индекс 3624

ЕРЕВАНСКИЙ ФИЗИЧЕСКИЙ ИНСТИТУТ

ЕФИ-717(32)-84

ЦЕНТРАЛЬНЫЙ НАУЧНО-ИССЛЕДОВАТЕЛЬСКИЙ ИНСТИТУТ
ИНФОРМАЦИИ И ТЕХНИКО-ЭКОНОМИЧЕСКИХ ИССЛЕДОВАНИЙ
ПО АТОМНОЙ НАУКЕ И ТЕХНИКЕ

R.A. ASATURIAN

IDENTIFICATION OF BINDING SITES IN Cu(II)-BSA COMPLEXES (Part II)

ЕРЕВАН-1984

Introduction

In our previous paper [1] the complex formation was investigated by means of the ESR method between Cu(II) and bovine serum albumin (BSA) versus pH. It was particularly shown that two types of complexes were then formed, distinguished by the stability of binding and relative concentration at various pH.

In the present paper experimental data are introduced allowing to identify binding sites of two types of complexes. Among such data are the values of g-tensor along the axes g_x, g_y and g_z, values of $g_{\parallel}$ and $g_{\perp}$ for axial-symmetrical tensor of type 1 complexes, the super-hyperfine structure of ESR spectra, the form of ESR spectra for each type of complexes etc.

Basing on the identification of binding sites in Cu(II)-BSA, an attempt is made to predict some structural data of EXAFS spectra, making use of the experimental results obtained by the ESR method. The materials and methods of the experiment were described earlier [1].

Results and Discussion

In Fig. 1 values of g-tensors for two types of complexes are presented. For the first type, values only for $g_{\parallel}$ and $g_{\perp}$ are available. For the second - three values of g-tensor, g_x,

g_y and g_z. Let us compare the values of $g_{\parallel}$ and g_z. It is seen that the values of g_z for type 2 complexes in the whole investigated pH range are much smaller than those of $g_{\parallel}$ for type 1 complexes. This apparently indicated that for type 2 complexes there is a more approximated to the central Cu(II) atom surrounding of protein atoms near the Z-axis[2], with a general tendency being observed for both $g_{\parallel}$ and g_z to decrease

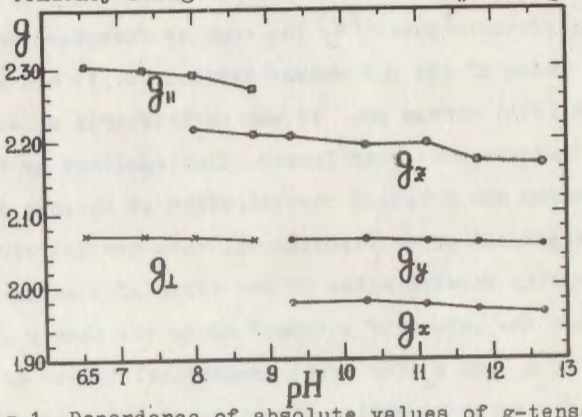

Fig.1 Dependence of absolute values of g-tensors for the binding sites of type 1 ($g_{\parallel}$ and $g_{\perp}$) and type 2 (g_x, g_y, g_z) on pH.

their absolute values. While for $g_{\parallel}$ these changes in the observed pH range take place monotonically, for g_z they proceed in two stages: the first in the pH range 8.0 to 11.1 and the second from 11.1 to 12.7.

The observed g-tensor values for the plane perpendicular to Z-axis have such a form that the values of $g_{\perp}$ for type 1 complexes and of g_y for type 2 complexes have almost the same values, which apparently should be an evidence of equal elec -

trostatical fields of these ligands[3]. With the increase in pH for both $g_{\perp}$ and g_y a tendency of their decrease is observed, though the rate of decrease is much smaller than in the case of $g_{\parallel}$ and g_z for type 1 and 2 complexes, respectively. The presence of $g_{\perp}$ serves for type 1 complexes as an evidence of the axial symmetry of environment, i.e. the Cu(II) atom should be in the center of the square, for which the distance from the central atom to its ligands both in X-and Y-axes should be the same [2]. Special experiments were performed to find out which atoms are ligand for the given type of complexes.

