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LOCAL STRUCTURE OF COPPER-ALBUMIN COMPLEXES : INVESTIGATIONS BY EPR AND EXAFS SPECTROSCOPY

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We have studied Cu(II)-BSA (bovine serum albumin) complexes by means of EPR and NMR spectroscopy in Refs [1-8]. The interest in them is due to their being a model system for most of active centres of copper-containing enzymes. Besides, it is important to learn the structure of these complexes to understand the biological role of copper in the BSA transportation function during the active life of organism.

The idea of applying the EXAFS method to the study of structures of these complexes has been made in Refs [1,2, 4-8]. In Ref. [4,5] the interaction between Cu(II) and BSA in a wide range of pH has been studied by the EPR method which revealed two types of bound centres. In one of them (type 1), having mainly an axial symmetry of surroundings, the central atom of Cu(II) is surrounded by two atoms of nitrogen His-9 and His-18 from the BSA molecule and two ones of oxygen from water molecule [3]. In the other centre (type 2), with rhombic symmetry of surroundings, Cu(II) is coordinated by 4 nitrogen atoms: one from amine group of asparagine acid, two - from peptide bonds and the fourth - His-3. Type 1 complexes were present at low pH, and type 2 ones - at $\text{pH} \geq 9$.

In Ref. [4] a relatively possible use of both methods has been supposed: when using the EXAFS method, serious difficulties arise in the identification of ligand atoms which surround the central atom of metal. This fault can fulfill the EPR method by means of which, via superhyperfine structure of its spectra, one can unambiguously identify the ligand atoms, though

the distance between the central atom of metal and the ligands remains indistinct. Besides, the absorption sensitivity of the EXAFS method is much poorer (some orders of magnitude) than that of the EPR method, which sometimes restricts its application.

One can say from the aforesaid, that these two methods as though supplement each other if the central atom of metal is a paramagnetic one. If it is a diamagnetic one, it should be replaced by a paramagnetic ion, though this restriction plays no role in the EXAFS method [4].

As copper is contained in the active centres of most enzymes, the developed in this work and in Ref.[4] approach can effectively be used in case of copper-containing enzymes too.

In Ref.[5] the behaviour of the radial distribution function of atoms (RDFA) for each bound centre in dependence with pH in solution is predicted based on the experimental spectra of EPR, registered at low temperature. Moreover, the necessity of development of methods for the separation of EXAFS spectra in systems in which two or more centres exist with the same type of absorbing atoms and a different ligand surrounding has been considered.

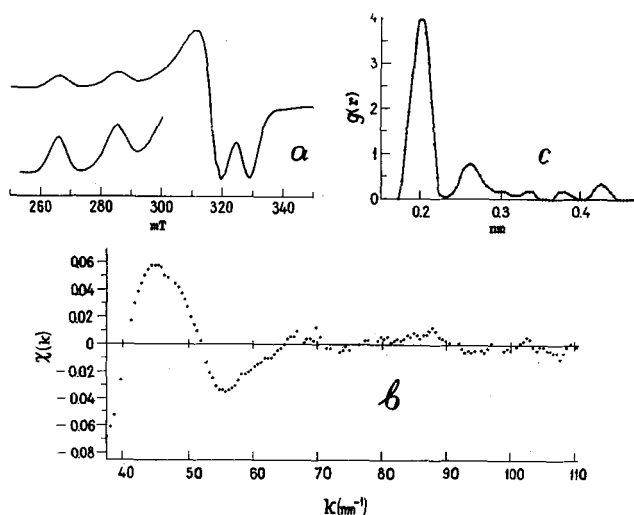
The aim of this work is to study the structure of Cu(II)-BSA complexes of type 2 by EPR and EXAFS spectroscopies.

In Fig.(a) the given EPR spectrum of Cu(II)-BSA complexes shows that only type 2 complexes with rhombic symmetry of surroundings are present. The superhyperfine structure of the EPR spectrum, found by us in Ref.[4], shows the presence of four ligand atoms of nitrogen. The EXAFS absorption spectrum of these complexes in freeze-dried form, registered by means of synchrotron radiation at room temperature in the Institute of Nuclear Physics of the Siberian Division of the USSR Academy of Sciences (Novosibirsk) displays a fine structure above the K - edge by which the short-range order of atoms can be determined.

