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

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Abstract. The interaction of both ferric (Fe\textsuperscript{3+}) and cupric (Cu\textsuperscript{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 are $1.7 \times 10^5$ M\textsuperscript{-1} and $-31.37$ kJ·M\textsuperscript{-1}, respectively. To obtain the binding parameters of metal ion-protein interaction over the whole range of Fe\textsuperscript{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.

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\textsubscript{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 voltman 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 Fe³⁺ solution was measured as described above except HSA was excluded.

Results and Discussion

The calculated heats for HSA interactions with cupric 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):

\[
q = q_{\text{max}}x_B^p - \delta_A^p(x'_A + x_B^pL_A) - (\delta_B^p - \delta_A^p)(x'_A + x_B^pL_A)\]

(1)

where q are the heats of Fe³⁺+HSA interactions and \(q_{\text{max}}\) represents the heat value upon the saturation of all HSA. The parameters \(\delta_A^0\) and \(\delta_B^0\) 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. \(x_B^p\) can be expressed as follows:

\[
x_B^p = \frac{px_B}{x_A + px_B}
\]

(2)

We can express \(x_B\) fractions, as the total Fe³⁺ concentrations divided by the maximum concentration of the Fe³⁺ upon the saturation of all HSA as follows:

\[
x_B = \frac{[Fe^{3+}]}{[Fe^{3+}]_{\text{max}}}, \quad x_A = 1 - x_B
\]

(3)

where \([Fe^{3+}]\) is the concentration of Fe³⁺ ions after every injection and \([Fe^{3+}]_{\text{max}}\) is the maximum concentration of the Fe³⁺ 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 Fe³⁺ ions in buffers, \(q_{\text{dilut}}\), as follows:

\[
L_A = q_{\text{dilut}} + x_B\left(\frac{\partial q_{\text{dilut}}}{\partial x_A}\right), \quad L_B = q_{\text{dilut}} + x_A\left(\frac{\partial q_{\text{dilut}}}{\partial x_B}\right)
\]

(4)

The heats of Fe³⁺+HSA interactions, \(q_{\text{dilut}}\), were fitted to Eq. 1 across the whole Fe³⁺ 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 \(\delta_A^0\) and \(\delta_B^0\) 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+Cu²⁺ interactions and the calculated data (lines) using Eq. 1. [Cu²⁺], the concentrations of [Cu(NO₃)₂] solution (the agreement between experimental and calculated data support the extended solvation model); \(q_{\text{dilut}}\) the heat of HSA+Cu²⁺ interaction in every concentration of Cu²⁺ ion; [HSA], the concentration of human serum albumin.

Figure 2. Comparison between the experimental heats (●) at 300 K, for Fe³⁺+HSA interactions and the calculated data (lines) using Eq. 1. [Fe³⁺], the concentrations of [FeCl₃] solution (the agreement between experimental and calculated data support the validity of Eq. 1); \(q_{\text{dilut}}\) the heat of HSA+Fe³⁺ interaction in every concentration of Fe³⁺ ion; [HSA], the concentration of human serum albumin.
values (0.999999) support the method. The binding parameters for Fe(III)+HSA and Cu(II)+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. \( \delta_A^\theta \) and \( \delta_B^\theta \) values for Fe(III)+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 \( \delta_A^\theta \) and \( \delta_B^\theta \) values to the antioxidant properties of HSA. Cuperic ions in solution interact strongly with human serum albumin as indicated by the large and positive \( \delta_A^\theta \) and \( \delta_B^\theta \) values (Table 1) and stabilized the HSA structure significantly. In other words, the stabilities indexes (\( \delta_A^\theta \) and \( \delta_B^\theta \) values), calculated from Eq. 1 for HSA+Cu(II) interactions is considerably positive, indicating that in the low and high concentrations of the Cu(II) ions, the HSA structure is stabilized drastically. The \( \delta_A^\theta \) and \( \delta_B^\theta \) values for HSA+Fe(III) interactions are negative (unstable HSA+Fe(III) complex), showing that the trace amounts of ferric ion can decrease the antioxidant property of HSA and increase oxidative stress. HSA+Cu(II) complex is much more stable than HSA+Fe(III) complex, resulting in a great increase of antioxidant activity of HSA as a result of its interaction with cupric ions. The large and positive \( \delta_A^\theta \) and \( \delta_B^\theta \) values show that the high stable HSA+Cu(II) complex has a strong tendency against the free radicals and prevent a subsequent Fenton reaction to produce the most harmful of hydroxyl radical (\( ^* \)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 \( \frac{\Delta q}{q_{\text{max}}} \) against \( \frac{1}{L_0} \) should be a linear plot by a slope of \( \frac{1}{g} \) 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)

