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Types of Paramagnetic Centers in Cu^{2+} Complexes with Model Neuromelanins

B. Pilawa, E. Chodurek, and T. Wilczok

Department of Molecular Biology, Biochemistry and Biopharmacy, School of Pharmacy, Medical University of Silesia, Sosnowiec, Poland

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Abstract. Electron paramagnetic resonance (EPR) spectroscopy was used to examine free radical properties of model neuromelanins obtained by oxidative polymerization of noradrenaline, adrenaline and dopamine. We compared the lineshape of the experimental spectra, type and concentration of free radicals in the analyzed samples. The effect of different concentrations of Cu^{2+} on free radicals in melanins was studied. The total concentration of free radicals (about 10^{18} to 10^{19} spin/g) in the studied melanins increases as follows: adrenaline-melanin < dopamine-melanin < noradrenaline-melanin. EPR spectra of dopamine-melanin and adrenaline-melanin were a single EPR line (ΔB_{pp}, 0.50 and 0.55 mT, respectively). o-Semiquinone free radicals with the characteristic g-value of 2.0040 exist in these melanins. EPR spectra of noradrenaline-melanin were a superposition of two lines (ΔB_{pp}, 0.45 and 0.81 mT). o-Semiquinone free radicals were responsible for the narrower component. Nitrogen free radicals with a g-factor of 2.0030 were probably responsible for the broader component. Paramagnetic copper ions quenched the EPR signals of melanin free radicals in the studied samples. For melanin-Cu^{2+} complexes, broad EPR lines (ΔB_{pp}, 10–32 mT) of copper ions with a g-value of about 2.1 appeared. The influence of the microwave power on the EPR spectra of these complexes demonstrated the fast spin-lattice relaxation in the copper system in melanins.

1 Introduction

Stable paramagnetism is characteristic for melanin biopolymers [1–9]. The properties of melanin free radicals strongly depend on the type of melanin. Metal ions (copper, zinc and iron) modify the free radical system in pigmented tissues [3–6]. The aim of this work was to compare free radical properties of model neuromelanins synthesized from different precursors. Electron paramagnetic resonance (EPR) spectroscopy was used to study the influence of paramagnetic Cu^{2+} ions on free radicals in these melanin polymers. The EPR spectra of paramagnetic Cu^{2+} ions were analyzed. EPR spectroscopy was used as a specific method to examine the binding of metal ions to melanin. The results obtained for model neuromelanins will be useful in the EPR analysis of natural neuromelanins. The

roles of the different free radicals of melanin from different precursors and copper ions in brain pathology is not known well so far.

2 Experimental

2.1 Samples

2.1.1 Preparation of Self-Precipitated Catecholamine Melanins

Melanins were synthesized by autooxidative polymerization of dopamine (DA), adrenaline (ADR), noradrenaline (NADR) (5 mM, Sigma) in Tris-HCl buffer (0.05 M, pH 7.4) for 48 h at room temperature [10]. The obtained sediment was separated by centrifugation (1500 × *g*, 15 min), washed with deionized water and dried over phosphorous pentoxide.

2.1.2 Preparation of Catecholamine Melanin Complexes with Copper Ions

DA, ADR or NADR (5 mM) was dissolved in 500 cm^3 of Tris-HCl buffer (0.05 M, pH 7.4) and mixed with 500 cm^3 of buffered solution of copper sulfate (2.5, 5, 10 mM). Melanin sediments formed after incubation for 48 h at room temperature were centrifuged (2500 × *g*, 15 min), washed with deionized water and dried over phosphorous pentoxide.

2.2 EPR Measurements

Catecholamine melanins and their complexes with Cu^{2+} ions were studied by EPR spectroscopy. The measurements at room temperature were performed on an X-band (9.3 GHz) EPR spectrometer with 100 kHz magnetic modulation. The microwave frequency was recorded.

The EPR spectra of melanin free radicals and Cu^{2+} ions were collected with a computer. The spectra were taken with a high attenuation of the microwave power, 20 dB (about 0.7 mW), to avoid signal saturation. The lineshape of the EPR spectra was numerically analyzed. Deconvolution for the complex EPR spectra was done. The parameters of the EPR spectra, the linewidth ΔB_{pp} and *g*-factor, were evaluated. The *g*-factor was calculated from the resonance condition

$$g = h\nu/\beta B_r,$$

where h is the Planck constant, ν is the microwave frequency, β is the Bohr magneton, and B_r is the resonance magnetic field.

