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# ANALYSIS OF LINESHAPE OF BLACK *Drosophila melanogaster* EPR SPECTRA

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**Melanin extracted from *Drosophila melanogaster* was examined by an X-band electron paramagnetic resonance (EPR) spectrometer. Unsymmetrical EPR spectra are measured for this biopolymer. Lineshapes of EPR spectra recorded at 125 K and 275 K were analysed. Influence of microwave power in the range 8-200 mW on asymmetry of spectra was tested. The performed analysis confirmed existence of two types of paramagnetic centers in melanin from *Drosophila melanogaster.* Effect of microwave power on amplitude and linewidth of the melanin's EPR spectrum at 125, 200, and 275 K were compared. Fastening of spin-lattice relaxation processes in melanin for higher temperatures was observed.**

## INTRODUCTION

Melanins are paramagnetic biopolymers (Sarna, Hyde & Swartz, 1976; Sarna, 1981; Pasenkiewicz-Gierula & Sealy, 1986; Sarna, 1987; Pasenkiewicz-Gierula, 1990; Wakamatsu & Ito, 2002; Pilawa, Chodurek & Wilczok, 2003; Matuszczyk, Buszman, Pilawa, Witoszyńska & Wilczok, 2004; Pilawa, Buszman, Gondzik, Wilczyński, Zdybel, Witoszyńska & Wilczok, 2005; Zecca, Zucca, Albertini, Rizzio & Fariello, 2006). o-Semiquinone free radicals with spin S = 1/2 which fulfill the Curie law (I = C/T) in melanina are well known (Sarna, Hyde & Swartz, 1976; Sarna, 1981; Pasenkiewicz-Gierula & Sealy, 1986; Sarna, 1987; Pasenkiewicz-Gierula, 1990; Różanowska, Sarna, Land & Truscott, 1999; Pilawa, Latocha, Buszman & Wilczok, 2003; Buszman, Pilawa, Zdybel, Wrześniok, Grzegorczyk & Wilczok, 2005). Four groups of paramagnetic centers with S = 1/2 were found in DOPA-melanin at different pH by Pasenkiewicz-Gierula and Sealy (Pasenkiewicz-Gierula & Sealy, 1986; Pasenkiewicz-Gierula, 1990). The second type of paramagnetic centers with spin S = 1 which does not obey the Curie law was recently detected (Pilawa, Latocha, Krzyminiewski, Kruczyński, Buszman & Wilczok, 2003; Kozdrowska, 2005). Evidence of existence of the group of paramagnetic centers with S = 1 in melanins is still searched.

Knowledge of the properties of paramagnetic centers in melanin biopolymers and their role in living organism are important in medicine and pharmacotherapy. Melanin's paramagnetic centers play an important role in binding of drugs (Pilawa, Latocha, Buszman & Wilczok, 2003; Buszman, Pilawa, Zdybel, Wrześniok, Grzegorczyk & Wilczok, 2005; Kozdrowska, 2005) and metal ions (Sarna, Hyde & Swartz, 1976; Szpoganicz, Gidanian, Kong & Farmer, 2002; Buszman, Pilawa, Witoszyńska, Latocha & Wilczok, 2003; Pilawa, Chodurek & Wilczok, 2003; Matuszczyk, Buszman, Pilawa, Witoszyńska & Wilczok, 2004; Buszman, Pilawa, Zdybel, Wrześniok, Grzegorczyk & Wilczok, 2005; Kozdrowska, 2005; Fasano, Bergamasco & Lopiano, 2006). Free radicals may be responsible for toxic effects in tissues (Pilawa, Latocha, Buszman & Wilczok, 2003; Buszman, Pilawa, Zdybel, Wrześniok, Grzegorczyk & Wilczok, 2005; Kozdrowska, 2005).

