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# Thermodynamic Studies of Copper(II)-Transport Site of Human Serum Albumin

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Human serum albumin specifically binds one Cu(II), and Cu(II) bound in this form is considered to be the transport form of Cu(II) in blood [1, 2]. The binding of Cu(II) involves the $NH_2$-terminal segment Asp-Ala-His- of human albumin. It is suggested that Cu(II) is ligated to terminal amino nitrogen, two intervening peptide nitrogens and the imidazole nitrogen of the histidine residue [3–5]. Recently, detailed studies have been reported on the Cu(II)-binding to the synthetic native sequence peptide, L-Asp-L-Ala-L-His-$NHCH_3$ (AAHNMA) [6, 7]. Furthermore, Cu(II)-binding studies have also been carried out with GlyGly-L-His$NHCH_3$ (GGHNMA) which is the simplification of the native Cu(II)-transport site of human albumin [8–10]. These studies have contributed significantly to our understanding of the structure and function of the transport site. However, the thermodynamic properties of this important site have never been investigated. Since such studies with an intact protein are difficult, we carried out an investigation with AAHNMA and GGHNMA to obtain the thermodynamic functions of the Cu(II)-transport site.

## Experimental

The peptides GGHNMA and AAHNMA were synthesized as previously reported [6, 8]. All solutions were prepared with twice distilled water. Acid washed glassware and reagent grade chemicals were used throughout the work. Copper(II) perchlorate solutions were prepared by neutralizing Cu(II) carbonate with perchloric acid. The ionic strength of all solutions was adjusted to 0.15 *M* by the addition of NaCl. The procedure followed to make up and to standardize all the above solutions was previously described [11].

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The calorimetric experiments were carried out at 25 ± 0.001 °C using a LKB 8700 precision calorimeter and a 8726-1 (100 ml) titration vessel. The change in the thermistor resistance during the titration was recorded by means of a Leeds and Northrup recorder (Speedomax W). The reproducibility and other details concerning the experimental system have already been reported [12]. In order to avoid systematic errors, the measurements were performed using NaOH or HCl in turn as titrants. To determine the protonation and the complexation enthalpies, at least seven calorimetric titrations were carried out for each system. The titration vessel was usually left in the thermostated bath (LKB Model 7603 A) overnight to allow the solution to be tested to reach a steady thermal equilibrium. In order to make sure that the ligand had not undergone decomposition, all solutions were tested by thin-layer chromatography. Chromatography showed no sign of a ligand decomposition. The enthalpy change values were calculated by means of the computer program DOEC [13] which minimizes the error squares sum,

$$U = \sum_i (Q_{corr, calc, i} - Q_{corr, exp, i})^2$$

The values of $\Delta H^\circ$ and $\Delta S^\circ$ are precise to ± 0.3 kcal/mol and ± 1 cal/(mol deg), respectively.

## Results and Discussion

The thermodynamic functions for the protonation of the two peptides are shown in Table I. The amino nitrogen of GGHNMA is less basic than the analogous glycine-$NH_2$ [14]. This is mainly due to the different thermodynamic contribution accompanying the protonation process, provided due allowance is made for the experimental conditions used. Such a difference can arise from the variations in the internal freedom of the two molecules. Although these differences play a very minor role in affecting the protonation $\Delta S^\circ$ in small molecules such as this peptide, nevertheless these differences have been shown earlier to play an important role [15, 16]. Also, the amino nitrogen of AAHNMA shows a lesser basicity in comparison to the same of aspartic acid [17]. In this case too, this is almost entirely due to the smaller entropic contribution caused by the larger 'stiffening' following the formation of the protonated species of the peptide compared to the amino acid. The imidazole nitrogen (pyridine type) of the two peptides has a basicity comparable to that of the corresponding histidine nitrogen. Also, there are no significant differences in $\Delta H^\circ$ and $\Delta S^\circ$ values [18]. This behaviour is con-

TABLE I. Thermodynamic Functions for Proton Association of Peptides and Related Ligands (L = GGHNMA; L' = AAHNMA).

