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A kinetic study on the distribution of Cu(II)-ions between albumin and transferrin

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Abstract

Serum albumin (human, bovine) has a specific Cu(II)-ion binding site, and is proposed to act as a copper transport protein in blood plasma. Human transferrin, normally about 30% saturated with iron *in vivo*, has two sites/molecule capable of complexing Cu(II); one more strongly than the other (Hirose et al. 1996). The present study shows that this binding site has a slightly stronger affinity for Cu(II) than that on the albumins. However, both human- and bovine albumin could take up part of the transferrin bound Cu(II), the second order rate constant for the reaction estimated to 12 mM^{-1} min^{-1} for both species. *In vivo* the albumin concentration is considerably higher than that of iron-free transferrin, and it seems unlikely that the latter can compete with albumin for non-ceruloplasmin cupric ions.

Introduction

Human- and bovine serum albumins (HSA, BSA) contain a high-affinity site for Cu(II), the binding involving both histidyl- and carboxyl groups (Breslow 1964; Lau & Sarkar 1971; Zgirski & Frieden 1990, Masuoka *et al*. 1993). Experiments strongly suggest that part of the non-ceruloplasmin copper fraction in blood plasma is bound to albumin, and that the protein functions as a copper transport protein *in vivo* (Owen 1965; Marceau & Aspin 1973; Wirth & Linder 1985; Gordon *et al.* 1987). In Wilson's disease patients the non-ceruloplasmin copper fraction may be markedly increased. At physiological pH log K-values between 12 and 13 were reported for the binding of the first copper ion to BSA (Giroux & Schoun 1981, Syvertsen *et al.* 1986, Zgirski & Frieden 1990). Later intrinsic log-K values of 11.12 and 11.18 were estimated for Cu(II)-BSA and Cu(II)-HSA, respectively (Masuoka *et al.* 1993).

The serum iron transport protein, transferrin (Tf), is bilobal with two binding sites for Fe(III), one in the N-terminal lobe, the other in the C-terminal lobe. Since human serum Tf in blood plasma is only about 30% saturated with iron, there is potential capacity for binding to other metal ions, like Cu(II). One of its metal binding sites binds Cu(II) more strongly than the other, the Cu(II)-Tf complex characterized by a log-K value of 12.3 (Hirose *et al*. 1996). In view of the fact that albumin and transferrin share the same environment in human blood plasma, it was of interest to study the distribution of Cu(II) ions between them.

Materials and methods

HSA and BSA (≥99% pure, crystallized and essentially free of globulins), human Tf (ironfree), Tris buffer, bathocuproine disulfonate and ascorbic acid were purchased from Sigma (St. Louis, Missouri, USA), $CuSO_4$ from Riedel-de Häen AG (Darmstadt, Germany), and $NaHCO_3$ from Matheson, Coleman & Bell (Cinncinati, Ohio, USA). Protein solutions were dialysed against 10 mM Tris buffer, pH 7.4, prior to use, in order to remove traces of small molecules. Tf concentration was calculated from the millimolar absorption, 93.0 mM^{-1} cm^{-1}, at 280 nm (Chasteen *et al*. 1977).

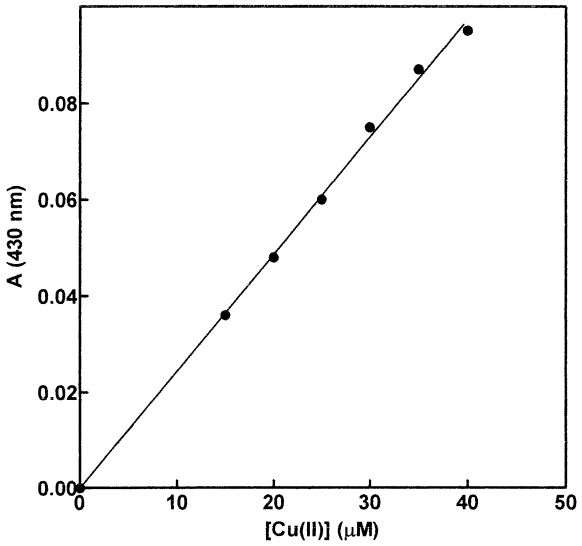

Fig. 1. The 430 nm absorbance of Cu(II)-Tf as a function of Cu(II) concentration. The solution contained Cu(II) (15–40 μM), 10 mM sodium bicarbonate and 70 μM transferrin in 20 mM Tris buffer, pH 7.4 (T = 25 °C).

