The effect of cupric and ferric ions on antioxidant properties of human serum albumin

Gholamreza Rezaei Behbehani, Katayon Gonbadi and Nasrin Eslami

Chemistry Department, Imam Khomeini International University, Qazvin, Iran

Abstract. The interaction of both ferric (Fe^{3+}) and cupric (Cu^{2+}) ions with human serum albumin (HSA) was assayed at a temperature of 27°C in aqueous solution using isothermal titration calorimetry. The association equilibrium constant and the molar enthalpy for one binding is 1.7×10^5 M^{-1} and -31.37 kJ·M^{-1}, respectively. To obtain the binding parameters of metal ion-protein interaction over the whole range of Fe^{3+} concentrations, the extended solvation model was applied. The solvation parameters obtained from this model were attributed to the structural change of HSA. The binding parameters obtained from the extended solvation model indicate that the stability of HSA was decreased as a result of its binding with ferric ions, which cause dampening the antioxidant property of HSA. Cuperic ion increases the stability of HSA considerably, indicating that the antioxidant property of human serum albumin are increased as a result of its interaction with cupric ion.

Key words: Human serum albumin — Isothermal titration calorimetry — Ferric ion — Cupric ion

Introduction

In the presence of catalytic amounts of transition metal ions, particularly iron and copper, reactive oxygen species (ROS) can generate the highly reactive hydroxyl radical by the Fenton reaction. ROS exert their detrimental effects at least partially, through endothelial dysfunction with alterations of vascular tone, increased cell adhesion, and vascular permeability (Parola et al. 2001; Young et al. 2001; Izzet et al. 2005; Otagiri et all. 2009; Sitar et al. 2013). The high levels of ROS can result in organ damages, the ROS amount being related to the severity of sepsis and mortality. Human serum albumin (HSA) molecule acts through its multiple-binding sites against ROS free radicals and trap them. Around 70% of the free radical-trapping activity of serum was due to human serum albumin (Atukeren et al. 2010). In physiological conditions, the function associated with changes in the redox state of HSA structure and its antioxidant properties can be changed. In plasma, most of the cupric ion is bound to caeruloplasmin, but a high percentage of the metal ion may exist bound to albumin.

Correspondence to: Gholamreza Rezaei Behbehani, Chemistry Department, Imam Khomeini International University, Qazvin, Iran
E-mail: grb402003@yahoo.com

HSA contains one high affinity site for cupric ion, the N-terminal tripeptide Asp-Ala-His, indicating that cupric ion prevent oxidative damages because stabilizes HSA structure. Ceruloplasmin is an important intravascular antioxidant and it protects tunica intima against free radical injury (Bourdon et al. 2005; Papatheodorou et al. 2007; Colombo et al. 2012; Kato et al. 2014). In the present work, we have found an accurate correlation between solvation parameters obtained from the extended solvation model and antioxidant properties of HSA.

Materials and Method

HSA was obtained from Sigma-Aldrich, and $FeCl_3$ was obtained from Merck. The buffer solution used in the experiments was 30 mM Tris, pH 7.0 (Merck). The isothermal titration microcalorimetric experiments were performed with the four channel commercial microcalorimetric system (Tehran University, Iran). The titration vessel was made from stainless steel. Ferric or cupric ions solution (2 mM) was injected by use of a Hamilton syringe into the calorimetric titration vessel, which contained HSA (90.32 μM). Thin (0.15 mm inner diameter) stainless steel hypodermic needles, permanently fixed to the syringe, reached directly into the calorimetric vessel. Injection of iron solution into

the perfusion vessel was repeated 29 times, with 10 μl *per* injection. The calorimetric signal was measured by a digital voltmeter that was part of a computerized recording system. The heat of each injection was calculated by the "Thermometric Digitam 3"software program. The heat of dilution of the Fe^{3+} solution was measured as described above except HSA was excluded.

Results and Discussion

The calculated heats for HSA interactions with cuperic and ferric ions were shown in Figures 1 and 2.

