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Structural studies on metal–serum albumin. IV. The interaction of Zn(II), Cd(II) and Hg(II) with HSA and BSA

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Abstract

There have been no detailed and reliable studies on the environment and configuration of Zn(II), Cd(II) and Hg(II) in the metal centers of human serum albumin and bovine serum albumin to date. In this paper the authentic evidence for the involvement of the cystinyl sulfur atoms in the ligation to the zinc group ions has been obtained from the X-ray photoelectron spectra. The belief that each of the zinc group ions possesses several similar binding sites in human- and bovine serum albumin and is bound to the deprotonated thiol group ($-RS^-$) of the cysteinyl residues to form tetrahedral and linear metal centers has been further confirmed by the treatment of ligand to metal charge transfer data with Jorgensen's method. According to these results, we have inferred that these binding sites may be located at the seventeen disulfide bridges, most likely at the seven pairs of adjacent disulfide bridges between positions 75 and 567, in the serum albumins.

Keywords: Serum albumin; Zinc group metal; XPS spectrum; UV spectrum; Optical electronegativity

1. Introduction

Both the biological functions and toxicities of Zn(II) have drawn People's attention. However, while Cd(II) and Hg(II) are common toxic elements, they have not been found to have biological functions up to now. In human serum, zinc bound to human serum albumin (HSA) accounts for 98% of the total exchangeable zinc [1], and albumin-bound zinc is believed to play a role in zinc transport [2]. Studies on the structure of the zinc group metal centers in serum albumin are the foundation of understanding their biological functions and toxicities.

Research in this area, however, is far from sufficient and not very reliable. In addition, studies on the interaction between Zn(II) and serum albumins have been hindered by the preconceived ideas originating from the earlier research on zinc-containing enzymes. X-ray structure data of some zinc-containing enzymes show that Zn(II) is always bound to two or three imidazole groups of histidyl residues [3]. This has led to the inference [4–6] that the imidazole group is a unique or primary ligand even when there is no unequivocal evidence to show that imidazole actually coordinates zinc or for the proposal [7–11] that imidazole is the only group involved in the ligation in

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the treatment of experimental data. In some studies, the imidazole group indeed coordinates the metal [12–14] when the *N*-terminal tripeptides or polypeptides containing histidine are used as model compounds of the serum albumin. However, some results cast doubts on whether or not Zn(II) and Cd(II) bind to the *N*-terminal end of bovine serum albumin (BSA) [4,6]. Rao et al. [15] believed that the coordinate group should be more active than the imidazole group and that imidazole and carboxyl groups were probably involved at the same time. Osterberg [16] deemed that the imidazole group did not participate in the ligation at all and that the highest affinity sites for Zn(II) and Cd(II) only involved the single thiol group. For Cd(II), peptide nitrogens are quite unlikely involved in the ligation [17]. It is universally acknowledged that Hg(II) binding sites are related to thiol groups [10,18]. Nevertheless, studies on Cd(II) and Hg(II) are much more insufficient [19].

This paper shows that complexes formed between Zn(II), Cd(II) and Hg(II) and HSA or BSA possess very similar XPS and UV spectra at physiological pH. Until the molar ratio of the metal to serum albumin reaches 6:1, the XPS (1*s* peaks) spectrum of elements C, N and O remains unchanged on the whole; and only the 2*p* peaks of the element S display distinct changes. These results clearly indicate that thiol groups of the cystinyl residues are involved in coordinating zinc group ions. There are no notable changes in the UV spectra of many systems, too, until the molar ratio is larger than 6:1. They all show a ligand to metal charge transfer (LMCT) band at 290 nm; sometimes another LMCT absorption at about 250 nm can also be seen. Electronegativity studies once again confirm that the ligation involves the RS^- group to form linear and tetrahedral metal centers. All of the above-mentioned evidence demonstrate that the interaction between zinc group ions and HSA or BSA is similar and that they all possess several similar primary binding sites. These binding sites may be located at the seventeen disulfide bridges, most likely at the seven pairs of adjacent disulfide bridges between positions 75 and 567, in the serum albumins. The matter of whether or not the imidazole group of histidine is involved in the ligation to metals is discussed in this paper. It is suggested that the possibility of the involvement of the imidazole group is minimal. However, it cannot be ruled out completely.

2. Experimental

HSA and BSA of not less than 95% purity, which were purchased respectively from the Beijing Red Cross Blood Center and the Tianjin Institute of Blood, were used without further purification. The major impurity was water. The contents of metal ions in the HSA (BSA) in ppm, were 2(60), 240(17), 18(29), 8.9(2.9), 1.7(0.4) and 14(15) for K^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, Cd^{2+} and Cu^{2+}, respectively. Mercury was not found in both HSA and BSA, whilst very little amounts of iron, manganese and nickel were present. The total contents of metals were less than 300 ppm and 150 ppm for HSA and BSA, respectively. This means that each protein molecule contains less than one metal ion on the average. The purity of Tris buffer was greater than 99.5%. NaCl, $ZnCl_2$, $CdCl_2$, $HgCl_2$, $CuCl_2$ and hydrochloric acid were all analytical grade reagents. Deionized water was used throughout.