Eumelanin mainly exist in organisms (Buszman, Latocha, Pilawa & Wilczok, 1995; Matuszczyk, Buszman, Pilawa, Witoszyńska & Wilczok, 2004; Liu, Hong, Wakamatsu, Ito, Adhyaru, Cheng, Bowers & Simon, 2005; Plonka, Michalczyk, Popik, Handjiski, Slominski & Paus, 2005; Lund & Timmins, 2007) and interactions of melanin with drugs or metal ions may be examined by the use of such model polymer. In this work we used melanin from black *Drosophila melanogaster* as the model sample. The aim of this work was to confirm our earlier hypothesis (Pilawa, Latocha, Krzyminiewski, Kruczyński, Buszman & Wilczok, 2003) about existence of two types of paramagnetic centers with S = 1/2 and S = 1 in melanin from *Drosophila melanogaster.* The new kind of paramagnetic centers with S = 1may be important in biochemical processes in cells and tissues.

## MATERIALS AND METHODS

*Samples*

Melanin was isolated from black strain of *Drosophila melanogaster*. Dry *Drosophila melanogaster* was degreased with ethyl ether, hydrolysed in 6 M HCl at 110° C for 24 h to remove the protein and washed with bidistilled water. The insoluble melanin was re-degreased with acetone and dried to a constant weight. The total efficiency of melanin isolation was 6.3%.

Dry powdered samples were put into thin-walled glass tubes and EPR measurements were done.

Additionally we tested by EPR the whole *Drosophila melanogaster* to check for the effects of melanin isolation procedure on the sample. The same EPR correlations for both melanin in *Drosophila melanogaster* and isolated melanin were observed. Melanin contained in the black strain of *Drosophila melanogaster* is mainly responsible for the EPR spectra. It indicates that strong changes in the paramagnetic structures of the melanin tested are not expected.

*Measurments*

EPR spectra were measured at the microwave frequency 9.3 GHz by BRUKER spectrometer. First derivatives of the EPR spectra were recorded at 125 K and 275 K with microwave power in the range 8-200 mW. The following shape parameters were analysed: $A_1/A_2$, $A_1$-$A_2$, $B_1/B_2$, and $B_1$-$B_2$. The parameters are defined and shown in Figure 1.

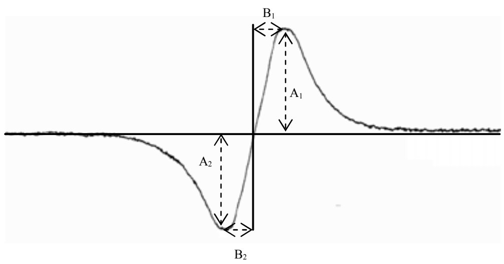


Fig. 1. EPR spectrum of melanin from black *Drosophila melanogaster* with marked the analysed parameters of its lineshape.

Influence of microwave power on the amplitude and linewidth of the melanin's EPR spectrum was tested. Microwave powers from 8 mW to 200 mW were used.

## RESULTS AND DISSCUSION

Strong EPR spectra were measured for natural melanin from black *Drosophila melanogaster* at all temperatures used (125, 200, and 275 K). Exemplary EPR spectrum of the melanin biopolymer studied is shown in Figure 1. First derivatives of the EPR curves clearly reveal unsymmetrical shape. The spectra are similar in shape to the simple EPR line of the model eumelanin – DOPA-melanin (Buszman, Pilawa, Witoszyńska, Latocha & Wilczok, 2003; Pilawa, Chodurek & Wilczok, 2003; Pilawa, Latocha, Buszman & Wilczok, 2003). It is expected, because in the black strain of *Drosophila melanogaster* mainly eumelanin exist.

This natural melanin may be used as reference in the studies of eumelanin. We tested paramagnetic centers system of the black biopolymer to obtain the information about paramagnetism of natural samples of this type. In our earlier work we proved the existence of o-semiquinone free radicals and biradicals in the synthetic model eumelanin – DOPA-melanin (Pilawa, Latocha, Krzyminiewski, Kruczyński, Buszman & Wilczok, 2003; Kozdrowska, 2005). During observations of temperature changes in the EPR spectra, the correlations typical of free radicals with spin S = 1/2 and biradicals with spin S = 1 were obtained for EPR lines of DOPA-melanin in the measuring temperatures from 100 K to 295 K.