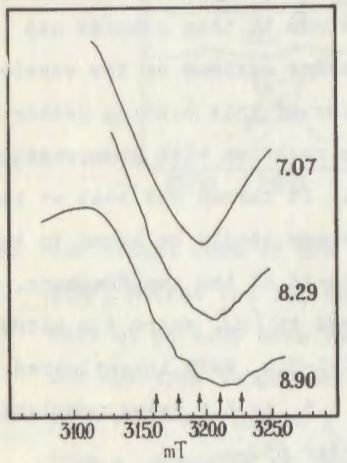

Fig. 2. Central part of ESR spectra of Cu(II)-BSA in 0.16 mol/l KCl at various pH values (figures on the left are for pH values; five arrows indicate the super-hyperfine splitting).

In Fig. 2 only the central part of ESR spectra of Cu(II)-BSA is presented at three pH values shown in the figure. As is seen, with the increase in pH there arise components respon - sible for the super-hyperfine structure, and while at pH 8.29 their number is rather difficult to establish, at the limiting pH value 8.90 it is easy to pick out five components, shown in the figure by arrows, with the splitting value being 1.6 mT.

Such a number of components should testify the presence of two ligand nitrogen atoms bound to Cu(II). This result is confirmed by the data of other papers. In [4] the Cu(II)-BSA complexes with the stoichiometry 2:1 were investigated. The ultraviolet absorption spectrum of a blue binding center was singled out on the wavelength 650mμ. In [5] the so-called Asp fragment was singled out, representing a peptide with a molecular weight 2808 and consisting of 24 amino acids of initial sequence of the BSA molecule. The minute study of the Cu(II) interaction with the Asp fragment carried out in [6] has shown that the ultraviolet absorption spectrum of this complex has at the same stoichiometry an absorbance maximum on the wavelenghth 650mμ. In [7] the identification of this binding center is performed by means of alkylation reaction with bromoacetate, spectrophotometric analysis etc. It turned out that at the above stoichiometry the Cu(II) atoms should be bound to the imidazole groups of His-9 and His-18 of the Asp fragment. This conclusion is once again confirmed in [8], where the ultraviolet absorption spectra of Cu(II)-L-His_2 were investigated. It turned out that in the pH range 5.5 to 8.0 these complexes too have an absorbance maximum near 650mμ.

Thus, as ligand atoms for the given binding site should serve nitrogen atoms of the BSA molecule from its two imidazole groups of His-9 and His-19, respectively. Both these nitrogen atoms should be located in the nodes of square-planar, perpendicular to the Z-axis, in whose two other nodes of two oxygen atoms should be located.

The individual form of the ESR spectrum of this type of

binding site at pH 6.55 with the values $g_{\parallel}$, $g_{\perp}$ and $A_{\parallel}$ at the same pH value is presented in Fig.3 (at the top). One may find there also the arrangement of X- and Y-axes in the plane of this square, in the center of which a Cu(II) atom should be located.

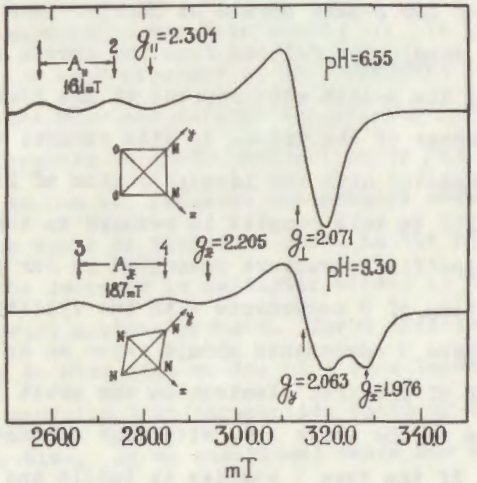

Fig.3 Individual form of ESR spectra of Cu(II)-BSA in 0.1 mol/l KCl: at the top the ESR spectrum is shown for type 1 site at pH 6.55 with values $g_{\parallel}$, $g_{\perp}$ and $A_{\parallel}$; below the ESR spectrum is presented for type 2 binding site at pH 9.30 with the values g_x, g_y, g_z, and A_z. Over each spectrum a symmetry of environment around a Cu(II) ion and appropriate atoms surrounding this ion are presented.