The serum albumin solutions were freshly prepared and their concentrations were determined spectrophotometrically [20]. The concentrations of $ZnCl_2$, $CdCl_2$, $HgCl_2$ and $CuCl_2$ solutions were determined by titration with EDTA. Prior to experiments, two groups of solutions were prepared by mixing the Zn(II), Cd(II) and Hg(II) solutions and the serum albumin solutions. In one group, the molar ratio of the metal ion to albumin was kept at 1:1 while the concentration of the albumin varied from 10^{-5} to 4 10^{-4} *M*, which is near up the physiological concentration. In the other group, the concentration of the serum albumin was held at 2 10^{-4} to 3 10^{-4} *M* while the ratios of the metal to albumin varied from 1:1, 1:2, up to 6:1. All the solutions contained 0.1*M* NaCl and were buffered with 0.1 *M* Tris–HCl (pH 7.43 ± 0.02). Both XPS and UV spectra were taken at room temperature.

The XPS measurements were performed on a Perkin-Elmer 5300 ESCA Spectrometer. A Mg K_α radiation source, operated at 250 W, was used for sample excitation. For these experiments, the electron energy analyzer was set to give a resolution of 0.8 eV; the electron binding energy error was ± 0.2 eV; and all electron binding energies were referenced to the Ag $3d_{5/2}$ peak. The sample solution was plated out and dried to form a film on the aluminum foil for XPS measurement.

The UV spectra of metal–albumin systems were recorded on a Shimadzu UV-240 Electronic Absorption Spectrograph, for albumin solutions with the same concentrations as the references.

3. Results and discussion

3.1 XPS analysis

The XPS spectra of all samples are similar to one another within the studied molar ratios. As shown in Fig. 1, the XPS spectra (1*s* peaks) of elements C, N and O of HSA and BSA are compared with those of the 6:1 M(II)-albumin (M = Zn, Cd, Hg) samples. Basically, the XPS spectra of N and O do not change regardless of whether or not the serum albumins interact with the zinc group ions. The binding energy is about 401.3 and 533.3 eV for N_{1s} and O_{1s} electrons,