\[
\frac{\Delta q}{q_{\text{max}}} M_o = \left( \frac{\Delta q}{q} \right) L_0 \frac{1}{g} - \frac{K_d}{g}
\]

(5)

where g is the number of binding sites, K_d is the dissociation equilibrium constant, M_o and L_0 are concentrations of biomacromolecule and ligand, respectively, \( \Delta q = q_{\text{max}} - q \), q represents the heat value in the certain metal ions concentration and q_{\text{max}} represents the heat value upon the saturation of all HSA. If q and q_{\text{max}} are calculated per mole of biomacromolecule then the molar enthalpy of binding for each binding site (g) will be \( \Delta H = q_{\text{max}}/g \). 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 (\( \Delta G \)) should be calculated according to the Eq. 6, which its value can use in Eq. 7 for calculating the change in standard entropy (\( \Delta S \)) of the binding process.

\[
\Delta G^* = -RT \ln K_d
\]

(6)

\[
\Delta G^* = \Delta H^* - T \Delta S^*
\]

(7)

![Figure 3](image)

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

### Table 1. Binding parameters for HSA+Fe(III) and HSA+Cu(II) interactions

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HSA+Fe(III)</th>
<th>HSA+Cu(II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \rho )</td>
<td>1 ± 0.01</td>
<td>1 ± 0.01</td>
</tr>
<tr>
<td>( \delta_A^\theta )</td>
<td>-2.98 ± 0.14</td>
<td>59.06 ± 0.18</td>
</tr>
<tr>
<td>( \delta_B^\theta )</td>
<td>-6.73 ± 0.17</td>
<td>58.34 ± 0.19</td>
</tr>
<tr>
<td>( K_d/M^{-1} )</td>
<td>(3.7 ± 0.032) \times 10^5</td>
<td>(3.7 ± 0.021) \times 10^5</td>
</tr>
<tr>
<td>( \Delta H (kJ/mol) )</td>
<td>-31.79 ± 0.07</td>
<td>-33.51 ± 0.09</td>
</tr>
<tr>
<td>( \Delta G (kJ/mol) )</td>
<td>-31.97 ± 0.06</td>
<td>-30.05 ± 0.09</td>
</tr>
<tr>
<td>( \Delta S (kJ/mol-K) )</td>
<td>0.0006 ± 0.00001</td>
<td>-0.01 ± 0.002</td>
</tr>
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</table>

The negative values of \( \delta_A^\theta \) and \( \delta_B^\theta \) indicate that the ferric ion dampened antioxidant activity of HSA. Positive and large \( \delta_A^\theta \) and \( \delta_B^\theta \) values indicate that Cu(II) ion stabilizes the HSA structure significantly and increases the antioxidant properties of HSA. \( \rho \), cooperativity index; \( \delta_A^\theta \) and \( \delta_B^\theta \) the structural changes of HAS indexes; \( \Delta H \), enthalpies of HSA+Fe(III) and HSA+Cu(II) and interactions; \( \Delta G \), free energies of HSA+Fe(III) and HSA+Cu(II) interactions; \( \Delta S \), entropies of the interactions.
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, $\delta_A^a$ and $\delta_B^a$ values can be correlated to the antioxidant properties of HSA. $\delta_A^a$ and $\delta_B^a$ values for $\text{Fe}^{3+}$+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 $\delta_A^a$ and $\delta_B^a$ values show that the high stable HSA+$\text{Cu}^{2+}$ 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**


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