Let us turn to the identification of type 2 complexes. In Fig.1 values of its g-tensor are presented consisting of three components g_x, g_y, and g_z, as distinct from type 1 complexes, for which $g_x = g_y = g_{\perp}$. While the symmetry of environment for

type 1 complexes is axial, the available three values of the component of g-tensor should indicate, in our opinion, the presence of rhombic symmetry of environment for type 2 complexes, and the distance between Cu(II) and the ligand atoms surrounding it along the X-axis should be shorter than along the Y-axis [2]. This conclusion follows from the growth of the ligand field along the X-axis with respect to the Y-axis which results in the decrease of the values g_x with respect to g_y [3]. The problem connected with the identification of ligand atoms around Cu(II) in this complex is reduced to the interpretation of super-hyperfine structure described in our preceding paper [1] and consisting of 9 components with the splitting value being 1.7 mT. These 9 components should serve as an evidence of the interaction of unpaired electron on the orbit $d_{x^2-y^2}$ of the Cu(II) atom in the state $3d^9$, with four nitrogen atoms.

Thus, if the type 1 complex is labile and at pH $\simeq$10 completely absent (see Fig.4 of our preceding paper [1]) the type 2 complex, being stable up to pH $\geq$12, should have a rhombic symmetry of environment with four nitrogen atoms being the ligand atoms in the rhombus plane.

Back in 1948 Klotz and Curme [3] found that the first Cu(II) ions are bound to BSA more rigidly than the following ones. This fact was confirmed also in subsequent papers [4,10] . This was followed by a stage of the identification of this stable complex, which we have called type 2 complex. Investigating at various pH the displacement of protons in Cu(II)-BSA, Breslow [10] found that at pH 9.0 and stoichiometry 1:1 the number of displaced protons should be $\simeq$2. This was an indication

that along with α-NH_2-group among the ligand atoms bound to Cu(II) there should be two more notrogen atoms of peptide bonds. In order to find out which atom is the fourth ligand atom, the peptide (1-24) was isolated from the BSA Asp fragment and its amino acid composition was determined [5]. In this paper, however, the amino acid sequence of this fragment for the first two amino acids only was determined: Asp-Thr-. The third amino acid was determined in the subsequent paper [4]. In it by adding Cu(II) to the Asp fragment the authors obtained the decrease in the slope of titration curve in the pH range 6 to 7 as well as the decrease in catalytic effect at the hydrolysis of p-nitrophenyl acetate by $\simeq$40%. These effects suggested that Cu(II) ought to bind also to the histidine imidazole group occupying the position 3 in the peptide chain of this fragments, i.e. Asp-Thr-His-. As an additional basis for such a statement served the ultraviolet absorption spectra. The authors of the same paper [4] have obtained the ultraviolet absorption spectra for the Cu(II) complexes with both BSA and Asp fragment at the stoichiometry 1:1. In both cases at pH 8 an absorbance maximum was obtained at the wavelength 525 mμ .

Thus, basing on these data, Peters and Blumenstock [4] proposed a structural model of this stable binding site. It included the α-NH_2 -group, an imidazole group of His-3 and two nitrogen atoms of peptide bonds located between them. The investigation of the isolated tetrapeptide Asp-Thr-His-Lys- from Asp fragment [7] , corresponding to the first four amino acids of the Asp fragments showed [6] that the equimolar Cu(II) complex with this tetrapeptide had ultraviolet absorption spec-

tra with a maximum at 525mμ and similar spectra of circular dichroism which the Cu(II)-BSA complexes yield at the stoichiometry 1:1. Besides, the ultraviolet absorption spectra of equimolar Cu(II)-HSA complexes have also exhibited an absorbance maximum at the wavelength 525mμ [8,11]. If one takes into account that the initial amino acid sequence in HSA consists of Asp-Ala-His-Lys [12] and for BSA of Asp-Thr-His-Lys [13], then the binding site model proposed in [4] will receive another confirmation in [6-8,11].

However, this model requires additional specification. The thing is that while proposing this binding site model, Peters and Blumenstock [4] have considered the symmetry of environment around Cu(II) to be axial and squareplanar. Our ESR data indicate at the presence of rhombic symmetry of environment instead of axial. Allowing for the abovestated, we have presented in Fig. 3 the form of the ESR spectrum of type 2 complexes at pH 9.3, for which the values of g_x, g_y, g_z and A_z are indicated as well as the structure of the binding site by our ESR data in Cartesian coordinates.