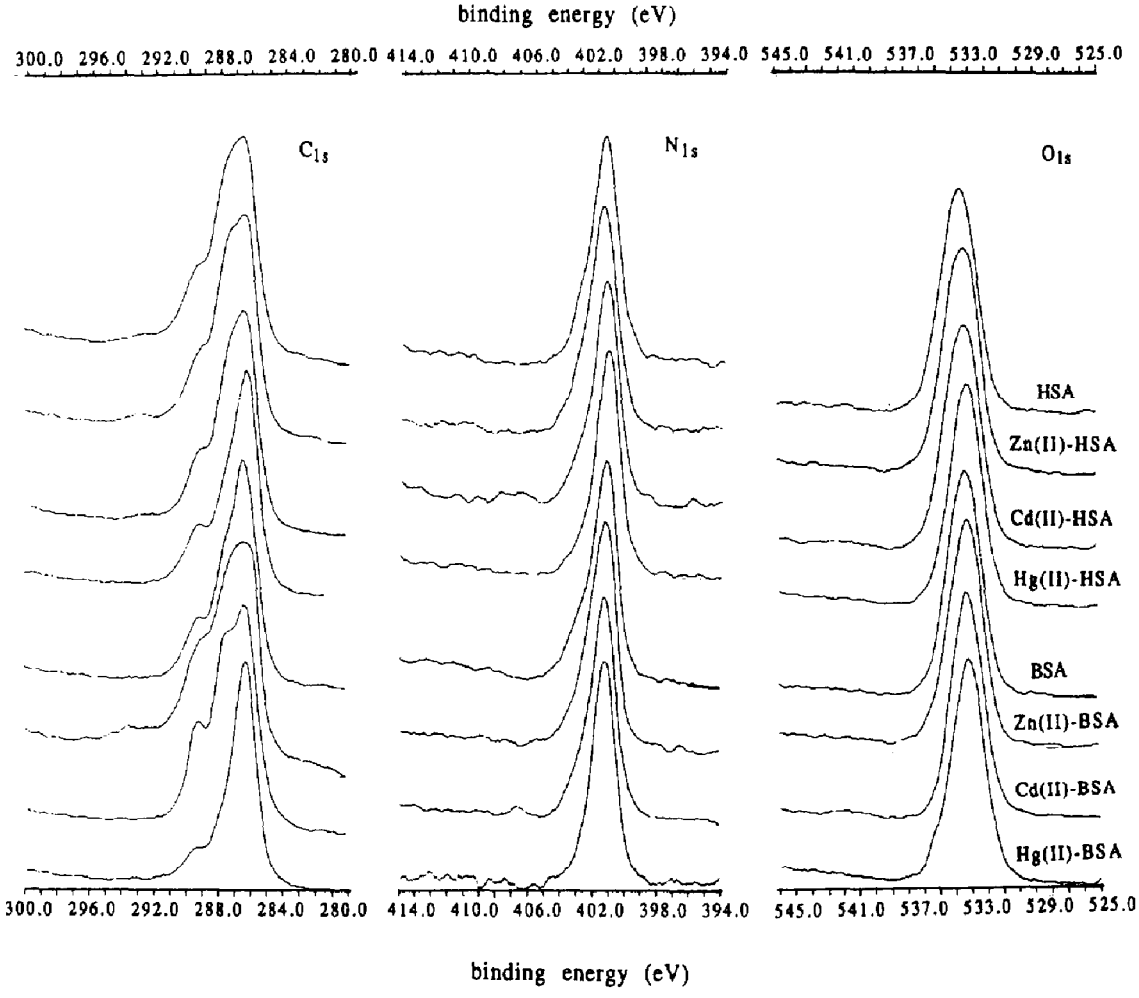

Fig. 1. C, N and O_{1s} XPS spectra of the albumins and the 6:1 M(II)–albumin systems (M = Zn, Cd and Hg).

respectively. In addition, it is hard to differentiate the multi-structure. Finally, the three C_{1s} peaks located at 286.5, 287.4 and 289.4 eV only change in their relative intensities, especially after their binding with Hg(II). However, the S ($2p$) XPS spectra, shown in Fig. 2, have undergone considerable changes by comparison. There is a pair of peaks with a relative intensity of 2:1 at 165.0, 166.2 and 164.8, 166.0 eV. in the spectra of HSA and BSA, respectively (the uppermost one in Fig. 2 (a) and (b)). Obviously, the pair of peaks arise from the $S_{2p_{3/2}}$ and $S_{2p_{1/2}}$ of the cystinyl residues (the ratio of intensity is determined by $2j+1$); the location and interval ($\sim$ 1.2 eV) are basically in accord with reported values [21,22]. After the albumin associates with the M(II), the pair of peaks show a slight shift while their relative intensities display a considerable change. There is an even more notable change in another pair of peaks at 168.5 and 170.0 eV for HSA and 169.4 and 171.0 eV for BSA. The chemical shifts, relative intensities and multi-structure of this pair have changed to varing degrees after the combination of the albumins with the divalent metal ions.

The binding energy of this pair of peaks is about 5 eV larger than that of the main peak ($2p$ peaks). Such a large chemical shift occurs only if the sulfur atom has a highly apparent positive charge (e.g. the sulfur in sulfates). It is unlikely that this type of sulfur atom is present in any significant amount in our systems. The pair of

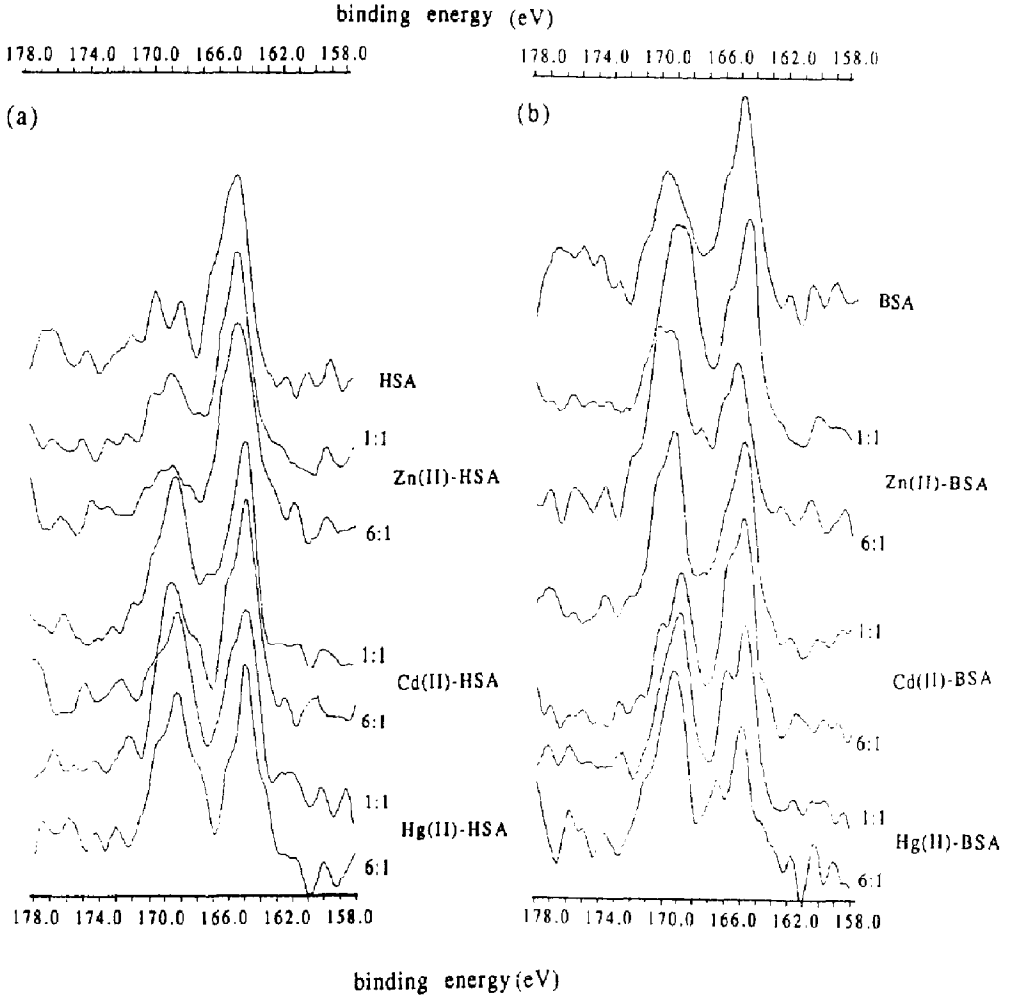

Fig. 2. Sulfur $2p$ XPS spectra of (a) HSA and M(II)–HSA and (b) BSA and M(II)–BSA (M = Zn, Cd and Hg).

