Current Topics in Biophysics 2010, 33 (suppl A), 271-275

EPR STUDIES OF *Cladosporium cladosporioides* MYCELIUM WITH FLUCYTOSINE

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Received August 5, 2010; accepted October 1, 2010; published online November 20, 2010.

Free radicals in *Cladosporium cladosporioides* cultured with flucytosine were studied by an X-band EPR spectroscopy. Different drug concentrations were used. The results were compared to data for the model eumelanin - DOPA-melanin. It was shown that similar paramagnetic properties characterizes complexes of *Cladosporium cladosporioides* mycelium with flucytosine and DOPA-melanin-flucytosine complexes.

INTRODUCTION

Mainly o-semiquinone free radicals exist in melanin biopolymers (Pilawa *et al.*, 2009; Krzywda *et al.*, 2008; Buszman *et al.*, 2006; Matuszczyk *et al.*, 2004; Pasenkiewicz-Gierula, 1990; Okazaki *et al.*, 1985; Sealy *et al.*, 1982; Sarna, 1981; Sarna *et al.*, 1976). EPR studies (Pilawa *et al.*, 2009; Buszman *et al.*, 2006; Buszman, 1994) indicate that free radicals of melanin interact with drugs during their complexation with this polymer in living organisms. These interactions may be responsible for toxic effects in tissues. Interactions of free radicals of melanin with many drugs are not well known so far.

The aim of this work is to determine effect of flucytosine on free radicals in *Cladosporium cladosporioides* mycelium. Melanin existing in *Cladosporium cladosporioides* was the model eumelanin's polymer for our studies. EPR line of eumelanin dominates in *Cladosporium cladosporioides* spectra (Pilawa *et al.*, 2009; Buszman *et al.*, 2006; Matuszczyk *et al.*, 2004). Free radical properties of the natural samples with those of synthetic DOPA-melanin (eumelanin) were compared.

EXPERIMENTAL DETAILS

Free radicals in mycelium of *Cladosporium cladosporioides* cultured with the synthetic antifungal drug as flucytosine were studied by an X-band (9.3 GHz) electron paramagnetic resonance spectroscopy. *Cladosporium cladosporioides* culture is shown in Figure 1.

Fig. 1. Culture of *Cladosporium cladosporioides.*

The chemical structure of flucytosine - the derivative of pyrimidine is presented in Figure 2 (Zejca & Gorczyca, 2004). The following two concentrations of flucytosine: 25 μg/ml culture medium and 50 μg/mg culture medium, were used. DOPA-melanin and DOPA-melanin-flucytosine complexes were also tested.

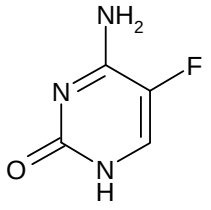

Fig. 2. Chemical structure of flucytosine.

Free radical concentration was determined as compared to the concentration in ultramarine as the reference. g-Factor, amplitude (A), integral intensities (I), and linewidth (ΔB_{pp}) were analysed. Influence of microwave power (M) in the range of 2.2-70 mW on the spectra was evaluated.

RESULTS AND DISSCUSION

Single EPR lines and complex EPR spectra (Fig. 3) were measured for the studied DOPA-melanin, and *Cladosporium cladosporioides* samples, respectively. EPR spectra of *Cladosporium cladosporioides* mycelium with flucytosine (50 μg flucytosine/ml culture medium) recorded at two different microwave powers are presented in Figure 3. EPR spectra of *Cladosporium cladosporioides* mycelium and the mycelium with flucytosine revealed complex character. They are superposition of the component line of eumelanin, the line of pheomelanin, and the line of the other part of organic matter of the mycelium.

Mainly EPR component of the eumelanin exist in the resonance absorption curve (Fig. 3). The resultant EPR spectrum of *Cladosporium cladosporioides* is superposition of EPR lines of eu- and pheomelanin. The same type of o-semiquinone free radicals are the source of EPR signals of eu- and pheomelanin, but different magnetic interactions are responsible for their different shape (Sarna, 1981; Sarna *et al.*, 1976). Unresolved hyperfine structure is visible in EPR spectrum of pheomelanin (Pilawa *et al.*, 2009; Buszman *et al.*, 2006; Matuszczyk *et al.*, 2004; Sarna, 1981; Sarna *et al.*, 1976).

