

Formation of complex compounds between iron ions and allomelanins

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Abstract

This paper presents and discusses the results of experimental studies on the complex formation of iron ions with melanins isolated from chaga growing on European beech (*Fagus sylvatica*) and from chaga growing on birch (*Betula*), which exhibit high antioxidant activity. The melanins were isolated by alkaline extraction followed by precipitation in an acidic medium. To identify the isolated pigments, their IR and EPR spectra were recorded. The complex formation of iron ions with these melanins was studied by gamma resonance (Mössbauer) spectroscopy (GRS). It was established that allomelanins are capable of efficiently binding iron ions both in the divalent and trivalent states. Importantly, allomelanins, similarly to melanins of animal and plant origin, can directly bind pro-oxidant Fe^{2+} ions and oxidize them to Fe^{3+} , which is inactive in terms of pro-oxidant activity, with subsequent complex formation. The activity of both processes increases with increasing pH of the reaction medium and upon illumination of the suspension with visible light.

Keywords: melanin; chaga; complex formation with iron ions; gamma resonance (Mössbauer) spectroscopy.

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1. Introduction

In recent years, the synthesis of biologically active ligand complexes of bimetals, the investigation of their structural properties, and the determination of correlations between chemical structure and biological activity have become the focus of

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growing interest among broad research communities. In our earlier works, the complex formation of pro-oxidant Fe^{2+} ions with synthetic L-DOPA melanin and with melanins of animal and plant origin was investigated [1–3]. Melanin pigments also play an important role in the microbial world, where their functions are mainly associated with protecting cells from various damaging environmental factors, primarily solar radiation. As in the case of melanins of animal and plant origin, the protective effect of allomelanin pigments is related both to passive shielding from solar radiation and to active suppression of photoinduced lipid peroxidation. As we have shown for melanins of animal and plant origin, their inhibition of lipid peroxidation is primarily associated with binding Fe^{2+} ions, which are catalysts of lipid peroxidation [5]. It can be assumed that this mechanism is also important for pigments of allo origin.

2. Materials and methods

In the present work, the results of experimental studies on the complex formation of iron ions with melanins isolated from chaga on European beech (*Fagus sylvatica*) and from chaga on birch (*Betula*), which exhibit high antioxidant activity, are presented and discussed. Melanins from beech chaga (FM1) and from birch chaga (FM2) were isolated according to the procedure described in [4,8] with a minor modification. Iron–melanin complexes were prepared by incubating freshly isolated melanin (18 mg dry weight) in 10 mL of a $^{57}\text{FeSO}_4$ solution ($c = 0.2 \text{ mg/mL}$) at room temperature according to the previously described method [4]. The incubation time was varied from 5 min to 2 h. The suspension was illuminated with an incandescent lamp (KGM-24) using a water filter. The illuminance at the surface was $7 \times 10^4 \text{ lx}$. To prevent oxidation of Fe^{2+} ions in a slightly acidic medium, hydroxylamine sulfate ($(\text{NH}_2\text{OH})_2 \cdot \text{H}_2\text{SO}_4$), a strong agent inhibiting the formation of iron oxides or hydroxides, was added to the initial solution. Preliminary experiments showed that hydroxylamine does not reduce melanin. The GRS spectra of the studied samples were recorded on a spectrometer operating in the constant-acceleration mode. A ^{57}Co source in a Cr matrix was used as the source of resonant gamma quanta. The spectrometer was calibrated using the GRS spectra of $\alpha\text{-Fe}$ at room temperature.

In order to identify the obtained pigments, their IR and EPR spectra were recorded. Analysis of the IR and EPR spectral parameters showed good agreement with analogous literature data [4,8,10]. This allows us to conclude that the pigments isolated by us are melanins.

3. Experimental results and their discussion

The GRS spectra of the studied samples at 80 K are shown in Fig. 1. The parameters of their GRS spectra are given in Table 1, and the distribution of iron ions among the

forms present in the studied systems, assuming equality of the recoilless fractions f/f' at 80 K, is presented in Table 2. The initial solution ($^{57}\text{FeSO}_4$) and the supernatants exhibit similar GRS spectra, representing a distinct doublet with parameters characteristic of aqua complexes of high-spin (HS) Fe^{2+} ions. The GRS spectra of the precipitates (complexes) are identical in shape. They are complex spectra consisting of at least four partial components: two doublets and two sextets with broadened lines of relaxation origin. The more intense broad doublet, whose contribution is 11–34% of the total spectral area depending on the type of melanin, is characteristic of HS Fe^{2+} complexes. The difference in their parameters, primarily ΔE_Q , from those of the initial solution indicates binding of Fe^{2+} ions by allomelanins. The central narrow doublet has parameters characteristic of paramagnetic HS Fe^{3+} complexes. The sextet components also have parameters characteristic of HS Fe^{3+} complexes.