We have shown previously that the heats of the ligand+HSA interactions in the aqueous solvent systems can be calculated *via* the following equation (Rezaei Behbehani et al. 2008, 2012, 2013; Barzegar et al. 2011):

$$q = q_{max} x'_B - \delta^{\theta}_A (x'_A L_A + x'_B L_B) - (\delta^{\theta}_B - \delta^{\theta}_A) \\ (x'_A L_A + x'_B L_B) x'_B \qquad (1)$$

where q are the heats of Fe^{3+}+HSA interactions and q_{max} represents the heat value upon the saturation of all HSA. The parameters δ^{θ}_A and δ^{θ}_B are the indexes of HSA stability in the low and high cupric or ferric ion concentrations, respectively. $p > 1$ or $p < 1$ indicate positive or negative cooperativity of a macromolecule for binding with a ligand, respectively; $p = 1$ indicates that the binding is non-cooperative. x'_B can be expressed as follows:

$$x'_B = \frac{p x_B}{x_A + p x_B} \qquad (2)$$

We can express x_B fractions, as the total Fe^{3+} concentrations divided by the maximum concentration of the Fe^{3+} upon the saturation of all HSA as follows:

$$x_B = \frac{[Fe^{3+}]}{[Fe^{3+}]_{max}}, \quad x_A = 1 - x_B \qquad (3)$$

where $[Fe^{3+}]$ is the concentration of Fe^{3+} ions after every injection and $[Fe^{3+}]_{max}$ is the maximum concentration of the Fe^{3+} ions upon the saturation of all HSA. L_A and L_B are the relative contributions due to the fractions of unbound and bound metal ions in the heats of dilution in the absence of HSA and can be calculated from the heats of dilution of Fe^{3+} ions in buffers, q_{dilut}, as follows:

$$L_A = q_{dilut} + x_B \left(\frac{\partial q_{dilut}}{\partial x_B} \right), \quad L_B = q_{dilut} + x_A \left(\frac{\partial q_{dilut}}{\partial x_B} \right) (4)$$

The heats of Fe^{3+}+HSA interactions, q, were fitted to Eq. 1 across the whole Fe^{3+} compositions. In the fitting procedure, the only adjustable parameter *(p)* was changed until the best agreement between the experimental and calculated data was approached (Fig. 1). The δ^{θ}_A and δ^{θ}_B values are recovered from the coefficients of the second and third terms of Eq. 1. The small relative standard coefficient errors and the high r^2
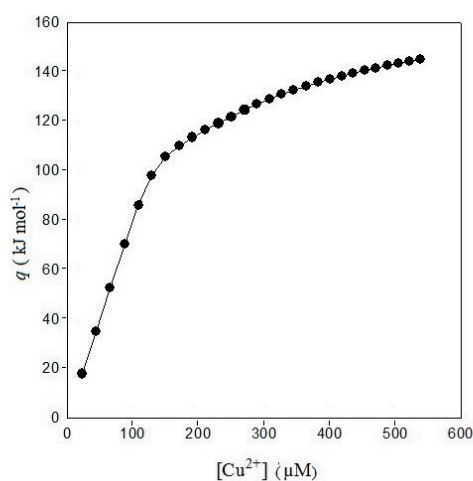

Figure 1. Comparison between the experimental heats (●) at 300 K, for HSA+Cu^{2+} interactions and the calculated data (lines) using Eq. 1. $[Cu^{2+}]$, the concentrations of $[Cu(NO_3)_2]$ solution (the agreement between experimental and calculated data support the extended solvation model); q, the heat of HSA+Cu^{2+} interaction in every concenteration of Cu^{2+} ion; [HSA], the concentration of human serum albumin.
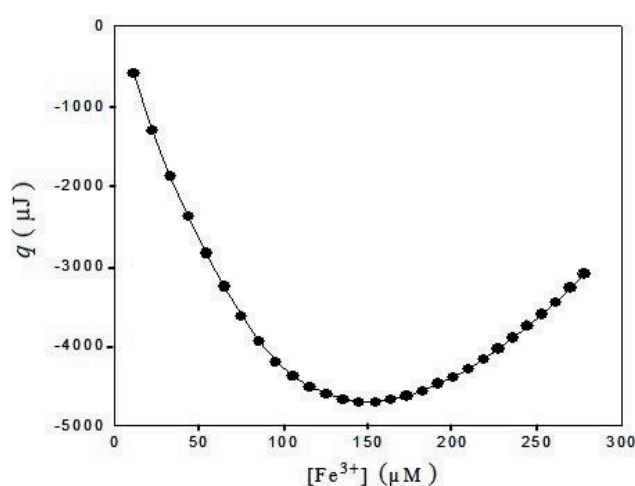