Our earlier EPR studies of melanin from black *Drosophila melanogaster* (Pilawa, Latocha, Krzyminiewski, Kruczyński, Buszman & Wilczok, 2003; Kozdrowska, 2005) indicate that its lines does do not fulfil the Curie law describing the temperature dependence of EPR intensities of free radicals (S = 1/2). We suggest that this effect results from the complex character of the paramagnetic centers system in this melanin. Because eumelanin similarly to DOPA-melanin with two types of paramagnetic centers (S: 1/2, 1) is the main constituent of *Drosophila melanogaster* biopolymer, we suggest that the same two-component paramagnetic system exists in this natural sample.

In this work we search for the arguments for the complex character of paramagnetic centers in *Drosophila melanogaster* melanin by analysis of the lineshape of its EPR spectra. It is expected that the lineshape of the EPR signal from the two-component paramagnetic system will strongly depend on the microwave power. Continuous microwave saturation of the EPR spectrum of two groups of paramagnetic centers (S: 1/2, 1) effects their lines differently. Different changes in the component with microwave power modify the shape of the resultant spectrum. The influence of microwave power on spectral parameters: $A_1/A_2$, $A_1$-$A_2$, $B_1/B_2$, and $B_1$-$B_2$ defined in Figure 1 is presented in Figures 2-5, respectively.

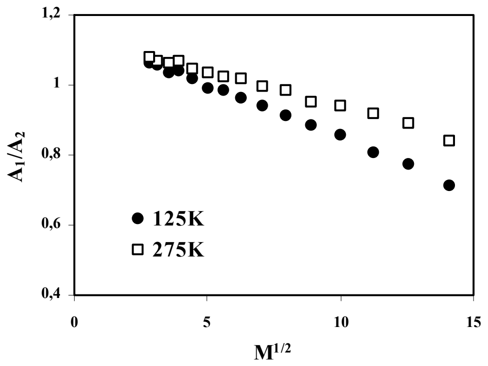


Fig. 2. Influence of microwave power M [mW] on the parameter $A_1/A_2$ of the melanin's EPR spectra measured at 125 and 275 K.

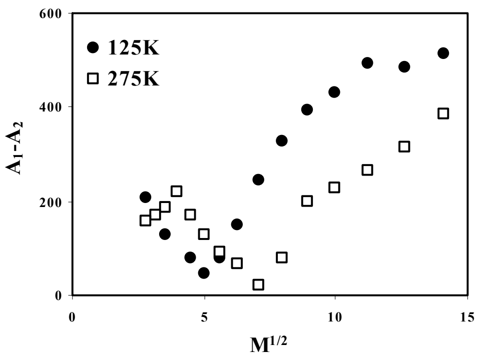


Fig. 3. Influence of microwave power M [mW] on the parameter $A_1$- $A_2$ [a. u.] of the melanin's EPR spectra measured at 125 and 275 K.

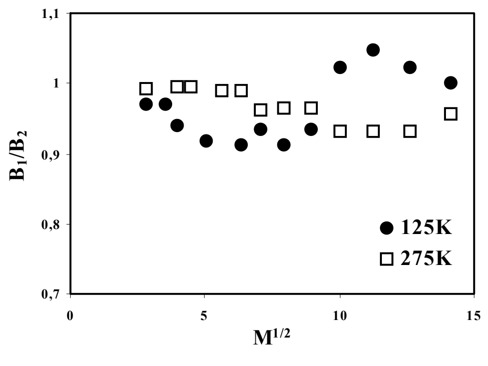


Fig. 4. Influence of microwave power M [mW] on the parameter $B_1/B_2$ of the melanin's EPR spectra measured at 125 and 275 K.