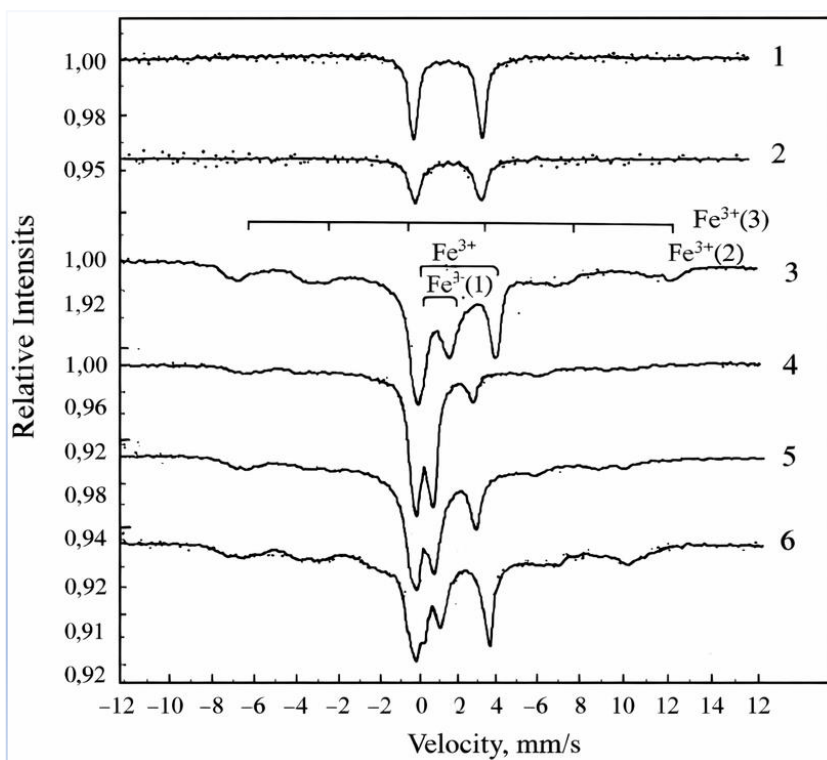


Fig. 1. Mossbauer spectra of the studied samples (pH 5.7) at 80 K. 1 – initial solution ($^{57}\text{FeSO}_4$), 2 – Supernatant after precipitation, 3 – complex $^{57}\text{FeSO}_4$ +L-DOPA melanin, 4 – $^{57}\text{FeSO}_4$ +MPQ, 5 – $^{57}\text{FeSO}_4$ +FM1, 6 – $^{57}\text{FeSO}_4$ +FM2.

As noted above, to further exclude possible formation of highly dispersed Fe^{3+} oxides or hydroxides, which could also yield a broadened sextet GRS spectrum, hydroxylamine was added to the initial solution. Washing of the precipitates (com-

plexes) and of Fe^{3+} oxides/hydroxides obtained under identical conditions with H_2SO_4 solution showed that Fe^{3+} oxides and hydroxides readily dissolve in H_2SO_4 at pH 2.5, whereas Fe^{3+} ions in the precipitate are not washed out even at pH 1.7. Finally, comparison of the GRS parameters of the precipitates with those of samples consisting of highly dispersed particles of Fe^{3+} oxides and hydroxides (superparamagnetism) showed that they differ sufficiently [11,12]. This suggests that the observed sextet partial spectra indeed belong to iron–allo melanin complexes. Thus, allomelanins, similarly to synthetic L-DOPA melanin and melanins of animal and plant origin, are capable of forming complexes with iron ions both in the divalent and trivalent states. Moreover, upon interaction with Fe^{2+} ions, allomelanins partially oxidize them to Fe^{3+} followed by complexation of both Fe^{2+} and Fe^{3+} . The parameters of all partial GRS spectra indicate an octahedral ligand environment of Fe^{2+} and Fe^{3+} ions in the complexes of $^{57}\text{FeSO}_4$ with allomelanins. In other words, in the studied complexes melanins act as weak-field ligands.