Figure 2. Comparison between the experimental heats (●) at 300 K, for Fe^{3+}+HSA interactions and the calculated data (lines) using Eq. 1. $[Fe^{3+}]$, the concentrations of $[FeCl_3]$ solution (the agreement between experimental and calculated data support the validity of Eq. 1); q, the heat of HSA+Fe^{3+} interaction in every concenteration of Fe^{3+} ion; [HSA], the concentration of human serum albumin.

values (0.99999) support the method. The binding parameters for Fe^{3+}+HSA and Cu^{2+}+HSA interactions obtained from Eq. 1 were listed in Table 1. The agreement between the calculated and experimental results (Fig. 2) is striking, and gives considerable support to the use of Eq. 1. δ_A^θ and δ_B^θ values for Fe^{3+}+HSA interactions is negative, indicating that in the low and high concentrations of the metal ions the HSA structure is destabilized, resulting in a decrease in its antioxidant properties of HSA (Table 1).

The more stable HSA can trap the free radical and decrease the oxidative stress. In the other hand, we can predict the stability of HSA accurately using Eq. 1 and attribute δ_A^θ and δ_B^θ values to the antioxidant properties of HSA. Cuperic ions in solution interact strongly with human serum albumin as indicated by the large and positive δ_A^θ and δ_B^θ values (Table 1) and stabilized the HSA structure significantly. In other words, the stabilities indexes (δ_A^θ and δ_B^θ values), calculated from Eq. 1 for HSA+Cu^{2+} interactions is considerably positive, indicating that in the low and high concentrations of the Cu^{2+} ions, the HSA structure is stabilized drastically. The δ_A^θ and δ_B^θ values for HSA+Fe^{3+} interactions are negative (unstable HSA+Fe^{3+}complex), showing that the trace amounts of ferric ion can decrease the antioxidant property of HSA and increase oxidative stress. HSA+Cu^{2+} complex is much more stable than HSA+Fe^{3+} complex, resulting in a great increase of antioxidant activity of HSA as a result of its interation with cupric ions. The large and positive δ_A^θ and δ_B^θ values show that the high stable HSA+Cu^{2+} complex has a strong tendency against the free radicals and prevent a subsequent Fenton reaction to produce the most harmful of hydroxyl radical ($^\bullet$OH), which prevent to the development of several age-related diseases by inducing oxidative damage.

For a set of identical and independent binding sites, according to the recent data analysis method, using Eq. 5, a plot of $(\frac{\Delta q}{q_{max}})M_0$ versus $(\frac{\Delta q}{q})L_0$ should be a linear plot by a slope of $1/g$ and the vertical-intercept of $\frac{K_d}{g}$, g and K_d can be obtained (Rezaei Behbehani et al. 2008, 2012, 2013; Barzegar et al. 2011)

$$\frac{\Delta q}{q_{max}}M_0 = (\frac{\Delta q}{q})L_0\frac{1}{g} - \frac{K_d}{g} \quad (5)$$

where g is the number of binding sites, K_d is the dissociation equilibrium constant, M_0 and L_0 are concentrations of biomacromolecule and ligand, respectively, $\Delta q = q_{max} - q$, q represents the heat value in the certain metal ions concenteration and q_{max} represents the heat value upon the saturation of all HSA. If q and q_{max} are calculated per mole of biomacromolecule then the molar enthalpy of binding for

