Comparative EPR studies of free radicals properties of DOPA-melanin-streptomycin complexes in air and in vacuum

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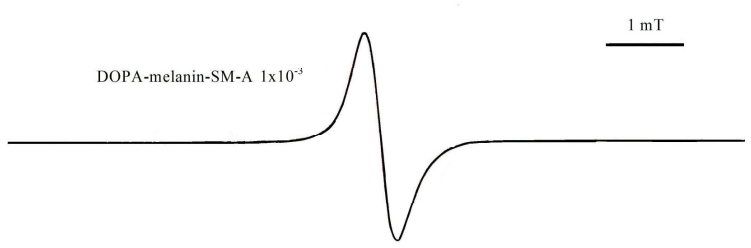
Melanin biopolymers play an important role during the drug binding by living organism. The precise nature of the binding of drugs to melanin has not been fully elucidated. It was obtained that o-semiquinone free radicals reactions occur during formation of melanin-drug complexes. The aim of this work was to study influence of streptomycin on free radicals in DOPA-melanin – the model eumelanin. The changes of free radicals concentrations in DOPA-melanin after binding of streptomycin was tested.

Oxygen level in the environment of melanin biopolymer in organism may change toxic effects resulted from free radicals formation in melanin-streptomycin complexes. Paramagnetic centers of biological structures interact with paramagnetic oxygen O_2 molecules at triplet states. These magnetic interactions of unpaired magnetic moments reveal physical character. Free radical reactions depend on oxygen effects in biopolymers and tissues. Susceptibility of different molecular units on oxygen is not well known. Influence of molecular oxygen on concentration and properties of free radicals in DOPA-melanin complexes with streptomycin was also studied.

Streptomycin, the oldest of the aminoglycosides, was isolated from *Streptomyces griseus* by Waksman and associates in 1944. Since then, has been used primarily against the aerobic gram-negative bacilli and staphylococci. Streptomycin bind to the 30S ribosome and inhibit bacterial protein synthesis. Although its antibacterial spectrum is very broad, serious limitation to the use of this antibiotic are side effects of ototoxicity and nephrotoxicity. Toxic effects may result from generation by the drug free radicals in melanin.

DOPA-melanin-streptomycin complexes with drug concentration $1x10^{-4}$, $5x10^{-4}$, $1x10^{-3}$, $5x10^{-3}$ and $1x10^{-2}$ [M] were examined by the use of electron paramagnetic resonance spectroscopy. Measurements were performed by EPR spectrometer at X-band (9.3 GHz). EPR spectroscopy was applied to examination of free radicals in melanin samples in contact with O_2 molecules and in vacuum (10^{-4} Torr). Microwave frequency was measured by MCM 102 recorder. The first derivative EPR lines were recorded. The following parameters of EPR spectra were determined: g-values, amplitudes (A), integral intensities (I) and linewidths (ΔB_{pp}). Effect of microwave power in the range 0.7-70 mW on EPR spectra was analysed. Continuous microwave saturation of EPR spectra was performed to study spin-lattice interactions. Changes of parameters of EPR lines with increasing of microwave power were analysed. Concentration of free radicals in the studied melanin samples was determined. Ultramarine was applied as the reference of free radicals concentration. Ruby crystal permanently placed in the resonance cavity was the second reference.
Single EPR lines were measured for DOPA-melanin and its complexes with streptomycin for samples in air and in vacuum. The exemplary spectra were compared in Figure 1.

a)



b)

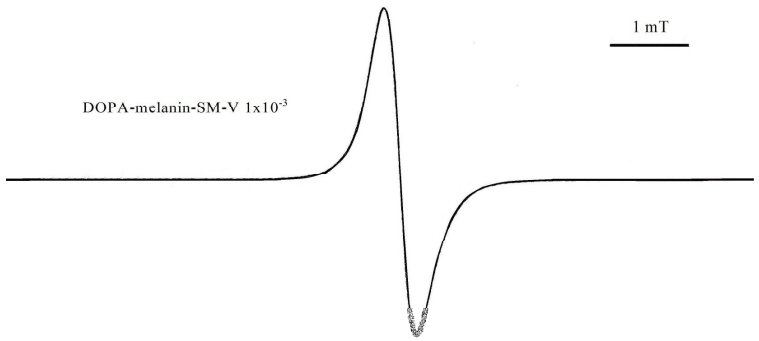


Fig. 1. EPR spectra DOPA-melanin-streptomycin complexes for samples in air (a) and in vacuum (b).

High concentrations of o-semiquinone free radicals (~10^{19} spin/g) were obtained for DOPA-melanin and its complexes with antibiotic. The existence of o-semiquinone free radicals with characteristic g 2.0035-2.0037 in the studied samples was confirmed. Strong dipolar interactions and slow spin-lattice relaxation processes characterize the studied polymer samples. Microwave saturation of EPR lines indicates homogeneous distribution of free radicals in molecular lattice of the samples. Free radical concentrations in DOPA-melanin-streptomycin complexes were lower than in DOPA-melanin.

Effect of oxygen on spin-spin and spin-lattice interactions in DOPA-melanin-streptomycin complexes was observed. Amplitude of the studied melanin samples in air and in vacuum increase with increasing of microwave power and after reaching the maximum it decreases. Formation of quasi-chemical bonds between melanin and oxygen molecules was proved. These complexes are responsible for decrease of free radical concentration in melanin polymer in air, because spin of unpaired electron of free radical of melanin is paired by spin of oxygen molecule. Quasi-chemical bonds are rupturing after oxygen removing from environment of melanin.

The obtained results indicate the interaction of the streptomycin with melanin, which may occur in the inner ear, and it may be one of the reasons of ototoxic effects of the analysed drugs. The results also show that toxic effects are stronger when oxygen level in tissue is low.