In addition we have calculated the spin-orbit coupling constants at pH values when a super-hyperfine structure is observed for type 1 (pH 8.90) and type 2 (pH 12.67) complexes, in accord with equation [14]

$$g_{\parallel} = 2 - \frac{8\lambda}{\Delta} ,$$

where Δ is the ligand field splitting of the relevant d-orbitals equal to 650mμ and 525mμ for type 1 and 2 com-

lexes, respectively, and $g_{\parallel}$ is 2.270 and 2.166 for type 1 and 2 complexes, respectively. It turned out that

$\lambda_1^{pH=8.90} = -515\ cm^{-1}$, and $\lambda_2^{pH=12.67} = -391\ cm^{-1}$.

These data and the data on the super-hyperfine structure of ESR spectra for corresponding complexes (see Fig.3 in our preceding paper [1] and Fig.2 of this paper) indicate a higher degree of covalent binding between Cu(II) and its ligand atoms for type 2 complexes with respect to type 1 complexes [15]. The performed investigation of Cu(II)-BSA may be supplemented by the structural data of EXAFS method [16] using the synchrotron radiation of electron accelerator.

As is known, after the Fourier transform, the EXAFS spectra represent the dependence of radial distribution function $\varphi(r)$ (or the probability of finding ligand atoms around the central atom) at the distance between the central atom and its ligands [16-18]. In accord with this, the dependence $\varphi(r)$ within the limits of the first coordination sphere for type 1 complexes having an axial symmetry of environment around Cu(II) should be presented, in our opinion, as a peak with a maximum equal to the distance between the central Cu(II) atom and ligand atoms of nitrogen and oxygen equidistant from it (see Fig.3). For the second binding site having a rhombic symmetry of environment, this dependence $\varphi(r)$ also in the limits of the first coordination sphere should be presented, apparently, in the form of two maxima: the first should correspond to the distance between the central Cu(II) atom and two nitrog
the X-axis (see Fig.3). and the second maximum

between Cu(II) and two other nitrogen atoms located on the Y-axis. With the pH increase 6.5 to 8.8. the single maximum of radial function for type 1 complexes should, in our opinion, shift a little to the left, which is caused by the small decrease in $g_{\perp}$ due to the growth of ligand field and emergence of experimentally observed super-hyperfine structure with the increase in pH 7.0 to 8.9 (see Fig.2).

For type 2 complexes, with the pH increase 11.1 to 11.8 a shift of hypothetic two-humped curve to the left should also be observed. It should, however, be more substential than that of the single-humped one of type 1 complexes with the pH increase 7.0 to 8.9 . As a basis for such a statement serves the detection of super-hyperfine structure at pH 11.8 at usual conditions of the ESR spectra recording. In addition, for type 2 complexes the shift to the left of radial function maximum corresponding to 2 nitrogen atoms along the X-axis, should, in our opinion, with the pH increase 11.1 to 11.8 be larger than that for two nitrogen atoms on the Y-axis. This follows from the growth of ligand field for g-tensor along the axes g_x and g_y , respectively (see Fig.1).

For convenience the possible changes of EXAFS spectra are described without consideration of the scattering of energy of the electron knocked out from the Cu(II) atom K-shell on atoms located near the Z-axis of appropriate complexes. Allowing for this type of scattering, there should apparently be observed additional peaks.

Besides, the separation of the ESR signals from two and more described types of complexes doesn't cause any essential difficulties. In the case of EXAFS spectra such a superposi-

tion of signals will apparently lead to additional difficulteis in interpretation. The fact that at pH 6.5, in the main, type 1 complexes are observed, and at pH $\geq$9.3 - type 2 complexes, raises to some extent the possibility of their separation.

One may expect some separation of EXAFS signals from various types of complexes also due to a different degree of covalent binding of Cu(II) with its ligands, especially at such pH values, when a super-hyperfine structure of ESR spectra is observed. Thus, after the Fourier transform the EXAFS signal from type 2 complexes at pH $\geq$12 may be slightly shifted to the left with respect to that of type 1 complexes at pH 8.9.

If we take into account the fact that there are several active sites in copper-containing enzymes, which may be investigated by both ESR and EXAFS methods, then the development of methods of the separation of two or more EXAFS spectra will permit to investigate in detail the behavior of all these sites versus pH, of denaturating agents, various anions etc.