Table 1. Binding parameters for HSA+Fe^{3+} and HSA+Cu^{2+} interactions

parameters	HSA+Fe^{3+}	HSA+Cu^{2+}
p	1 ± 0.01	1 ± 0.01
δ_A^θ	−2.98 ± 0.14	59.06 ± 0.18
δ_B^θ	−6.73 ± 0.17	58.34 ± 0.19
K_a / M^{-1}	$(3.7 ± 0.032) \times 10^5$	$(3.7 ± 0.021) \times 10^5$
ΔH (kJ/mol)	−31.79 ± 0.07	−33.51 ± 0.09
ΔG (kJ/mol)	−31.97 ± 0.06	−30.05 ± 0.09
ΔS (kJ/mol·K)	0.0006 ± 0.00001	−0.01 ± 0.002

The negative values of δ_A^θ and δ_B^θ indicate that the ferric ion dampened antioxidant activity of HSA. Positive and large δ_A^θ and δ_B^θ values indicate that Cu^{2+} ion stabilizes the HSA structure significantly and increases the antioxidant properties of HSA. p, cooperativity index; δ_A^θ and δ_A^θ the structural changes of HAS indexes; ΔH, enthalpies of HSA+Fe^{3+} and HSA+Cu^{2+} and interactions; ΔG, free energies of HSA+Fe^{3+} and HSA+Cu^{2+} interactions; ΔS, entropies of the interactions.

each binding site (g) will be $\Delta H = q_{max}/g$. The related plot for the binding of Iron ions by HSA is shown in Figure 3.

To compare all thermodynamic parameters in metal binding process for HSA, the change in standard Gibbs free energy (ΔG) should be calculated according to the Eq. 6, which its value can use in Eq. 7 for calculating the change in standard entropy (ΔS) of the binding process.

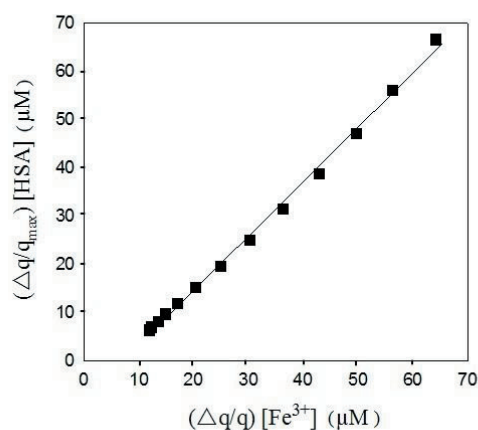$$\Delta G° = -RT \ln K_a \quad (6)$$

$$\Delta G° = \Delta H° - T\Delta S° \quad (7)$$

Figure 3. A representation of approaching to the best linear plot of $(\frac{\Delta q}{q_{max}})[HSA]$ (■) against $(\frac{\Delta q}{q})[Fe^{3+}]$, using $q_{max} = -5100$ μJ at T = 300 K for HSA+Fe^{3+} interaction. [HSA], the concentration of human serum albumin.

where K_a is the association binding constant (the inverse of the dissociation binding constant, K_d). The thermodynamic parameters were listed in Table 1.

Conclusion

The solvation parameters obtained from the extended solvation model (Eq. 1) model were attributed to the structural change of HSA. The more stable HSA can trap the free radical and decrease the oxidative stress. In the other hand, the stability of HSA is predicted simply using Eq. 1, therby, $\delta_A{}^\theta$ and $\delta_B{}^\theta$ values can be correlated to the antioxidant properties of HSA. $\delta_A{}^\theta$ and $\delta_B{}^\theta$ values for Fe^{3+}+HSA interactions is negative, indicating that in the low and high concentrations of the feric ions, the HSA structure is destabilized, which dampened the antioxidant property of HSA. The large and positive $\delta_A{}^\theta$ and $\delta_B{}^\theta$ values show that the high stable HSA+Cu^{2+} complex has a strong tendency against the free radicals formation, resulting in a great increase of antioxidant property of HSA as a result of its interation with cupric ions.