peaks, therefore, are not caused by the chemical shift. Furthermore, there have been no reports about this kind of satellites for sulfur atoms. However, strong satellites have been observed at 5–6 eV apart on the high energy side of the main peak in the 1*s* XPS spectra of C, N and O bonding to transition metals [23,24]. They are usually assigned to *shake-up* peaks and are believed to be relevant to the binding to metals. Wendin, after reviewing a great deal of experimental and theoretical studies in detail [25], believed that these peaks occur commonly and that they arise from large relaxation shifts or from shake-up caused by an orbital perturbation, which is brought about by a photoionized hole localized on a certain molecular orbital, through a so-called molecular giant Coster–Kronig fluctuation process. Here we still call them shake-up peaks for the sake of convenience. They are sensitive to the chemical environment because valence electrons that are able to shield the energy level of the hole are involved in this process. It is worth noticing that the relative intensity of the shake-up peaks is generally larger for BSA systems than for HSA systems in our investigations.

Two important facts have emerged from our studies. One fact is that there are considerable changes in the sulfur XPS spectra of the albumins, including the changes in the relative intensity of the main $2p_{1/2}$ and $2p_{3/2}$ peaks after the combination of the albumins with the metals, the changes in the relative intensity and multi-structure of the shake-up peaks, and that these changes in the multi-structure are more complicated for 6:1 systems than for 1:1 systems. (Fig. 2). All of these changes demonstrate that the bonding and orbitals of sulfur have changed in different degrees after the combination of the albumins with the metal ions. The other fact is that the 1*s* XPS spectra of C, N and O in Fig. 1 remain unchanged or only show a small degree of change (limited to C 1*s* peaks). These two facts provide strong evidence for the involvement of sulfur atoms in bonding to zinc group elements in serum albumins. However, they are probably not enough to rule out the involvement of the nitrogen and oxygen atoms in bonding to the metals. That is because the number of nitrogen and oxygen atoms are much more than that of sulfur atoms in the protein molecule. Even though a few of the nitrogen and oxygen atoms may have bonded to the metal ions, it is possible that the 1*s* XPS spectra of O and N will not show distinguishable changes.

In order to test the sensitivity of the XPS method to the coordinating atoms, similar experiments have been done with a high concentration of Cu(II)–HSA and Cu(II)–BSA (dry film). In these experiments, the Cu(II) only bonds to nitrogen atoms [26,27]. It is found that the XPS spectra of C, N and O (the 1*s* peaks) and of S (the 2*p* peaks) do not show any distinguishable change until the molar ratio of Cu(II) to albumin goes up to 3:1. This indicates that only when the coordinating atoms reach a considerable proportion can they be distinguished with the XPS method. Since the number of the coordinating nitrogen atoms involved in Cu(II)–serum albumin only takes a very small proportion of the total nitrogen atoms in the protein, they cannot be distinguished. On the other hand, it is known that the XPS spectra (2*p* peaks) of the sulfur atoms those are not involved in coordination to metals remain unchanged. Therefore, the considerable changes observed in the S (2*p*) XPS spectra of the metal–serum albumin systems are irrefutable evidence for the involvement of the sulfur atoms in coordination to the metals.

3.2 UV Spectra

The UV difference spectra of all samples are very similar. When the concentration of albumins is larger than 10^{-4} *M*, every system shows an absorption at about 290 nm; and some systems also give another absorption at about 250 nm. Each system, however, displays a few differences.

The Zn(II)–albumin systems possess the simplest spectra. Both 1:1 Zn(II)–HSA and 1:1 Zn(II)–BSA show two peaks at about 240 nm and 280 nm (Fig. 3 (a)) when the concentration is less than $7\ 10^{-5}$ *M*. There is only one peak occurring at 290 nm as the concentration increases (Fig. 3 (b)). As the molar ratio varies, the spectra of the Zn(II)–HSA systems do not display notable changes in comparison with that of 1:1 system until Zn(II)–HSA ratio reaches 4:1. For the