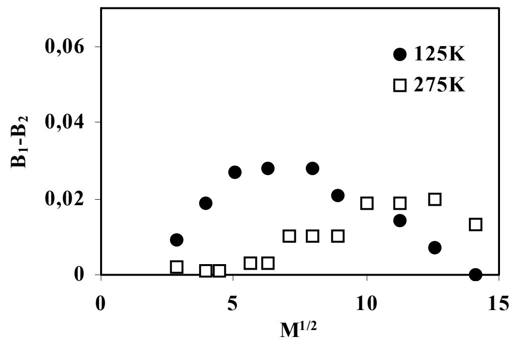


Fig. 5. Influence of microwave power M [mW] on the parameter $B_1$-$B_2$ [mT] of the melanin's EPR spectra measured at 125 and 275 K.

As one can see, all these parameters change strongly with the microwave power at 125 K and 275 K. The character of these changes is illustrated in Figures 2-5. Such changes cannot be obtained from only one group of paramagnetic centers in the sample. Our expectations about the complex paramagnetic centers system in melanin from black *Drosophila melanogaster* are confirmed.

To characterize the paramagnetic centers in more detail in the melanin used we applied the continuous microwave saturation of its EPR spectra at exemplary three temperatures (125, 200, and 275 K). Changes in the amplitude with increasing microwave power at these temperatures are shown in Figure 6. The influence of the microwave power on the linewidths of the EPR spectra is illustrated in Figure 7.

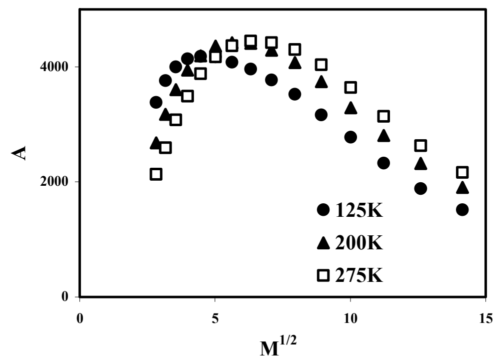


Fig. 6. Changes of amplitude A [a. u.] of EPR spectrum of *Drosophila melanogaster* melanin with microwave power M [mW].

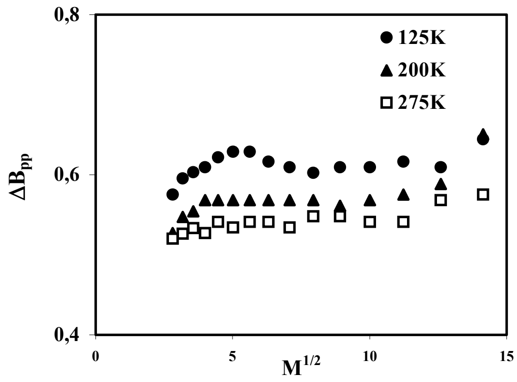


Fig. 7. Changes of linewidth $\Delta B_{pp}$ [mT] of EPR spectrum of *Drosophila melanogaster* melanin with microwave power M [mW].

The amplitude of the EPR spectra measured increases with increasing microwave power, and after reaching the maximum it decreases for higher microwave powers (Figure 6). The saturation of the EPR lines took place at low microwave powers, so it can be concluded that slow spin-lattice relaxation processes characterize the studied melanin. Microwave power of saturation of EPR line shift to higher values with increasing temperature from 125 K to 275 K (Figure 6). The above correlation indicates that spin-lattice relaxation processes at higher temperatures are faster. Broadening of the EPR lines with increasing microwave power was observed at 125, 200, and 275 K (Figure 7). Such a correlation points out that the isolated spin packet does not exist in the melanin sample analysed.

## CONCLUSIONS

Lineshape analysis of EPR spectra of melanin from black *Drosophila melanogaster* indicates that:

1) Lineshape of EPR spectra of this melanin change with temperature.
2) Lineshape of EPR spectra of the melanin at the given temperature depends on microwave power.
3) Complex paramagnetic centers system exist in the studied melanin. Mainly free radicals (S = 1/2) exist in this polymer, but triplet character (S = 1) some paramagnetic centers is suggested.
4) Slow spin-lattice relaxation processes characterize the melanin biopolymer.
5) Fastening of spin-lattice relaxation occur at higher temperatures.

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