References

Atukeren P., Aydin S., Uslu E., Gumustas M. K., Cakatay U. (2010): Redox homeostasis of albumin in relation to alpha-lipoic acid and dihydrolipoic acid. Oxid. Med. Cell Longev. **3,** 206–213
http://dx.doi.org/10.4161/oxim.3.3.11786

Barzegar L., Rezaei Behbehani G., Saboury A. A. (2011): A thermodynamic study of zinc ion interaction with bovin carbinic anhydrase II at different temperatures. J. Solution Chem. **40,** 843–848
http://dx.doi.org/10.1007/s10953-011-9686-2

Bourdon E., Loreau N., Lagrost L., Blache D. (2005): Differential effects of cysteine and methionine residues in the antioxidant activity of human serum albumin. Free Radic. Res. **39,** 15–20
http://dx.doi.org/10.1080/10715760400024935

Colombo G., Clerici M., Giustarini D., Rossi R., Milzani A., Dalle-Donne I. (2012): Redox albuminomics: oxidized albumin in human diseases. Antioxid. Redox. Signal. **17,** 1515–1527
http://dx.doi.org/10.1089/ars.2012.4702

Izzet T., Osman K., Ethem U., Nihat Y., Ramazan K., Mustafa D., Hafize U., Riza K. A., Birsen A., Habibe G., Seval A., Gonul S. (2005): Oxidative stress in portal hypertension-induced rats with particular emphasis on nitric oxide and trace metals. World J. Gastroenterol. **11,** 3570–3573

Kato R., Akiyama M., Kawakami H, Komatsu T. (2014): Superoxide dismutase activity of the naturally occurring human serum albumin–copper complex without hydroxyl radical formation. Chem. Asian J. **9,** 83–86
http://dx.doi.org/10.1002/asia.201301285

Otagiri M., Chuang V. T. (2009): Pharmaceutically important pre- and posttranslational modifications on human serum albumin. Biol. Pharm. Bull. **32,** 527–534
http://dx.doi.org/10.1248/bpb.32.527

Papatheodorou L., Weiss N. (2007): Vascular oxidant stress and inflammation in hyperhomocysteinemia. Antioxid. Redox. Signal. **9,** 1941–1958
http://dx.doi.org/10.1089/ars.2007.1750

Parola M., Robino G. (2001): Oxidative stress-related molecules and liver fibrosis. J. Hepatol. **35,** 297–306
http://dx.doi.org/10.1016/S0168-8278(01)00142-8

Rezaei Behbehania G., Saboury A.A., Taleshi E. (2008): Determination of partial unfolding enthalpy for lysozyme upon interaction with dodecyltrimethylammonium bromide using an extended solvation model. J. Mol. Recognit. **21,** 132–135
http://dx.doi.org/10.1002/jmr.881

Rezaei Behbehani G., Mirzaie M., Mehreshtiagh M., Barzegar L., Saboury A. A., Mohammadi Gorgi S. (2012): Inhibitory effects of p-phenylene-bis and phenyl dithiocarbamate on mushroom tyrosinase J. Solution Chem. **41,** 581–588
http://dx.doi.org/10.1007/s10953-012-9821-8

Rezaei Behbehani G., Mehreshtiagh M., Barzegar L., Saboury A. A. (2013): A calorimetric investigation for the bindings of mushroom tyrosinase to p-phenylene-bis dithiocarbamate and xanthates. J. Serb. Chem. Soc. **78,** 255–263
http://dx.do^õ/_õ/aõ/bõ/&ö/

Sitar M. E., Aydin S., Cakatay U. (2013): Human serum albumin and its relation with oxidative stress. Lab. **59,** 945–952

Young I. S., Woodside J. V. (2001): Antioxidants in health and disease. J. Clin. Pathol. **54,** 176–186
http://dx.doi.org/10.1136/jcp.54.3.176