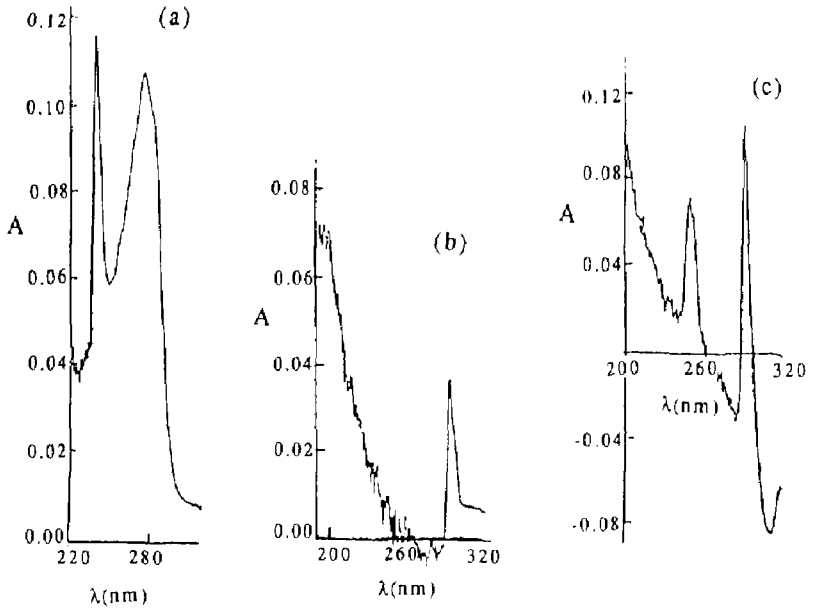

Fig. 3. UV absorption spectra of the Zn(II)–albumin systems: (a) 1:1 Zn(II)–HSA, [HSA] = 5.00 10^{-5} *M*; (b) 1:1 Zn(II)–HSA, [HSA] = 3.03 10^{-4} *M*; (c) 3:1 Zn(II)–BSA, [BSA] = 2.00 10^{-4} *M*.

Zn(II)–BSA system, however, the spectrum of the 2:1 system shows only one peak at 290 nm when the BSA concentration is held at 2.00 10^{-4} *M*. The 3:1 system gives another peak at 248 nm in addition to the one at 290 nm (Fig. 3 (c)), which is similar to the spectrum of systems with lower concentrations. When the molar ratio increases again, it becomes difficult to examine the spectrum because of the frequent negative absorptions.

The spectra of the Cd(II)–albumin systems are similar to those of the Zn(II)–albumin. When the concentration is less than 6 10^{-5} *M*, the 1:1 system shows two bands at about 240 and 280 NT, the latter splits apart into multiple peaks. As the concentration becomes higher, only one band

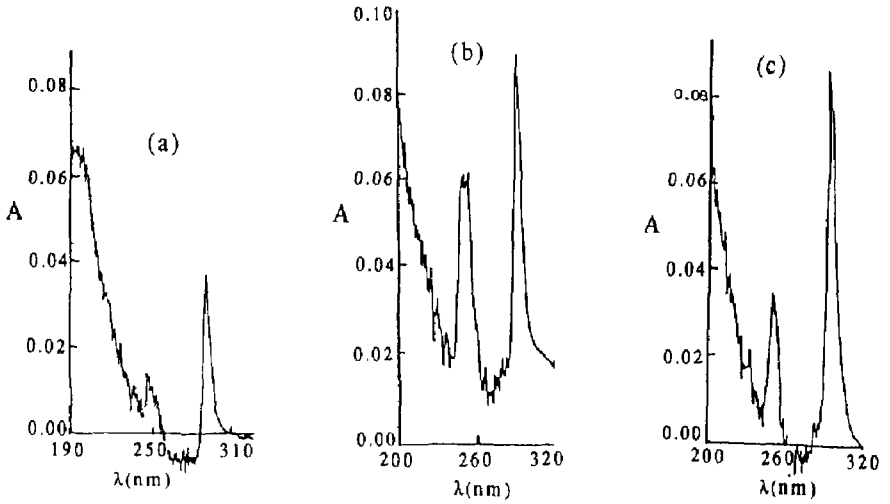

Fig. 4. UV absorption spectra of the Cd–albumin systems with [albumin] = 2.00 10^{-4} *M*: (a) 1:1 Cd(II)–HSA; (b) 6:1 Cd(II)–HSA; and (c) 2:1 Cd(II)–BSA.

occurs at 290 nm for the 1:1 system (Fig. 4 (a)). If the albumin concentration is maintained at 2.00 10^{-4} *M*, the spectrum of the 2:1 Cd(II)–HSA system is still similar to that of the 1:1 system, while for systems from 3:1 to 6:1 a twin peak also appears at about 250 nm in addition to the band at 290 nm (Fig. 4 (b)). The 2:1 Cd(II)–BSA system gives a new absorption at 248 nm (Fig. 4 (c)). As before, the negative absorption often turns up when the molar ratio is increased further.