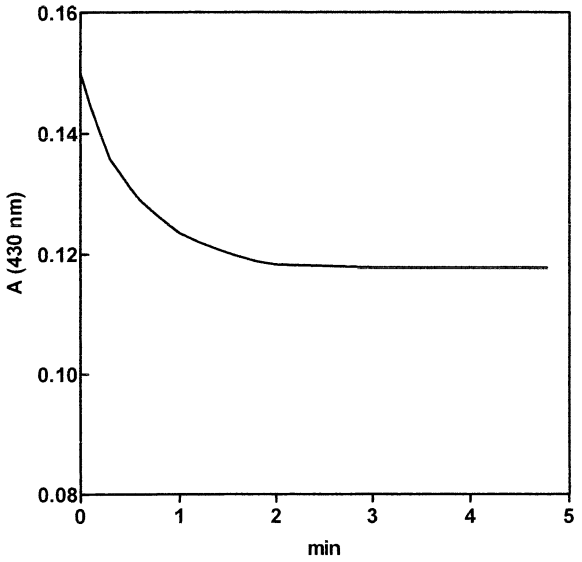

Fig. 2. The change in the 430 nm absorbance of Cu(II)-Tf after addition of 30 μM HSA to 70 μM Cu(II)-Tf (total Tf concentration = 80 μM) in 20 mM Tris buffer, pH 7.4, containing 10 mM sodium bicarbonate (T = 25 °C).

A Cecil 292 instrument, connected to a Radiometer REC 80 recorder, was used for spectrophotometric measurements. Equilibrium dialysis was performed with a Kontron Diapack 4000 apparatus.

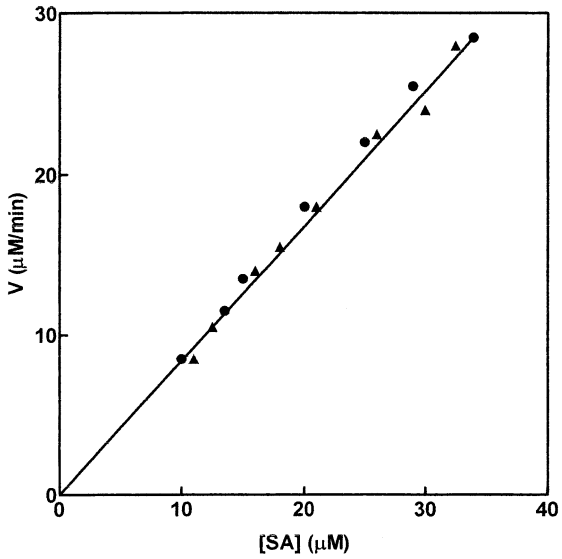

Fig. 3. Initial rate of Cu(II) transfer from Tf to HSA (•) and BSA (▲) as a function of albumin concentration. The reaction solutions contained 70 μM Cu(II)-Tf initially (total Tf concentration = 80 μM), 10 mM sodium bicarbonate and albumin (10–34 μM) in 20 mM Tris buffer, pH 7.4 (T = 25 °C).

Table 1. Distribution of Cu(II)-ions between albumin and transferrin after dialysis.

