Short communication

Cu(II)-BSA Complexes and Their Identification by the ESR Method

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In 1948 Klotz and Curme (1948) began investigating interactions between Cu(II) and bovine serum albumin (BSA), aiming at clarifying the role of Cu(II) in the transport function mechanism of this protein. A review of further investigations of this problem may be found e. g. in Österberg (1974). Results mainly obtained using spectrophotometric methods, potentiometric titration, proton displacement reaction, equilibrium dialysis, etc., have been reduced to the fact that at Cu(II)-BSA complex formation two basic binding sites are formed. One of them, with optical absorption at 650 nm, is labile, the other one, with an absorption peak at 525 nm, is stable; both these sites behave differently depending on pH. Although there have been numerous papers on the topic, the ESR method has so far not been used to treat this problem.

The purpose of the present paper has been to investigate the structure of Cu(II)-BSA by the ESR method.

Analysis of the structure-pH relationship of Cu(II)-BSA has revealed the existence of two binding sites, each of them having a specific ESR spectrum pattern (see Fig. 1).

The upper ESR signal in Fig. 1 is best developed at low pH values, and it is labile. The optical absorption spectrum at 650 nm, reported in the literature, corresponds to this ESR signal (Österberg 1974). The value of the hyperfine structure splitting A_{11} at pH 6.55 is 16.1 ± 0.1 mT. With increasing pH it raises to approximately 17.0 mT. With increasing pH the $g_{\|}$ monotonously decreases from 2.304 to 2.270 with an accuracy of ± 0.001. At pH 6.55, $g_{\perp}$ is 2.071. At a pH value of about 9, the value of $g_{\perp}$ remains practically unchanged. At pH 8.9, five components of the superhyperfine structure of ESR spectra with a splitting value of 1.6 ± 0.1 mT are observed. Besides these data, the structure of the type I complexes may be represented as a flat square with two nitrogen and two oxygen atoms located in its nodes. These results were also confirmed by Bradshaw et al. (1968) who used the method of alkylation reaction with bromoacetate and the spectrophotometric method to show that one of the nitrogen atoms of these complexes is His-9, the other being His-18 of the imidazole group.

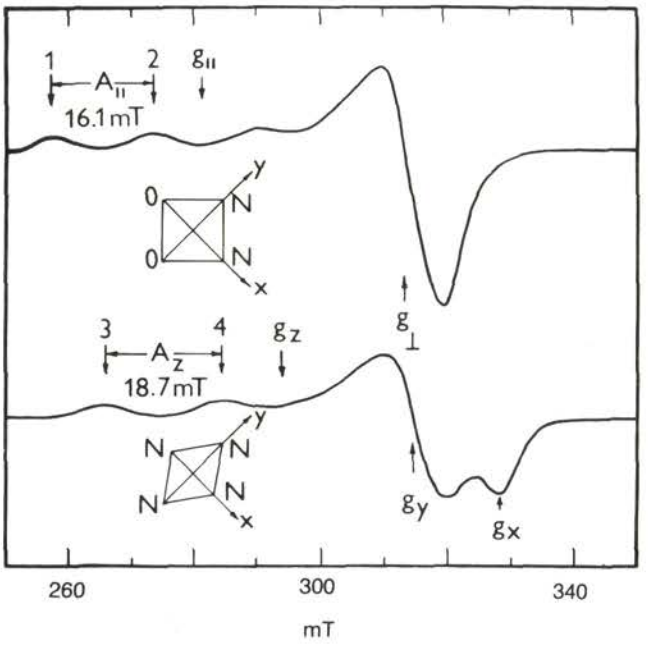

Fig. 1. Individual form of ESR spectra of Cu(II)-BSA complexes in 0.16 mol/l KCl at −190°C. *Top:* ESR spectrum for the type I binding site at pH 6.55; values of $g_{\parallel} = 2.304$ and $g_{\perp} = 2.071$. *Bottom:* ESR spectrum for the type II binding site at pH 9.30 with respective values of $g_x = 1.976$, $g_y = 2.063$ and $g_z = 2.205$. Symmetry of environment around Cu(II) and the corresponding ligands is shown below the traces.

A recentely performed detailed investigation of the type I complex structure by the NMR method has shown that two ligand oxygen atoms are attached to water molecules (Asaturian 1983).

The other ESR spectrum (Fig. 1, *bottom*) is typical of the stable site. It starts appearing in the summary ESR spectrum at pH ≥7.3. With pH increasing from 7.3 to 10.3, its relative proportion increases with a subsequent drop; it remains practically at a constant level thereafter. As compared to the type I ESR spectrum, as many as three different values of the g-tensor are observed in this spectrum: g_x, g_y and g_z. In the pH range of 8 to 12.7, values of g_z are smaller than those of $g_{\parallel}$ of the type I complexes. In both complexes the values of g_y are almost identical. The presence of the g_x component suggests a rhombic symmetry of the environment (Ingram 1969). The hyperfine splitting constant A_z for this type complexes is larger that that for the type I complexes in all the pH ranges studied. It changes as a function of pH: in the pH range from 7.3 to 9.0 it decreases from 19.8 ± 0.1 mT

to 18.8 mT; a further increase in pH results in an increase of this value to as high as 20.3 mT, then at pH > 10.3 peculiar periodic changes of A_z are observed up to pH 12.6. At pH 12.6, nine components of superhyperfine structure of ESR spectra are observed with the splitting value of 1.7 ± 0.1 mT. These 9 components might suggest an interaction of the unpaired electron, rotating on the orbit $d_{x^2-y^2}$ of the Cu(II) atom in the state $3d^9$, with four nitrogen atoms.

Thus, while type I complexes are labile and completely lacking at pH $\simeq$ 10, type II complexes, that stable up to pH $\geq$ 12, should have a rhombic symmetry of the environment with four nitrogen atoms being the ligand atoms in the rhombus plane.

A model of a stable binding site of the type II complexes studied by the spectrophotometric method has been proposed earlier by Peters and Blumenstock (1967). It was a flat square with four nitrogen atoms in its nodes. It included an α-NH_2-group, an imidazole group of His-3, and two nitrogen atoms of peptide bonds located between them. Though this model has been well supported by subsequent papers (Lau and Sarkar 1971; Shearer et al. 1967), as well as by the present paper, the axial asymmetry of the environment in the form of a flat square should be ruled out. Our ESR data have suggested the presence of a rhombic symmetry of the environment rather than an axial one.

We calculated values of the constants of spin-orbital interactions for each binding site at pH values, at which the super-hyperfine structure of ESR spectra appears. Thus, for type I complexes the value of λ_1 at pH 8.90 is -515 ± 3 cm^{-1}, and that of λ_2 at pH 12.67 is -391 ± 2.7 cm^{-1}. These data as well as values of the super-hyperfine structure of ESR spectra suggest a higher degree of covalent binding of Cu(II) and its ligand atoms for type II complexes as compared with type I complexes.

Identification of ligand atoms only is insufficient for a full interpretation of data on the binding site structure.

Recently, in connection with the wide use of synchrotron radiation, the EXAFS method (Doniach et al. 1980) has successfully been applied in biology. This method supplies information on distances between the central atom of the metal and the surrounding ligands for each type of binding sites. However, the identification of ligand atoms by this method is difficult. This shortcoming may successfully be overcome by the ESR method.

As it may be seen, the above two methods may be considered mutually complementary if the central metal atom is paramagnetic. This idea has been first proposed by Asaturian (1982). A detailed description of this problem and of the relation between ESR spectra and EXAFS may be found in subsequent papers by Asaturian (1984a, b) using Cu(II)-BSA complexes as an example.

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