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SPECTROSCOPIC AND EPR STUDIES OF THE ACTIVE SITES AND ANTIOXIDANT PROPERTIES OF COPPER-CONTAINING PROTEIN - CERULOPLASMIN

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Ceruloplasmin (Cp), a blue copper oxidase, is the major copper-containing protein of the mammalia blood plasma.
This multifunctional protein is possessed of characteristic electron paramagnetic and optical spectra.
EPR, spectroscopic and luminescent properties of Cp in Na-acetic and phosphate buffers have been investigated in the presence of the inhibitor of Cp oxidase activity NaN and without it.#VA3
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Absorption, fluorescent, phosphorescent and EPR #VA<
spectra of Cp and model systems have been registered.
Phosphorescence lifetime of ceruloplasmin at 77 K has been measured.
The main instruments were: РЭ 1306 and Bruker BER 418 (electron paramagnetic resonance analysis), Specord UV VIS and КСВУ-23М (spectrophotometric analysis), Hitachi 850 and MPF-4 (luminescent analysis), helium-neon and nitrogen lasers, ultraviolet and electric filament lamps.
Complicated character of the absorption and luminescent spectra of Cp in the visible and near UV regions is conditioned by the presence of great number of aromatic amino acids residues in this protein.
Cp absorption bands lie in UV as well as in visible light regions of spectrum.
Photochemical reactions were initiated by the N laser emission (= 337 nm), simultaneously the#VA3
2#VA8
intensity of photoluminescence gradually decreased#VA<
and EPR spectrum changed.
Antioxidant activity of Cp was studied under illumination and in darkness.
Donor-acceptor electron transfer by Cp with the cooperative participation of all three types Cp copper centres was also studied under the both conditions of darkness and illumination.
I type centres state changes were estimated by the absorption and EPR spectra, II type centres - by the EPR spectrum, III type centres - by the luminescence spectrum.
Cp activity in electron transfer was estimated by measuring its absorption properties.
Elementary processes of electron transfer from amines (p-phenylene diamine - PPD and diphenyl amine - DPA), to Cp in phosphate buffer solution were investigated at room temperature.
When ascorbic acid was used as a reducing agent variations of the I type copper active site EPR spectra were observed with the corresponding decrease of absorption at 610 nm.
The process of electron transfer was accompanied by the decrease of the luminescence intensity of the III type copper centre in Cp (= 330 nm,#VA3

exc#VA8
= 415 nm).#VA3
lum#VA8
When Cp was reduced by PPD, EPR signal of PPD#VA<
radical cation appeared and it disappeared during
several minutes.
Heterogeneous systems were used in this experi-
ments: microporous glass plate with DPA adsorbed
was placed in Cp solution.
Process of single electron transfer from DPA to Cp
was studied by the absorption spectral analysis of
adsorbates (DPA and Cp) as well as by EPR spectrum
analysis.
Blood irradiation by He-Ne laser (= 632.8 nm) in
doses used was not found to produce activation of
peroxidation, moreover, it decreases the level of
peroxidation processes and promotes plasma lipids
stability.
The data obtained show that He-Ne laser irradia-#VA8
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tion of the blood in the doses of 0.1 - 0.3 J/cm#VA<
causes photobiological action on the enzyme system
of antioxidant protection increasing its activity.
This effect is confirmed by inhibition of lipid
peroxidation under these conditions.

Literature:

1. Яковлева Т.Ю., Васильев В.Б., Холмогоров В.Е. и др.
   Исследование активного центра церулоплазмина методом люминесцентной спектроскопии.
   Тезисы докладов на IV Всесоюзной конференции по биологии клетки. Тбилиси. 1985. Ч.II. Стр. 798-800.
2. Васильев В.Б., Нейфах С.А., Русаков Д.В., Яковлева Т.Ю. и др.
   Спектральные исследования механизма оксидазной активности церулоплазмина.
   Биохимия. 1988. Т. 53. Вып. 4. Стр. 620-625.
3. Яковлева Т.Ю., Холмогоров В.Е.
   Спектральные исследования окислительно-восстановительных реакций церулоплазмина.
   Тезисы докладов на VI Конференции по спектроскопии биополимеров. Харьков. 1988. Стр. 328-330.