Short communication

Interaction of Mn(II) ions with Human Serum Albumin

E. N. CHIKVAIDZE

Department of Physics, Tbilisi State University
380028 *Chavchavadze Ave. 3, Tbilisi, USSR*

Investigation of nonspecific interactions of metal ions with proteins and studies of biological function of such complexes are of importance (Breslow 1978; Friedberg 1975). Identification of the binding centers of metal ions with proteins and determination of the binding constants are necessary to explain the transfer mechanisms of these ions by proteins.

Earlier it has been shown (Lau et al. 1971; Friedberg 1975) that serum albumin is the major carrier of metal ions in the blood. The present paper is a report of a comparative spin resonance (ESR) investigation of Mn(II) ion binding with human serum albumin (HSA) and lysozyme, and the effect of pH

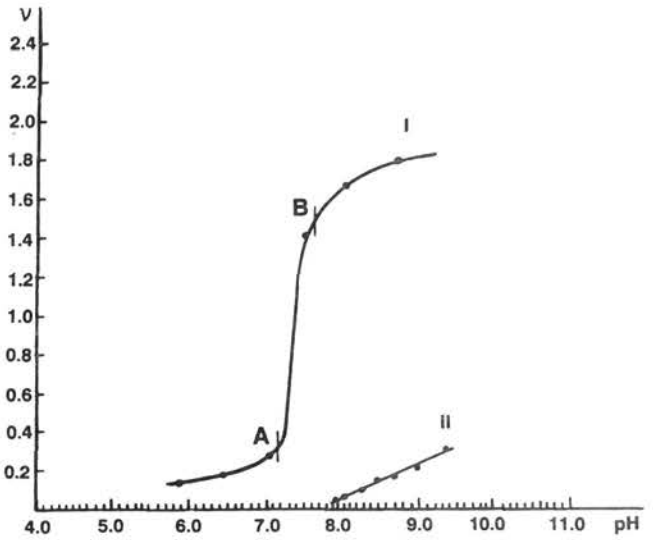

Fig. 1. Plots of $\nu = \frac{[\mathrm{Mn(II)}]_B}{[P]}$ vs pH for HSA (I) and lysozyme(II). $[P]$ — total protein concentration, 10^{-4} mol/l; $[\mathrm{Mn(II)}]_B$ — concentration of Mn(II) bound to protein, [Mn(11)] — total Mn(11) concentration, 1.9×10^{-4} mol/l; Buffer: Tris-HCl 0.025 mol/l. AB — range of blood pH in vivo. $t = 20\,°C$.

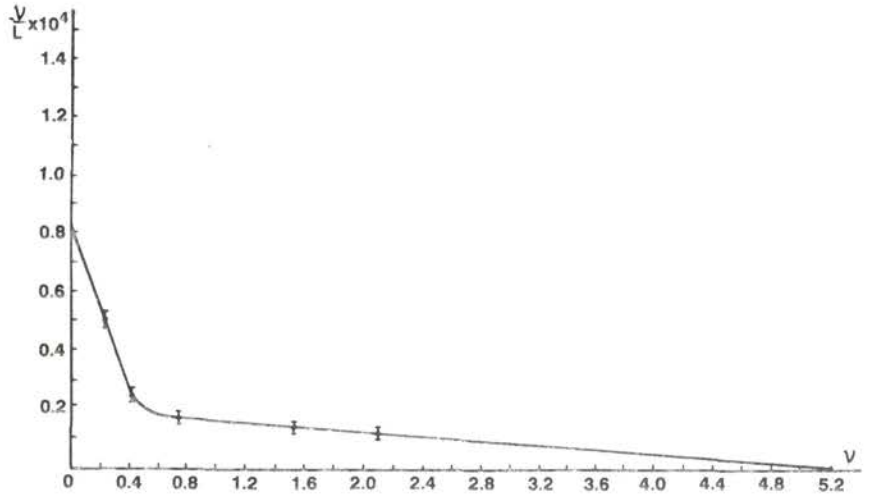

Fig. 2. The Scatchard-Klotz plot of $\frac{\nu}{[L]}$ vs ν for HSA, pH = 7.7. Buffer: Tris-HCl 0.25 mol/l, [Mn(II)] = 1.9×10^{-4} mol/l, $[P] = (10^{-5} \div 8 \times 10^{-4})$ mol/l, $[L]$ – concentration of unbound Mn(II) in solution. $t = 20\,°C$.

of the medium on this binding. The two proteins showing different spatial structures and having different functions are expected to interact differently with the metalions. This should allow to define the binding specificity of Mn(II) ions to albumin, the possible carrier of the metal ions in the blood, and on the other hand, the study of Mn(II) ion- HSA binding may provide important information concerning the transfer mechanisms of these ions.