Compound	Amount of Cu(II) bound* (%)
HSA	60 ± 1.8
BSA	62 ± 1.8

*Mean ± SE (n = 5)
Each cell contained 30 μM Cu(II)-protein initially (total protein concentration = 80 μM) and 10 mM sodium bicarbonate in 20 mM Tris buffer, pH 7.4. (T = 22 °C). Total volume/cell was 0.8 ml. After dialysis copper was determined as Cu(I)-bathocuproine at 480 nm (ϵ = 12.3 mM^{-1} cm^{-1}) by adding 0.1 ml bathocuproine disulfonate (5 mM), 0.1 ml sodium acetate buffer, pH 6.0 and and 0.1 ml ascorbic acid (40 mM, as a reducing agent), to 0.7 ml of the Cu(II)-protein solution.

Results and discussion

The visible absorption spectrum of the Cu(II)-Tf complex is characterized by a broad band around 430 nm (Hirose *et al.* 1996). A millimolar extinction coefficient (ϵ) of 2.4 mM^{-1} cm^{-1} for the complex between Cu(II) and the first binding site on Tf was calculated from Figure 1, which shows the absorption at 430 nm as a function of Cu(II) concentration, keeping [Cu(II)] < [Tf].

Figure 2 shows that addition of 30 μM HSA to 70 μM of the Cu(II)-Tf complex resulted in a rapid

Table 2. Equilibrium constant logarithms.

HSA added (μM)	Cu(II)-HSA formed (μM)	log K*	BSA added (μM)	Cu(II)-BSA formed (μM)	log K**
13.5	8	0.41	10	5.5	0.36
38	16	0.35	21	12	0.48
42	18	0.40	26	13	0.40
48	20	0.43	42	19	0.47

*Mean ($\pm$ SEM) $= 0.40 \pm 0.02$.
**Mean ($\pm$ SEM) $= 0.43 \pm 0.03$.
Various amounts of albumins were mixed with a solution of 70 μM Cu(II)-Tf (total Tf concentration $= 80$ μM) and 10 mM sodium bicarbonate in 20 mM Tris buffer, pH 7.4 (T $= 25$ °C).

decrease in the 430 nm absorption. After approx 2 min a steady-state level was reached. An identical curve was obtained when BSA was used instead of HSA. The results suggest that both HSA and BSA are able to take up a fraction of the Tf bound Cu(II)-ions.

Equilibrium dialysis experiments confirm this suggestion. When Cu(II)-HSA, or Cu(II)-BSA, was dialyzed against an initially equimolar concentration of Cu(II)-Tf at physiological pH, some of the Cu(II) was transferred across the membrane from Tf to HSA (BSA) (Table 1).

The rate, V, of Cu(II)-ion transfer from Tf to HSA and BSA,

$$\text{Cu(II)} - \text{Tf} + \text{SA} \xrightarrow{\text{k}} \text{Cu(II)} - \text{SA} + \text{Tf} \qquad (1)$$

could be calculated from the initial part of the curve shown in Figure 1. Figure 3 shows that there is a linear correlation between V and the concentration of albumin, and that the reaction rate was the same for both albumins investigated. From the plot the second order rate constant, k = V/([Cu(II) − Tf][SA]), was estimated to 12 mM^{-1} min^{-1}.

By measuring the optical absorption of Cu(II)-Tf at 430 nm at equilibrium, after addition of various amounts of HSA, or BSA, it was possible to estimate the concentration of Cu(II)-Tf and Cu(II)-SA. The equilibrium constants were calculated from the equation,

$$\text{K} = \frac{[\text{Cu(II)} - \text{SA}][\text{Tf}]}{[\text{Cu(II)} - \text{Tf}][\text{SA}]} \qquad (2)$$

and listed in Table 2.

The data suggest that the specific binding site on HSA and BSA has approximately the same affinity for Cu(II), in accordance with the report of Masuoka *et al.* (1993). The rate constant, k′, characterizing the reaction between Cu(II)-HSA and Tf, was calculated from the equation, k′ = k/K, to be 30 mM^{-1} min^{-1}.

The present study suggests that the first Cu(II) binding site on transferrin has a slightly better affinity for Cu(II) than HSA and BSA at physiological pH. In human blood plasma, however, the albumin concentration is considerably higher than that of metal free transferrin, and it seems unlikely that the latter would compete favourably with albumin for non-ceruloplasmin bound copper ions.

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