The concentration of paramagnetic centers in the samples was determined with ultramarine-containing stable free radicals as the reference. A ruby crystal, per-

manently placed in the spectrometer cavity, served as an internal reference. The concentration of paramagnetic centers in the sample was calculated as

$$N = n_u \frac{W_u A_u / P_u}{P/WAm},$$

where n_u is the number of paramagnetic centers in ultramarine ($1.2 \cdot 10^{19}$ spin), W and W_u are the receiver gains for the sample and ultramarine, respectively; A and A_u are the amplitudes of the ruby signal for the sample and ultramarine, respectively; P and P_u are the areas under the absorption curves for the sample and ultramarine, respectively; m is the mass of the sample. Double integration of the first-derivative EPR spectrum was performed to determine the area under the absorption curve.

The influence of microwave power on the EPR spectra of melanin samples was studied.

3 Results and Discussion

Concentrations of paramagnetic centers, N, g-factors and linewidths ΔB_{pp} of EPR lines of the studied melanins are presented in Table 1. The EPR data for copper ions in melanin-Cu^{2+} complexes are also shown.

The total concentration of free radicals increases as follows: ADR-melanin < DA-melanin < NADR-melanin.

A single symmetrical Lorentzian EPR line (Fig. 1) was observed for DA-melanin and ADR-melanin. o-Semiquinone free radicals with the characteristic g-values of 2.0039–2.0040 are responsible for these lines.

Table 1. Concentration of paramagnetic centers, N, g-factor and linewidths ΔB_{pp} of EPR lines of model neuromelanins synthesized from NADR, ADR and DA and their complexes with Cu^{2+}.

Sample	N (10^{-19} spin/g)	ΔB_{pp} ($\pm$0.02 mT)	g ($\pm$0.0002)
NADR-melanin I	4.1	0.45	2.0042
NADR-melanin II	2.3	0.81	2.0030
NADR-melanin-Cu^{2+} (2:1)	0.2	10.42	2.1004
NADR-melanin-Cu^{2+} (1:1)	0.6	10.85	2.1112
NADR-melanin-Cu^{2+} (1:2)	2.9	20.15	2.1045
ADR-melanin	0.2	0.55	2.0039
ADR-melanin-Cu^{2+} (2:1)	3.0	24.80	2.1358
ADR-melanin-Cu^{2+} (1:1)	1.6	24.54	2.1042
ADR-melanin-Cu^{2+} (1:2)	1.8	24.97	2.1450
DA-melanin	1.2	0.50	2.0040
DA-melanin-Cu^{2+} (2:1)	0.1	10.50	2.1065
DA-melanin-Cu^{2+} (1:1)	2.6	25.58	2.1279
DA-melanin-Cu^{2+} (1:2)	4.2	22.39	2.1326

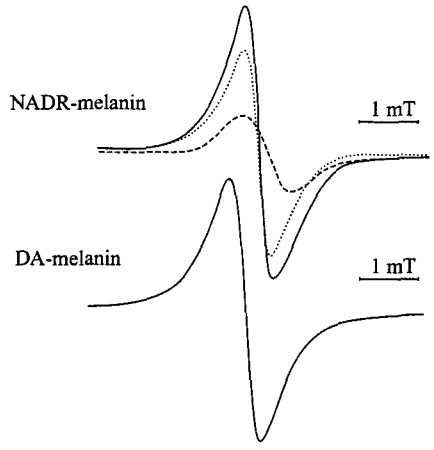

Fig. 1. Two-component EPR spectrum of NADR-melanin and single EPR line of DA-melanin. The solid line shows the experimental spectrum, the doted line and the dashed line show component lines I and II, respectively. The EPR spectra at room temperature were recorded at about 0.7 mW.

The shape of the EPR spectrum of NADR-melanin differs from that of other melanin EPR spectra (Fig. 1). The EPR spectrum of NADR-melanin was very asymmetric and its shape changed with changes of the microwave power. It was fitted by a superposition of two lines (Lorentzian line I, $\Delta B_{pp} = 0.45$ mT, $g = 2.0042$; and Gaussian line II, $\Delta B_{pp} = 0.81$ mT, $g = 2.0030$). The Gaussian line saturated at a relatively higher microwave power than the Lorentzian line. The g-value of the line I indicated that it results from o-semiquinone free radicals generally existing in melanin. The g-value of the line II differed from the value characteristic for o-semiquinone free radicals in melanin (higher than 2.0040). It is expected that the g-value of nitrogen free radicals is lower than the g-value of oxygen free radicals located at a similar structure due to the relatively lower spin-orbit coupling for the nitrogen atom [11]. We propose that nitrogen free radicals in the NADR-melanin are responsible for the line II. The concentration of free radicals with the unpaired electron localized on the nitrogen atom in NADR-melanin was lower than the concentration of o-semiquinone free radicals (Table 1).