Table 1. Parameters of the GRS spectra of frozen solutions of the studied samples at 80 K (A: initial solution; B: supernatant after precipitation; C: complex (precipitate))

№	Sample	Fe^{2+}		$\text{Fe}^{3+}(1)$		$\text{Fe}^{3+}(2)$			$\text{Fe}^{3+}(3)$		
		δ , mm/s	ΔEQ , mm/s	δ , mm/s	ΔEQ , mm/s	δ , mm/s	ΔEQ , mm/s	B_{eff} , T	δ mm/c	ΔEQ , mm/s	B_{eff} , T
$^{57}\text{FeSO}_4 + \text{L} - \text{DOPA} - \text{melanin}$, pH 5.6											
1	A	1.27	3.38	-	-	-	-	-	-	-	-
2	B	1.29	3.39	-	-	-	-	-	-	-	-
3	C	1.34	3.14	0.54	0.82	0.68	0.19	50.0	0.53	0.38	54.8
$^{57}\text{FeSO}_4 + \text{MPQ}$, pH 5.8											
4	A	1.30	3.40	-	-	-	-	-	-	-	-
5	B	1.33	3.41	-	-	-	-	-	-	-	-
6	C	1.32	3.09	0.51	0.83	0.68	0.21	50.1	0.51	0.36	55.0
$^{57}\text{FeSO}_4 + \text{FM1}$, pH 5.7											
7	A	1.29	3.39	-	-	-	-	-	-	-	-
8	B	1.27	3.35	-	-	-	-	-	-	-	-
9	C	1.31	3.11	0.50	0.88	0.65	0.22	50.7	0.53	0.34	55.1
$^{57}\text{FeSO}_4 + \text{FM2}$, pH 5.7											
10	A	1.29	3.39	-	-	-	-	-	-	-	-
11	B	1.26	3.33	-	-	-	-	-	-	-	-
12	C	1.32	3.08	0.52	0.86	0.64	0.24	50.2	0.52	0.32	55.4

MPQ – melano-protein granules of the pigment epithelium of the bovine eye

Binding of iron ions by allomelanins, as in the case of synthetic melanins as well as melanins of animal and plant origin, occurs mainly within the first 5 min of incubation. With further incubation, the fraction of iron ions bound to allomelanins increases only slightly.

Illumination of the suspension with visible light led to a minor additional binding of iron ions. Comparative analysis of the GRS parameters of the samples obtained in the dark and under illumination showed that they are almost identical. Therefore, it can be assumed that illumination with visible light does not cause any substantial change in the mode of coordination of iron ions with melanins and does not distort the chelate site. It should also be noted that the parameters of the partial GRS spectra in the iron complexes with allomelanins FM1 and FM2 do not differ markedly. However, significant differences were found in their iron-binding capacities and in the ratios of different iron-complex forms in the samples (Table 2). Compared to FM1, FM2 binds iron ions to a lesser extent and oxidizes Fe^{2+} to Fe^{3+} to a lesser extent.

Table 2. Distribution of iron among the forms present in the studied systems according to GRS (A: initial solution $^{57}FeSO_4$; B: $^{57}FeSO_4$ + L-DOPA melanin, pH 5.6; C: $^{57}FeSO_4$ + MPQ, pH 5.8; D: $^{57}FeSO_4$ + FM1, pH 5.7; E: $^{57}FeSO_4$ + FM2, pH 5.7)

№	Sample	In the system			In solution			In precipitate				
		ΣFe	ΣFe^{2+}	ΣFe^{3+}	ΣFe	Fe^{2+}	Fe^{3+}	ΣFe	Fe^{2+}	ΣFe^{3+}	$Fe^{3+}(\Pi)$	$Fe^{3+}(M)$
In the dark												
1	A	1.00	0.99	0.01	-	-	-	-	-	-	-	-
2	B	1.00	0.52	0.48	0.52	0.51	0.01	0.48	0.16	0.32	0.10	0.22
3	C	1.00	0.55	0.45	0.50	0.49	0.01	0.50	0.06	0.44	0.26	0.18
4	D	1.00	0.57	0.43	0.40	0.39	0.01	0.60	0.18	0.42	0.13	0.29
5	E	1.00	0.58	0.42	0.41	0.40	0.01	0.59	0.18	0.41	0.08	0.33
Under illumination												
6	A	1.00	0.99	-	-	-	-	-	-	-	-	-
7	B	1.00	0.53	0.47	0.39	0.38	0.01	0.61	0.15	0.46	0.22	0.24
8	C	1.00	0.53	0.47	0.43	0.42	0.01	0.57	0.09	0.45	0.12	0.31
9	D	1.00	0.54	0.46	0.38	0.37	0.01	0.62	0.17	0.45	0.13	0.32
10	E	1.00	0.55	0.45	0.39	0.38	1.01	0.61	0.17	0.44	0.09	0.35