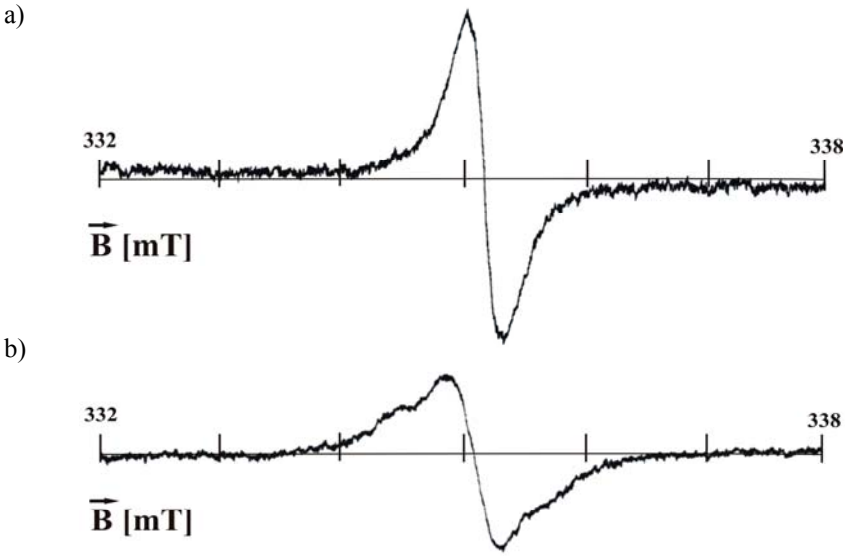

Fig. 3. EPR spectra of *Cladosporium cladosporioides* mycelium with flucytosine recorded at 2.2 mW (a) and 70 mW (b), respectively. B – induction of magnetic field.

The line of pheomelanin is visible in the spectra of *Cladosporium cladosporioides* mycelium and the mycelium with flucytosine measured with the relatively higher microwave powers (Fig. 3). Concentrations of free radicals and the parameters of the spectra of the examined samples are compared in Table 1. Influence of microwave power on amplitudes (A) and linewidth (ΔB_{pp}) of EPR lines of the studied samples is shown in Figures 4-7, respectively.

Table 1. Free radical concentration (N), g-factor, and linewidth (ΔB_{pp}) of EPR spectra of *Cladosporium cladosporioides* mycelium and the mycelium with flucytosine.

Sample	N x 10^{18} (spin/g)	g (±0.0002)	ΔB_{pp} (±0.02 mT)
Cladosporium cladosporioides mycelium	0.3	2.0034	0.39
Cladosporium cladosporioides mycelium with flucytosine (25 μg flucytosine/ml culture medium)	0.4	2.0035	0.37
Cladosporium cladosporioides mycelium with flucytosine (50 μg flucytosine/ml culture medium)	0.3	2.0037	0.41
DOPA-melanin	8.5	2.0037	0.45
DOPA-melanin-flucytosine complex (50 μg flucytosine/mg DOPA-melanin)	8.4	2.0038	0.46

g-Values (2.0034-2.0038) characteristic for o-semiquinone free radicals were obtained for *Cladosporium cladosporioides* mycelium and DOPA-melanin samples (Table 1). Broad EPR lines were measured for both mycelium (ΔB_{pp}: 0.37-0.41 mT) and DOPA-melanin (ΔB_{pp}: 0.46 mT) samples (Table 1). Dipolar interaction may be responsible for line broadening. Homogeneous broadening of EPR line was observed (Fig. 4-7). Slow spin-lattice relaxation processes exist in all the examined samples (Fig. 4, 6).