Saturation of EPR lines of both types of free radicals at low values of microwave power indicates slow spin-lattice relaxation in model neuromelanins. A spin-lattice relaxation time of 10^{-6}–10^{-5} s was measured in melanins [12]. The influence of microwave power on the intensity and linewidth of EPR spectra of DA-melanin is presented in Fig. 2. The changes of the parameters of the two components of the EPR spectrum of NADR-melanin with changes of the microwave power were discussed earlier [9]. The faster spin-lattice relaxation is characteristic for the nitrogen free radicals in NADR-melanin.

Paramagnetic copper ions quenched the EPR signals of melanin free radicals in the studied model neuromelanins. A similar effect was observed for 3,4-dihydroxyphenylalanine-melanin-Cu^{2+} complexes [6, 12]. The observed quench-

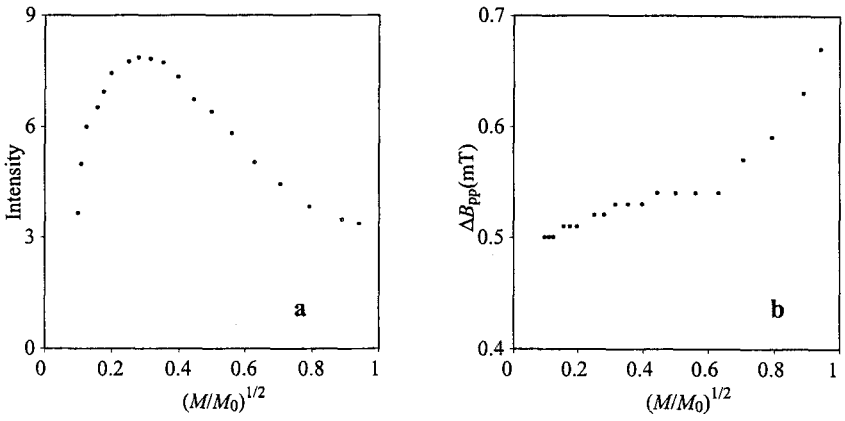

Fig. 2. **a** Influence of microwave power on intensities of DA-melanin EPR spectra recorded at room temperature. M_0 and M are the total microwave power produced by klystron (about 70 mW) and the microwave power used during the measurement, respectively. **b** Influence of microwave power on linewidths of DA-melanin EPR spectra recorded at room temperature.

ing of the melanin EPR signal by paramagnetic Cu^{2+} ions results from the dipole-dipole interaction between free radicals and metal ions with a short spin-lattice relaxation time. The effective metal-ion binding sites in melanins are phenolic, hydroxyl, carboxyl, and amine groups [6].

Dipolar-broadened EPR lines (ΔB_{pp}, 10–32 mT) with g-value of about 2.1 were measured for copper ions in melanins. Intensities of Cu^{2+} EPR lines increase for higher concentrations of copper in DA- and NADR-melanins. Such an effect was not observed for ADR-melanin. As was obtained for 3,4-dihydroxyphenylalanine-melanin earlier [12], probably the formation of bipolarons in ADR-

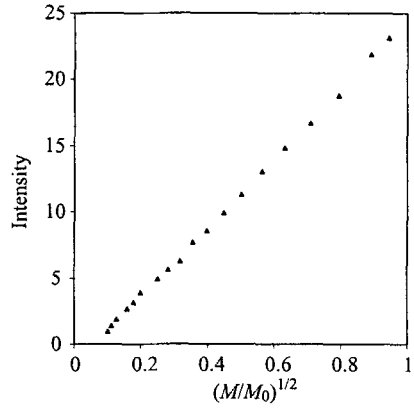

Fig. 3. Influence of microwave power on intensities of EPR spectra of DA-melanin-Cu^{2+} (1:2) complexes. The EPR spectra were recorded at room temperature. Notations as in Fig. 2.

melanin is responsible for the decrease of copper EPR signal with increasing of Cu^{2+} concentration in the studied ADR-melanin-Cu^{2+} complexes.

The influence of the microwave power on the intensity of EPR spectra of DA-melanin-Cu^{2+} (1:2) complexes is shown in Fig. 3. EPR lines of Cu^{2+} were not saturated in the used range of microwave power. Fast spin-lattice relaxation processes were characteristic for the copper system in melanins.

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Authors' address: Barbara Pilawa, Department of Medical Physics, School of Pharmacy, Medical University of Silesia, Narcyzów 1, 41-200 Sosnowiec, Poland
E-mail: bpilawa@slam.katowice.pl