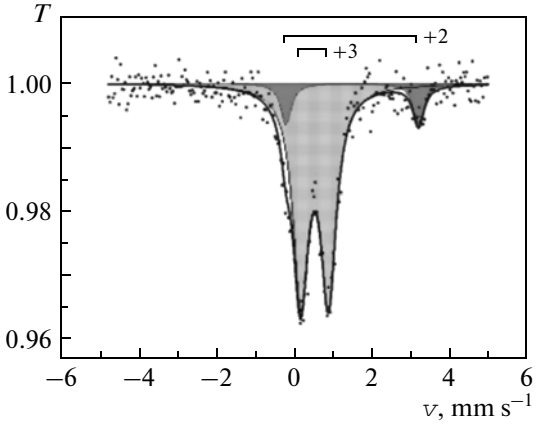

Fig. 3. Mössbauer spectrum of aqueous ethanol (20 vol % ethanol) $^{57}Fe^{III}$-containing solution of 4-*n*-hexylresorcinol (molar ratio, 1 : 3, respectively; $Fe_{total} = 0.016$ M) held at pH 3.7 for 2 min, in which pH was subsequently adjusted to 2.3, and then rapidly frozen after 70 min (the spectrum was measured at 80 K). Notations and shaded regions are described in the caption to Fig. 2 (see also table).

This indicates resumption of the oxidation of 4-*n*-HR, though at a considerably slower rate, by iron(III). The latter obviously exists in this system in the form of polynuclear hydroxide compounds and colloidal entities (the parameters of the Fe(III) quadrupole doublet in this case actually coincide with those of mixtures held at pH 3.7 and 4.5; see table) [9, 20]. This in particular could explain the slower redox process in this case (see Fig. 3), compared to the conditions at initial pH values of ~1.5 and ~3 (see Fig. 2a, 2b).

UV absorption for soluble reactive products of the mixture $^{57}Fe^{III}$ + 4-*n*-HR held at pH ~3 (with subsequent separation of solid products by centrifugation and dilution of the supernatant in order to reduce absorption in the UV range to values acceptable for measurements) was measured via spectrophotometry 5, 10, and 30 min after mixing the reagents. It can be seen from the spectra in Fig. 4a that their shape (with the maximum at ~279 nm characteristic of 4-*n*-HR) remains nearly unchanged, but a drop in the intensity of UV absorption over time is observed and is especially rapid in the first 10 min (Fig. 4b).

For the sake of comparison, note that upon the radiative oxidation of aerated solutions of 5-methylresorcinol (γ-radiation, ^{60}Co; dosage, up to 30 kGy), an increased absorption was observed in the region of the maxima corresponding to 5-methylresocinol (λ_{max} = 273 and 279 nm) and in the regions of the wings (around 260 and 285–290 nm) in [15]. A similar initial increase in UV absorption (by about 25%) was also determined for the $^{57}Fe^{III}$ + 5-methylresorcinol mixture in water at pH ~3 (characterized by a considerably slower rate of the redox process than in the system $^{57}Fe^{III}$ + 4-*n*-HR) with a subsequent drop in UV absorption [12]. The initial increase in absorption was attributed to the oxidation (additional hydroxylation [21]) of the aromatic nucleus, while the subsequent

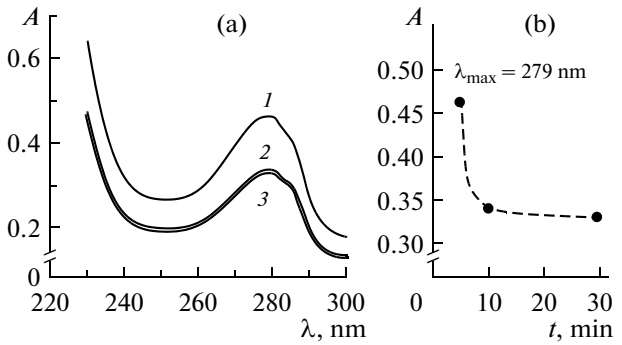

Fig. 4. Absorption spectra in the UV region (a) and optical density A at the maximum as a function of time t at wavelength $\lambda_{max} = 279$ nm (b) for an aqueous ethanol (20 vol % ethanol) Fe^{III}-containing solution of 4-*n*-hexylresorcinol (initial molar ratio, 1 : 3, respectively; pH = 3.0; initial content of $Fe^{III} = 0.016$ M) with 100x dilution after centrifuging the mixture (*1*) 5, (*2*) 10, and (*3*) 30 min after mixing.

drop in UV absorption could be related to the partial decomposition of chromophoric groups (an aromatic cycle) with subsequent oxidation and/or the formation of insoluble complexes of iron with the oxidation products removed while centrifuging the reactive mixture [12].

Based on these data, we may assume that in our $^{57}Fe^{III}$ + 4-*n*-HR system characterized by a high rate of the redox process at pH ~3 [12] (Fig. 2b and table), the measurements of UV spectra (after the separation of suspended solid particles by centrifugation) after 5 min and longer obviously include only the subsequent stages of the process, accompanied by a decrease in the intensity of UV absorption. (According to the data in [12], more than 30% of the Fe^{III} was reduced to Fe^{II} at pH ~3 after just 5 min in the presence of 4-*n*-HR.) After 10 min, the main redox process is obviously largely terminated and its rate falls sharply (the drop in the intensity of UV absorption accordingly slows dramatically; see Fig. 4b), corresponding in general to the MS data [12].

CONCLUSIONS

We studied the interaction between iron(III) and 4-*n*-HR in the pH range of 1.5–4.5 in aqueous media (frozen solutions) by means of MS. The concomitant oxidation of 4-*n*-HR by iron(III) was monitored via UV spectrophotometry. It was shown that the redox process occurs at a high rate at pH 1.5–3.0, but slows dramatically at pH > 3 and actually terminates at pH ~4 and higher (it nevertheless resumes, though at a comparatively moderate rate, after reacidifying the medium to pH < 3). In real systems (acid soils, aquifers [13, 14]), similar processes resulting in oxidative degradation of the most reactive alkylresorcinols with definite structure that participate in the autoregulation processes in microorganisms can substantially affect the exchange of microbial molecular signals by reducing their concentration in a medium, depending in particular on the acidity (pH) of the medium.

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