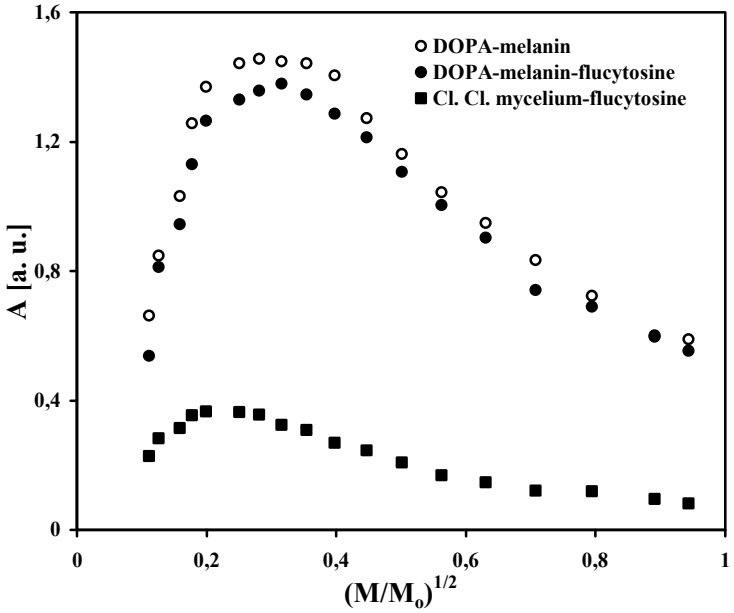

Fig. 4. Influence of microwave power (M/M_o) on amplitude (A) of EPR spectra of DOPA-melanin, *Cladosporium cladosporioides* mycelium, and their complexes with flucytosine. M – microwave power used during the measurement, M_o – total microwave power produced by klystron (70 mW). Samples with 50 μg flucytosine/ml culture medium and 50 μg flucytosine/mg DOPA-melanin were tested.

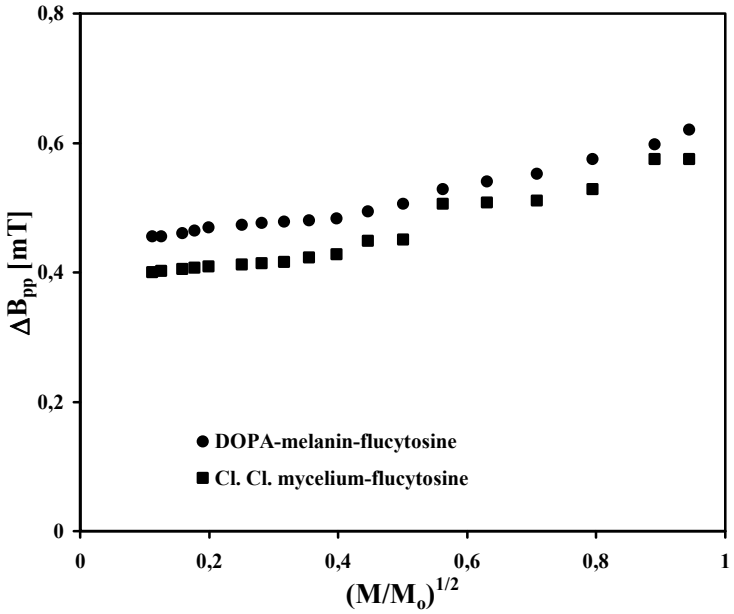

Fig. 5. Influence of microwave power (M/M_o) on linewidth (ΔB_{pp}) of EPR spectra of DOPA-melanin and *Cladosporium cladosporioides* mycelium with flucytosine. M – microwave power used during the measurement, M_o – total microwave power produced by klystron (70 mW). Samples with 50 μg flucytosine/ml culture medium and 50 μg flucytosine/mg DOPA-melanin were tested.

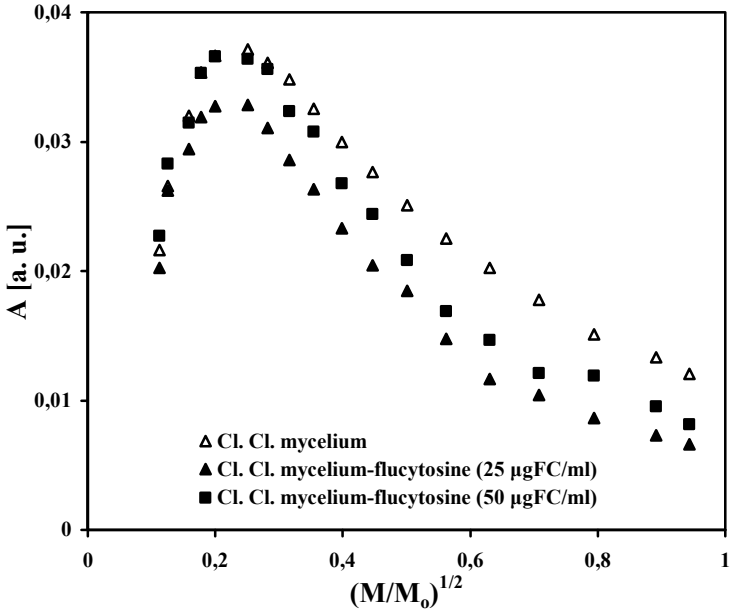

Fig. 6. Influence of microwave power on amplitude (A) of EPR spectra of *Cladosporium cladosporioides* mycelium and its complexes with flucytosine. M – microwave power used during the measurement, M_o – total microwave power produced by klystron (70 mW).