At low concentrations, all of the 1:1 Hg(II)–albumin systems show a strong peak at 240 nm, together with a multiple peak at the long wavelength side (Fig. 5 (a)). When the concentration is higher than 10^{-4} *M*, the strong peak displays a red-shift to 245 nm and the multiple peak converges into a new peak at 290 nm (Fig. 5 (b)). The

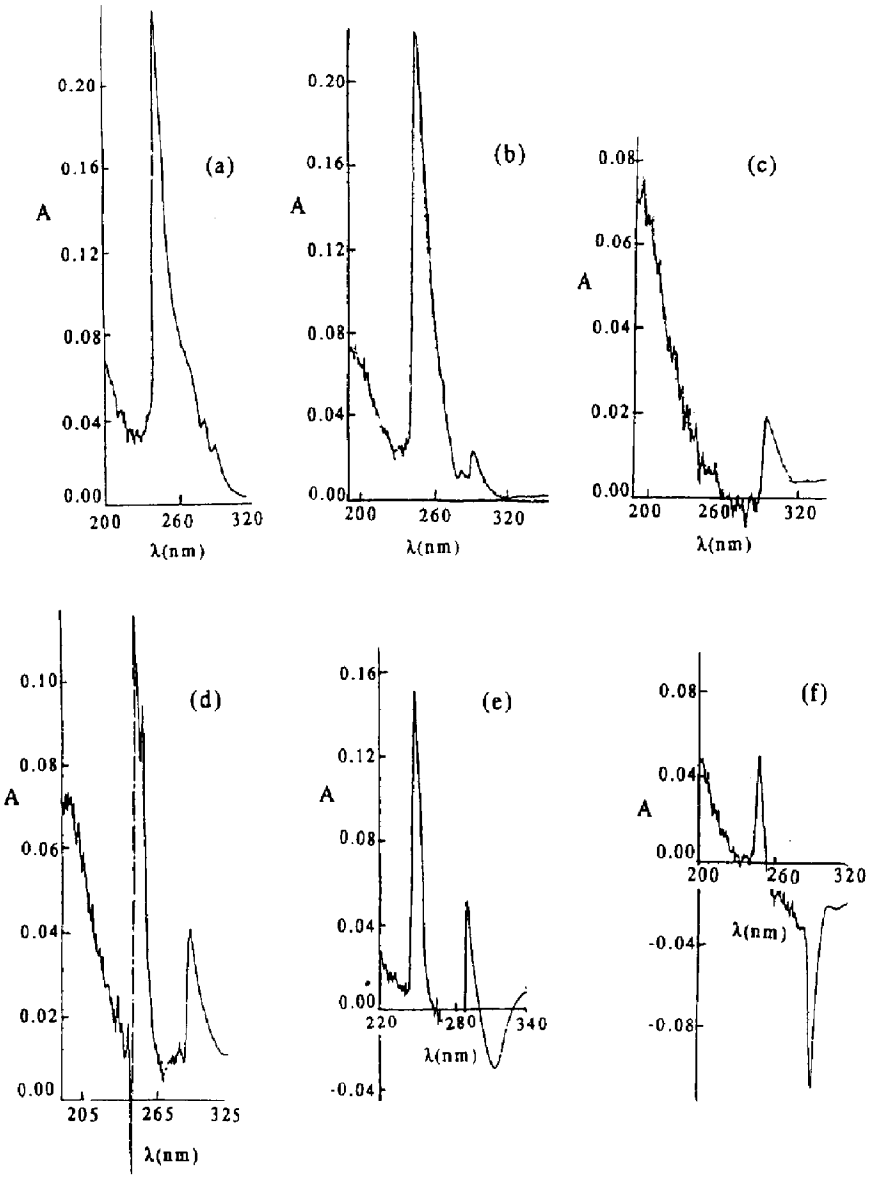

Fig. 5. UV absorption spectra of the Hg(II)–albumin systems: (a) 1:1 Hg(II)–HSA, [HSA] = 8.00 10^{-4} *M*; (b) 1:1 Hg(II)–HSA, [HSA] = 1.00 10^{-4} *M*; (c) 1:1 Hg(II)–HSA, [HSA] = 3.00 10^{-4} *M*; (d) 4:1 Hg(II)–HSA, [HSA] = 2.00 10^{-4} *M*; (e) 4:1 Hg(II)–BSA, [BSA] = 2.00 10^{-4} *M*; (f) 5:1 Hg(II)–BSA, [BSA] = 2.00 10^{-4} *M*.

strong peak disappears and only the peak at 295 nm remains if the concentration is higher than 3 10^{-4} M (Fig. 5 (c)). When the albumin concentration is kept at 2.00 10^{-4} M, the 2:1 and 3:1 Hg(II)–HSA systems show two peaks at 250 and 290 nm, respectively. For a 4:1 system, the peak at 250 nm splits up, and the peak at 290 nm is slightly split (Fig. 5 (d)). For the 2:1 to 4:1 Hg(II)–BSA systems, each gives two absorptions, one at 248 and the other at 290 nm (Fig. 5 (e)). When the molar ratio increases further, a negative absorption again emerges (Fig. 5 (f)).

In general, negative absorptions referred to a reference solution are common in electronic absorption spectra. The inverse peak emerging at the absorption position in some of our systems (Fig. 5 (f)), however, has not been reported to date. And the causes are still left to be explored. A preliminary observation indicates that an increase in the ion strength or a decrease in pH value can be advantageous in the suppression of the negative absorption.

3.3 UV Spectral analysis

The absorptions we have observed in the ultraviolet region are all produced after the combination of the albumins with the metal ions. All of them should therefore arise from LMCT transitions. We now intend to analyze the relevant data with the method proposed by Jorgensen [28]. For this reason, we need first to know the optical electronegativity of some relevant orbitals of the metal and donor atoms.