As is known, allomelanins, similarly to melanins of animal and plant origin, are complex polymeric materials composed of monomer units of different structure. They contain ortho-quinone and ortho-hydroquinone groups, amino and imino groups, as well as carboxyl and carbonyl groups, each of which can participate in binding iron ions by the polymer [8, 9, 10].

4. Conclusion

The simultaneous presence of magnetic and doublet partial GRS spectra in a sample is apparently related to an inhomogeneous distribution of iron-binding sites within the melanin polymer. For two or more closely spaced Fe^{3+} ions (for example, they may be part of polynuclear ($n \geq 2$) clusters), due to fast relaxation caused by efficient spin–spin interaction, doublet partial GRS spectra will be observed. For Fe^{3+} ions sufficiently separated in space, spin–spin interaction is strongly weakened, and relaxation GRS spectra with blurred hyperfine structure will be observed for such structures. The sextet with an effective magnetic field of ~ 55 T likely corresponds to Fe^{3+} ions bound to carboxyl groups. The sextet with a smaller field (~ 50 T) corresponds to structures in which, in addition to COO^- groups, amino or imino groups of the melanin polymer also participate in Fe^{3+} coordination. The aforementioned aspects can be schematically illustrated as follows:

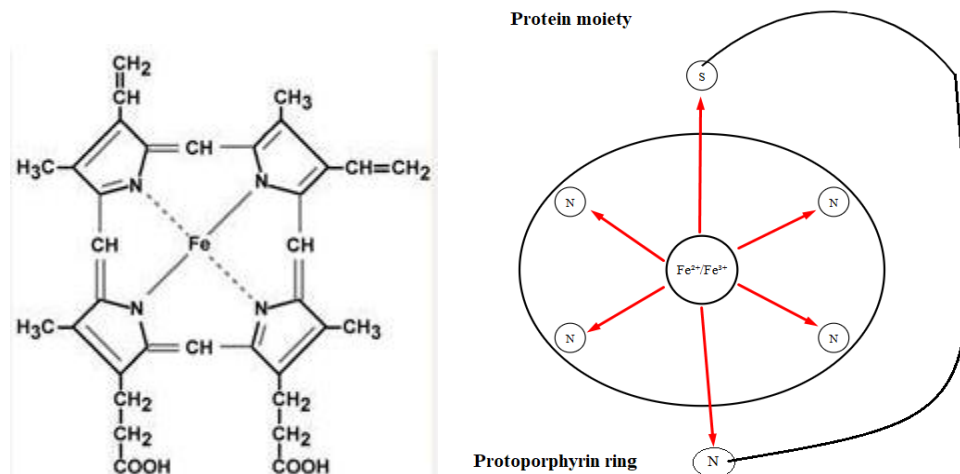


Fig. 2. Possible coordination of iron ions with the functional groups of allomelanins in complex formation.

Based on the obtained data, it can be assumed that all these melanins contain similar functional groups with respect to iron-ion binding. The observed differences in the GRS spectra of iron complexes with these ligands are apparently related to structural features of the melanins. In molecules of animal melanins, indole monomer units usually predominate, whereas in plant and allomelanins pyrocatechol-type units predominate [6, 7].

Thus, the results obtained indicate that allomelanins are capable of efficiently binding iron ions both in the divalent and trivalent states. Importantly, allomelanins, similarly to melanins of animal and plant origin, can directly bind pro-oxidant

Fe^{2+} ions and oxidize them to Fe^{3+} , which is inactive in terms of pro-oxidant activity, with subsequent complex formation. The activity of both processes increases with increasing pH of the reaction medium and upon illumination of the suspension with visible light.

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