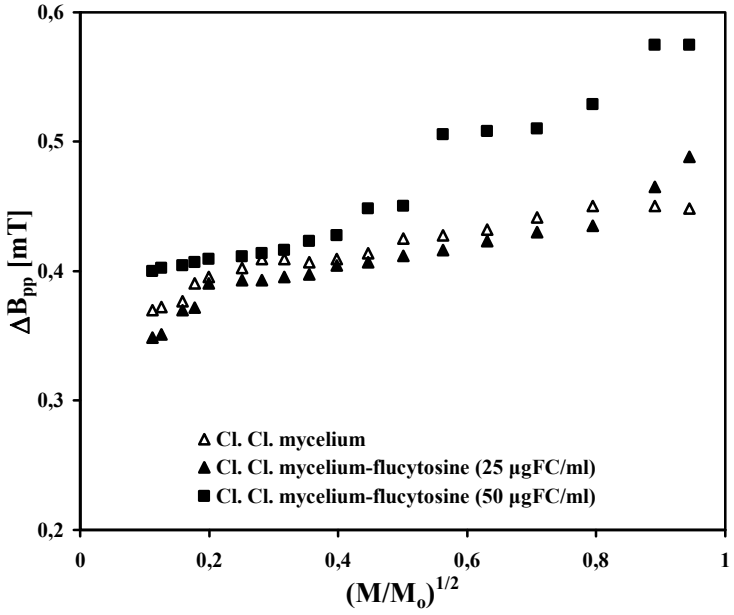

Fig. 7. Influence of microwave power on linewidth (ΔB_{pp}) of EPR spectra of *Cladosporium cladosporioides* mycelium and its complexes with flucytosine. M – microwave power used during the measurement, M_o – total microwave power produced by klystron (70 mW).

Addition of flucytosine to *Cladosporium cladosporioides* mycelium and to DOPA-melanin did not change microwave saturation of their EPR spectra (Fig. 4). EPR lines of *Cladosporium cladosporioides* samples saturated at relatively lower microwave power than EPR lines of synthetic eumalnin (Fig. 4). Effect of flucytosine concentration on changes of amplitude (A) and linewidth (ΔB_{pp}) of *Cladosporium cladosporioides* EPR spectra with increasing of microwave power is compared in Figures 6 and 7, respectively. Probably flucytosine does not change spin-lattice relaxation processes in mycelium and DOPA-melanin samples. The pulse spectroscopic studies in this matter are necessary.

Flucytosine does not change free radicals concentration in *Cladosporium cladosporioides* mycelium and in DOPA-melanin (Table 1). Such effect was not observed earlier for dry *Cladosporium cladosporioides* mycelium complexed with flucytosine (Pilawa *et al.*, 2009). It can be then concluded that different free radical reactions exist in the liquid fungal culture with flucytosine and in the sample of flucytosine added to dry mycelium. It is expected that in *Cladosporium cladosporioides* mycelium the interactions between free radicals of its melanin biopolymer and flucytosine appeared. Because of the main content of eumelanin in *Cladosporium cladosporioides* mycelium, the changes of free radical concentrations in DOPA-melanin after complexing with flucytosine were not observed similar to the natural sample (Table 1). The role of melanin's free radicals in binding of drugs to this polymer was described in the works (Pilawa *et al.*, 2009; Wrześniok, 2004; Buszman, 1994). The performed EPR studies pointed out that properties of the whole paramagnetic centers system remain unchanged after formation of melanin – flucytosine complexes in *Cladosporium cladosporioides* mycelium. These result differ from the data obtained for melanin complexes with the others drugs (Wrześniok, 2004; Buszman, 1994).

CONCLUSIONS

The performed EPR studies pointed out that interactions of flucytosine with *Cladosporium cladosporioides* mycelium containing melanin biopolymers differ from interactions of model eumelanin with the others drugs. Flucytosine does not changed free radical concentrations in the natural sample. The influence of fungal organic structures on these interactions may be responsible for this effect.

ACKNOWLEDGEMENTS

This study was supported by Medical University of Silesia in Katowice (KNW-1-096/10, KNW-1-126/10).

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