The equation for the normalized oscillating part of the K - spectrum of absorption of the copper atom in the Cu(II)-BSA complex, in approximation that the absorbing atom is surrounded only by those of nitrogen, has the form [9] :

$$\chi_{Cu}(\kappa) = 4\pi\rho_0 C_N \frac{|f_N(\kappa, \pi)|}{K} \int_0^{\infty} g_{Cu-N}(z) \exp\left(-\frac{2zm}{\hbar^2 \kappa} \eta\right) \sin[2\kappa z + \varphi(\kappa)] dz,$$

where ρ_0 is the mean atomic density, C_N is the concentration of the nitrogen ligand atoms, $|f_N(\kappa, \pi)|$ is the amplitude of scattering on ligand atoms, K is the wave number, $g_{Cu-N}(z)$ is the RDFA partial function defined as the density of probability



- a) EPR spectrum of Cu(II)-BSA complexes at pH 10 and $-190^{\circ}C$; the concentration of Cu(II) is 1.28 mM and that of BSA is 0.32 mM. The g - tensor values for these complexes are: $g_x = 1.971$, $g_y = 2.053$, $g_z = 2.197$ and $A_{||} = 19.4$ mT. In the bottom left of the spectrum its repeated registration at 3.1-fold amplification is given.

- b) The normalized oscillating part of the absorption coefficient $\chi(k)$ obtained at preliminary processing of initial data.

- c) Radial distribution function of atoms $g_{Cu-N}(z)$ of Cu(II)-BSA complexes obtained from the function $\chi(k)$ using a regular algorithm [9].

of nitrogen atoms being located z distant from the Cu atom, a and b are the final limits of integration which are introduced from conditions $g_{Cu-N}(z) = 0$ at $z \leq a$ and $\exp(-\frac{2em}{A^2k}\eta) \ll 1$ at $z > b$, m is the electron mass, η is a constant corresponding to imaginary part of the complex potential of electron-electron interaction and determining the damping of photoelectron wave, $\varphi_{Cu-N}(k) = 2\delta_{Cu}(k) + \varphi_N(k,*)$ is the total phase shift of the scattering on Cu and N atoms.

The normalized oscillating part $\chi_{Cu}(k)$ obtained as a result of preliminary processing of initial data, the algorithm of which is described in Ref. [10], is presented in Fig.(b). For the K-edge of absorption of Cu a tabular value of $E_K = 8985.5$ eV has been used, the parameter $E_0 = 14.5$ eV according

to EXAFS spectrum of crystalline copper [10]. The even background of atom absorption μ_p has been approximated in the wave-number space κ by a polynomial of tenth order. The normalizing Victoreen $\mu_e = a\lambda^3 - b\lambda^4$ with coefficients $a = 176.0$ and $b = 48.3$ [11], has been brought to the value of absorption K-edge jump by a factor of 0.0015.

A regular algorithm has been used to solve the inverse problem of determination of the partial RDF $g_{Cu-N}(z)$. The amplitude $|f_N(\kappa, \theta)|$ and phases $2\delta_{Cu}^p(\kappa)$, $\psi_N(\kappa, \theta)$ are tabulated in Ref. [12]. Other parameters: $\rho_0 C_N = 80 \text{ atoms/nm}^3$ and $\eta = 1.6 \text{ eV}$. Initial data - the function $\chi_{Cu}(\kappa)$ has been given in the interval from 38 to 151 nm^{-1} , while the limits of the integration of the solution $g_{Cu-N}(z)$ are chosen to be from $a = 0.175 \text{ nm}$ to $b = 0.475 \text{ nm}$. The smoothness of the solution and its derivative as well as its non-negativeness $[g_{Cu-N}(z) \geq 0]$ have been taken into account. The result obtained in these conditions is presented in Fig.(c).

The partial RDF $g_{Cu-N}(z)$ is characterized by a narrow peak of distribution, corresponding to the first coordination sphere of ligand atoms around the metal atom. The position of the distribution maximum determines the most probable interatomic distance Cu - N, which is equal to $0.204 \pm 0.001 \text{ nm}$. This result well agrees with literature data for different biological objects in which this distance varies from 0.195 nm to 0.207 nm .

The observed peak represents itself the mean value of distances between the metal's central atoms and the ligands, since the EXAFS spectrum has been registered at room temperature. Record at room temperatures must show up a doublet splitting of this peak, as, according to EPR data, at -190°C the metal is located in the centre of the rhomb in the sites of which four nitrogen atoms are arranged.

It is impossible to give certain values to other details of the solution shown in Fig.(c) in the framework of the present paper. Hence, it should be noted that the studied complex is quite a multicomponent system and to determine the partial interatomic distances in them by the EXAFS method a new approach developed in Ref. [13] is necessary.

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