Fig. 1 shows the plot of parameter ν vs. pH of the medium (where ν is the ratio of moles of Mn(II) ions bound with the protein to those of the protein). As the plot shows, Mn(II) ions bind to HSA already within the range of acidic pH. "Strong" binding of Mn(II) to HSA is observed at pH = 7.0, with a maximum at pH – 8.5. With lysozyme, Mn(II) ions start binding at pH = 8.0. At identical pH, the concentrations of Mn(II) ions bound to HSA are several times greater than those bound to lysozyme.

To determine the number of binding sites and the association constants for Mn(II) and HSA, Scatchard-Klotz curves were constructed for pH = 7.7 (Fig. 2) (Klotz and Hunston 1971). Two sites were defined for Mn(II) binding to HSA at pH = 7.7: one strong binding site with $n_1 = 1$, $k_1 = (0.63 \pm 0.06) \times$ $\times 10^4$; and a "weak" binding site with $n_2 = 5$, $k_2 = (0.40 \pm 0.05) \times 10^3$.

Studies of Cu(II) ion binding to bovine serum albumin (BSA) revealed two

major binding sites for the metal ion: "a labile" complex is formed at acid pH; it includes the imidazolyl group of histidine and the carboxylic group of the side chain. The "strong" binding center consists of the α-NH_2 group, the imidazolyl group His-3, and of the two nitrogen atoms of the peptide group localized between them (Peters and Blumenstock 1967; Breslow 1978). Appleton and Sarkar (1971) have established that BSA and HSA have similar binding sites at neutral pH values of the medium. This was confirmed by investigations of the absorption spectra of Cu(II) + HSA and Cu(II) + BSA in the visible range of the spectrum (Breslow 1964; Lau et al. 1971).

Our recent measurements (Chikvaidze 1988) showed that at neutral pH values, Cu(II) ions block Mn(II) ion binding to HSA. Thus, Cu(II) ions compete with Mn(II) ions for one binding site. Actually, it should be considered that the binding constant of Mn(II)-HSA binding is $k = 0.63 \times 10^{-4}$, whereas it is several orders of magnitude higher for Cu(II), $k = 1.5 \times 10^{16}$ (Lau and Sarkar 1971).

As the plot in Fig. 1 shows, at neutral pH values, the binding parameter ν increases sharply and reaches a maximum at pH = 8.5. At this pH the "strongly" binding α-NH_2 group is obviously participating in the complex formation, resulting in markedly increased concentrations of Mn(II) ions bound to HSA. The participation of histidine amino acid residues in the complex formation at neutral pH confirms agrees with the observation of no Mn(II) binding to lysozyme at this pH, since lysozyme contains only a single histidine amino acid residue per molecule. HSA, which contains 16 histidine residues, binds Mn(II).

Differences in the values of the binding constants for Mn(II) and HSA obtained in our experiments and those reported by Nandedkar et al. (1973) can be explained by differences in pH and other conditions between our measurements and those of the above authors.

It is generally accepted that HSA is the major carrier of metal ions in the blood; less is known however about the transfer mechanisms and functional significance of the protein-metal ion complexes and the effect of pH. We could show (Fig. 1) that pH affects the equilibrium of the complex-forming reaction. Upon increasing pH the equilibrium is shifted towards the complex-formation. It is noteworthy that maximum binding of Mn(II) ions to HSA occurs exactly at pH values at which in vivo HSA is functioning in the blood (pH = 7.2 ÷ 7.6). An increase of the pH value to 7.6 leads to enhanced binding of Mn(II) ions to HSA, and a decrease of the pH value results in "a drop" of a part of the ions bound. As intracellular pH is 7.2 this may represent the mechanism underlying Mn(II) transfer by HSA from the blood into the cells.

References

Appleton D. W., Sarkar B. (1971): The absence of specific copper (II)-binding site in dog albumin. J. Biol. Chem. **246,** 5040—5046

Breslow E. (1964): Comparison of cupric ion-binding sites in myoglobin derivatives and serum albumin. J. Biol. Chem. **239,** 3252—3259

Breslow E. (1978): Complexes of metals with proteins. In: Inorganic Biochemistry (Ed. G. L. Eichhorn) vol. 1. pp. 274—299. "Mir" Moscow (in Russian)

Chikvaidze E. N. (1988): Intraction of bivalent metal ions with human serum albumin. Biofizika, **33,** 723—725 (in Russian)

Friedberg F. (1975): Albumin as the major metal transport agent in blood. FEBS Lett. **59,** 140—141

Klotz I. M., Hunston D. L. (1971): Properties of graphical representations of multiple classes of binding sites. Biochemistry **10,** 3065—3069

Lau S., Sarkar B. (1971): Ternary coordination complex between human serum albumin, copper (II) and L-histidine. J. Biol. Chem. **246,** 5938—5943

Nandedkar A. K., Nurse C. E., Friedberg F. (1973): Mn^{2+} binding by plasma proteins. Int. J. Peptide Prot. Res. **5,** 279—281

Peters T. Jr., Blumenstock F. A. (1967): Copper-binding properties of bovine serum albumin and its aminoterminal peptide Fragment. J. Biol. Chem. **242,** 1574—1578

Final version accepted February 15, 1990