Thus, the investigations carried out by the ESR method make it possible, in our opinion, to predict some data of EXAFS spectra, which suppliment the results obtained by the ESR method, structural parameters required for the understanding of the roll of the Cu(II) ion in the mechanism of BSA transporting function.

I wish to express my deepest gratitude to A.Ts.Amatuni and Ts.M.Avakian for their interest in the work.

Thanks are also due to M.A.Simonian, Miss T.A.Nalbandian and A.A.Muradian for providing us with an ESR spectrometer Varian E-4 as well as for their help.

References

1. Asaturian R.A. ESR Spectra of Cu(II)-BSA Complexes Versus pH (part I).- Preprint Yerevan Phys.Inst, 716(3I)-84.

2. Ingram D.J.E.(1969): Biological and Biochemical Applications of Electron Spin Resonance. Adam Hilger LTD, London.

3. Ballhausen C.J. (1962): Introduction to Ligant Theory. McGraw-Hill, New York.

4. Peters T.Jr., Blumenstock F.A. Copper-Binding Properties of Bovine Serum Albumin and its Amino-Terminal Peptide Fragment.-J. Biol.Chem. 1967, vol.242, p.1574-1578.

5. Peters T.Jr., Hawn C.Isolation of two Large Peptide Fragments from the Amino-and Carboxyl-Terminal Positions of Bovine Serum Albumin.- J.Biol.Chem. 1967, vol.242, p.1566-1573.

6. Shearer W.T., Bradshaw R.A., Gurd F.R.N. The Amino Acid Sequence and Copper (II)-Binding Properties of Peptide (1-24) of Bovine Serum Albumin.-J.Biol.Chem. 1967, vol.242,p.5451-5459.

7. Bradshaw R.A., Shearer W.T., Gurd F.R.N. Sites of Binding of Copper (II) Ion by Peptide (1-24) of Bovine Serum Albumin.-J. Biol.Chem. 1968, vol.243,p.3817-3825.

8. Lau S., Sarkar B. Ternary Coordination Complex Between Human Serum Albumin, Copper (II), and L-Histidine. - J.Biol.Chem. 1971, vol.246, p.5938-5943.

9. Klotz I.M., Curme H.G. The Thermodynamics of Metalloprotein Combinations, Copper with Bovine Serum Albumin.- J.Amer. Chem. Soc. 1948, vol.70, p.939.

10. Breslow E. Comparison of Cupric Ion-Binding Sites in Myoglobin Derivatives and Serum Albumin.- J.Biol.Chem.1964,vol.239, p.3252-3259.

11. Appleton D.W., Sarkar B. The Absence of Specific Copper (II)-Binding Site in Dog Albumin.- J.Biol.Chem.1971,vol.246, p.5040-5046.

12. Brown J.R. (1977) Serum Albumin: Amino Acid Sequence. In: Albumin: Structure, Function and Uses (Ed V.M. Rosenoer, M. Oratz, and M.A. Rothschild), p.27-51, Pergamon Press,Oxford.

13. Brown J.R.(1977): Structure and Evolution of Serum Albumin In: Albumin: Structure, Biosynthesis, Function, vol.50, Colloquium B9(Ed.T.Peters and I.Sjöholm), FEBS 11th Meeting Copenhagen, p.11-20, Pergamon Press, Oxford.

14. Malkin R., Malmstrom B.G. The State and Function of Copper in Biological Systems.-Adv.Anzymol. 1970,vol.33,p.177-244.

15. Orton J.W.(1968): Electron Paramagnetic Resonance. Iliffe Books, London.

16. Doniach S., Eisenberger O., Hodgson K.O. (1980):X-ray Absorption Spectroscopy of Biological Molecules. In: Synchrotron Radiation Research (Ed.H.Winick and S.Doniach), p.425-458, Plenum Press, New York and London.

17. Ashley C.A., Doniach S. Theory of Extended X-ray Absorption Edge Fine Structure (EXAFS) in Crystalline Solids.-Phys. Rev.1975, vol.B11, p.1279-1288.

18. Lee P.A.Pendry J.B. Theory of the Extended X-ray Absorption Fine Structure.- Phys.Rev.1975, vol.11, p.2795-2811.

The manuscript was received 7 March 1984