Since Zn^{2+}, Cd^{2+} and Hg^{2+} all have $nd^{10}(n+1)s^0$ configuration, the receptor orbital for the LMCT transition should be the vacant $(n+1)s$ orbital. Kennedy and Lever [29] have assigned a set of absorptions of the tetrahedral complexes, $Zn(Py2SH)_4^{2+}$ at 26,850 cm^{-1}, $Cd(Py2SH)_2Cl_2$ at 27,550 cm^{-1}, $Hg(Py2SH)_2Cl_2$ at 27,700 cm^{-1} and $Co(Py2SH)_4^{2+}$ at 22,720 cm^{-1}, to the $\pi S \rightarrow M(II)$ LMCT transitions and reported the optical electronegativity, $\chi(Py2SH)$ to be 2.4. For the first three complexes, the transition energy is in accord with the simple formula $E(cm^{-1}) = 30{,}000\,[\chi(L) - \chi(M)]$. With this equation, the optical electronegativity, $\chi(M)$, of Zn(II), Cd(II) and Hg(II) is calculated to be in the narrow range of 1.48 to 1.51 when $\chi(L) = 2.4$ is used. The ligands Py2SH and Cl^- in this set of complexes are quite close to each other in the spectrochemical series. Both possess a typical spectrum with tetrahedral symmetry. Some reports [30,31] declared that Zn(II), Cd(II) and Hg(II) had different optical electronegativities. We will discuss this later.

The optical electronegativity of the cysteinyl thiol π-donor orbital, $\chi(L)$, has been reported to be 2.6 [32]. Since there are different values reported in the literature [33], we will prove the reliability of this result. The band shown at 340 nm for many of the Zn(II)-containing enzymes substituted by Co(II) has been unequivocally assigned to a LMCT transition from the cysteinyl thiol group to tetrahedral Co(II) [33–35]. For Co(II) complexes, the transition energy should be corrected on the basis of d-orbital splitting and the change in the spin pairing energy. From the work of Kennedy and Lever [29], we can have the following equation,

$$30{,}000\chi(L)(\text{transition energy}) = 30{,}000\chi(Co) + (\text{correction term}) = 49{,}280\ cm^{-1}.$$

Therefore the optical electronegativity of the cysteinyl thiol π-donor orbital, corresponding to the transition at 340 nm, is 2.62, which agrees with the literature value [32].

All of our samples showed absorption at 290 or 295 nm. We have assigned it to the $\pi S \rightarrow M(II)$ LMCT transition. With the use of the data mentioned above, $\chi(L)$ will be equal to 2.61–2.63 if $\chi(M)$ is taken 1.48; the $\chi(M)$ will be equal to 1.47–1.49 if $\chi(L)$ is taken 2.62. These values are completely in accord with those reported. This indicates that the zinc group metal centers are likely to be tetrahedrally coordinated by the thiol groups of cysteinyl residues. It is difficult for XPS to distinguish between cysteinyl sulfur and cystinyl sulfur, while UV spectra show that the cysteinyl sulfur is involved in the coordination.

Some of our samples also showed another band at about 250 nm. Boyer [36] confirmed that linear dithiolate mercury had a characteristic band at 250 nm. It is most likely that the absorption at

250 nm observed in our samples results from the two-coordinate linear –S–M–S– species or from some linear fragments existing in the distorted tetrahedral configuration. The existence of an approximately linear structure in tetrahedral Hg(II) compounds has been verified [37]. This shows that zinc group metals still have an approximately equal optical electronegativity when they take linear configurations. Therefore, the χ(M) calculated on the basis of the band at 250 nm should be 1.27–1.29. Vasak et al. [31] reported that Zn(II), Cd(II) and Hg(II) had different optical electronegativities. The values given in those references (1.15–1.2 for Zn(II), 1.27 for Cd(II) and 1.50 for Hg(II)) are in accord with the values obtained: 1.27–1.29 for linear configuration and 1.48–1.51 for the tetrahedral structure. The metal centers they studied may not be all in the tetrahedral configuration. When these two configurations are considered, there just exists an allowed $\pi S \rightarrow M$ transition derived from the common molecular orbital energy diagram for each of the configurations: $\pi_u \rightarrow \sigma_g^+$ for the linear structure and $t_2 \rightarrow a_1$ for the tetrahedral structure. It can be observed from the UV spectra that the peak at about 250 nm occurs only in the dilute ($< 2\ 10^{-4}$ M) 1:1 system or in some other systems containing higher ratios of metal ions. This indicates that the linear configuration does not exist universally and that the dominant configuration is tetrahedral.

3.4 The possibility of the involvement of imidazole groups in the ligation

Although the possibility of the involvement of many groups in ligation to zinc group ions can be completely ruled out on the basis of the two main bands at 250 and 290 nm, the situation of imidazole groups of the His-residues should be thought over carefully. The involvement of imidazole groups in the ligation have been inferred by a number of studies [4–15]. In this paper, however, the XPS evidence strongly comfirms that the sulfur atoms coordinate the metal ions, but it does not give any clear evidence of whether or not some other kinds of atoms are involved in the ligation.

There has been no reports about the optical electronegativity of the imidazole π orbitals to date. Krogh-Jespersen and Schugar [38] assigned the two LMCT transitions of $[Ru(NH_3)_5Im]^{3+}$ at 23,300 cm^{-1} and 33,400 cm^{-1} to π_1(Im-N) $\rightarrow t_{2g}$ (Ru) and π_2 (Im-N) $\rightarrow t_{2g}$ (Ru), respectively. It can be calculated that χ(Im-N, π_1) = 2.6–2.7 and χ(Im-N, π_2) = 3.0–3.1 if the optical electronegativity of the low spin octahedral Ru(III) complex is taken as 2.0–2.1 [30] and if the correction term for the spin pairing energy is taken as approximately 5000 cm^{-1}. Thus, the band at 290 nm in this study may be considered as an electron transition from Im-N π_1 orbital to the $(n+1)s$ orbital of the metal atom or a portion of this band is from the transition. Unfortunately, the other similar transition from the π_2 orbital, which should be seen at about 220 nm, does not appear. Therefore, the evidence presented in this paper does not suggest the involvement of the imidazole nitrogen in the ligation. The possibility of the involvement of nitrogen, however, cannot be ruled out only by the evidence we have here. It is still open to investigation.

3.5 The binding sites

There are 35 Cys residues both in HSA and BSA [39]. It is generally believed that they form seventeen disulfide bridges through their thiol groups and the remaining one exists as a single cysteinyl residue. The positions of these Cys residues are the same in HSA and BSA. Three of the disulfide bridges in positions 53–62, 265–282 and 279–289 are relatively isolated and fourteen others constitute seven pairs by the combination of adjacent bridges, distributed in the section 75–567. Our studies have shown that there are quite a few similar binding sites for zinc group ions in HSA and BSA, respectively, and that the metal ions are most likely to be linearly or tetrahedrally coordinated by the cysteinyl thiol groups. The linearly coordinated sites, however, might be parts of the distorted tetrahedral configuration or they are transformable with the tetrahedral structure. Therefore it is possible that the zinc group ions are bound at the seventeen disulfide bridges, and it is most likely that these ions are dis-

tributed at the seven pairs of disulfide bridges between positions 75 and 567, because both linear and tetrahedral coordination can be formed at these positions.

In order to coordinate metals through the deprotonated thiol groups of the cysteinyl residues, the disulfide bridges or the pairs of adjacent disulfide bridges would have to undergo cleavage and a change in oxidation level. These questions remain to be addressed. At present, what can be pointed out is: (1) there is no direct evidence for the formation of the seventeen disulfide bridges from the 35 Cys residues. Their occurrence is merely a reasonable inference. The possibility that all or some of them exist as lone cysteinyl residues all the while is not excluded; (2) it has been verified in the study of metallothioneins that Zn(II), Cd(II) and Hg(II) are all bound to thiol groups of cysteinyl residues to form tetrahedral coordination [31]. When the metal is removed, disulfide bridges can be formed spontaneously. Although a chemical reduction is needed when the disulfide bridge re-coordinates metal in the form of thiol groups [40], the spontaneous cleavage of the disulfide bridge has been observed in some other systems [41,42].

4. Conclusions

The XPS spectra show that before and after HSA or BSA coordinate Zn(II), Cd(II) and Hg(II) only the $2p$ peaks and shake-up peaks of the sulfur display considerable changes, while the electron binding energy and the shape of the peaks of all the other atoms with potential coordinating ability remain basically unchanged. These spectral characteristics are maintained until the ratio of metal to albumin reaches 6:1. This reliable evidence indicates that the cystinyl sulfur atoms in HSA or BSA are indeed involved in the ligation to the zinc group ions. Because the sensitivity of the XPS method in differentiating coordinating atoms from the rest is limited, it cannot be ruled out whether N and O atoms are involved in the ligation.

All of the systems studied show an absorption at about 290 nm in the UV spectra, while some of them give another band at about 250 nm. The data analysis based on Jorgensen's method indicates that the optical electronegativity of the ligand orbital $\chi(L)$ is equal to 2.61–2.63, which is in good accord with the data reported for the cysteinyl thiol group. The band at 290 nm is assigned to the LMCT transition of the tetrahedral metal center MS_4 corresponding to $\chi(M) = 1.48–1.51$. The band at 250 nm is assigned to the LMCT transition of the linear metal center MS_2 corresponding to $\chi(M) = 1.27–1.29$. The data are also in accord with those reported in the literature. From these results, we infer that the seventeen disulfide bridges in HSA or BSA may be the possible binding sites, while the seven pairs of adjacent disulfide bridges between positions 75 to 567 are most likely to be the binding sites.

The optical electronegativity calculated for the π_1 orbital of the imidazole group when it coordinates metals is 2.6–2.7. It is in accord with the 290 nm band. Since a similar transition from the π_2 orbital is not observed, the possibility of the involvement of the imidazole group in the ligation is minimal although it cannot be definitely excluded. The possibility of the involvement of carboxyl groups can be ruled out because the strong absorption that should appear based on its optical electronegativity (3.22) [27] was not found.

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