| Reaction | Site | log K | ΔG° (kcal/mol) | ΔH° (kcal/mol) | ΔS° (cal/(mol deg)) | References |
|---|---|---|---|---|---|---|
| $L + H \rightleftarrows LH$ | $NH_2$ | 8.00 | −10.9 | −9.4 | 5.0 | This work |
| $LH + H \rightleftarrows LH_2$ | N | 6.47 | −8.8 | −6.4 | 8.0 | This work |
| $L' + H \rightleftarrows L'H$ | $NH_2$ | 7.73 | −10.5 | −8.8 | 5.8 | This work |
| $L'H + H \rightleftarrows L'H_2$ | N | 6.56 | −9.0 | −6.6 | 8.0 | This work |
| $L'H_2 + H \rightleftarrows L'H_3$ | O | 2.98 | −3.87 | −0.4 | 12.4 | This work |
| $Gly + H \rightleftarrows GlyH$ | $NH_2$ | 9.60 | −13.0 | −10.2 | 9.5 | 14 |
| $Asp + H \rightleftarrows AspH$ | $NH_2$ | 9.70 | −13.1 | −9.4 | 12.0 | 17 |
| $His + H \rightleftarrows HisH$ | $NH_2$ | 9.12 | −12.4 | −10.4 | 6.0 | 18 |
| $HisH + H \rightleftarrows HisH_2$ | N | 6.16 | −8.3 | −7.0 | 5.0 | 18 |

TABLE II. Thermodynamic Functions for the Formation of Copper(II) Complexes (L = GGHNMA; L' = AAHNMA; A = Butanoic-3-amino acid; A' = Aspartic acid).

| Reaction | log β | ΔG° (kcal/mol) | ΔH° (kcal/mol) | ΔS° (cal/mol deg)) | References |
|---|---|---|---|---|---|
| $Cu + L' \rightleftarrows [CuL']$ | 8.39 | −11.41 | −8.85 | 8.70 | This work |
| $Cu + L \rightleftarrows [CuH_{-2}L] + 2H$ | −0.48 | 0.65 | −1.25 | −6.2 | This work |
| $Cu + L' \rightleftarrows [CuH_{-2}L'] + 2H$ | −0.55 | 0.75 | −1.18 | −6.7 | This work |
| $Cu + A \rightleftarrows [CuA]$ | 7.12 | −9.68 | −4.94 | 16.0 | 22 |
| $Cu + A' \rightleftarrows [CuA']$ | 8.80 | −11.9 | −6.10 | 21.0 | 17 |

sistent with the above explanation about the protonation of the amino nitrogen. In fact, in the protonation reaction of the imidazole group in which the nitrogen forms a part of a rigid ring, it has no significant influence on the rest of the molecule.

As previously reported [6, 8], Cu(II) forms only one major species $[CuH_{-2}L]$ with GGHNMA in aqueous solution whereas with AAHNMA it forms three complex species, $[CuL']$, $[CuH_{-2}L']$ and $[CuH_{-1}L'_2]$, in the pH range 4–10. We have determined the formation enthalpies of all the aforesaid species (Table II) with the exception of that concerning $[CuH_{-1}L'_2]$, the degree of formation of which was not high.

The results obtained for the species $[CuL']$ are of considerable importance since this is the species which is formed prior to the formation of the main physiological species $[CuH_{-2}L']$. This species was detected with human albumin and Cu(II) at pH 5.5–6.5 having a characteristic visible spectrum with a $\lambda_{max}$ = 730 nm [19, 20]. The same species was also detected in the Cu(II)–AAHNMA system [6]. But little could be discerned about this species from the earlier studies. Like many Cu(II)–amino acid complexes [21], this species is enthalpically and entropically stable. The complex has high ΔG° and ΔH° values which are not consistent with a β-alanyl-like coordination. The literature reports for six membered chelate rings of Cu(II) show ΔG° and ΔH° values fairly low [22]. Formation of a macrochelate ring involving the imidazole nitrogen of the histidine residue, the carboxyl group of the aspartyl side chain and the amino nitrogen is most unlikely. Such a species should be entropically unstable. On the basis of all the available data, it is likely that in $[CuL']$, the Cu(II) is chelated to the nitrogen of the amino group, the β-carboxyl side chain and the adjacent peptide carbonyl oxygen. This array of ligand binding to Cu(II) is consistent with a $\lambda_{max}$ of 730 nm. This hypothesis is further strengthened by the ΔG° values (see Table II) relative to the Cu(II)-binding to aspartic acid equilibrium [17]. The lower ΔH° and the higher ΔS° value of the Cu(II)–amino acid species with respect to $[CuL']$ can be ascribed to the different extent of interaction with the solvent. Since the carboxylic oxygen is able to neutralize the metal ion charge to a greater extent in comparison with the peptide carbonyl oxygen, the complexation of the aspartic acid to the Cu(II) will result in a desolvation of the metal ion larger than that following the complexation of the peptide. Thus Cu(II)–Asp will have a ΔS° value higher than that of $[CuL']$ and, at the same time, a lower ΔH° value. This is due to the larger number of metal ion–water bonds to be weakened or broken during its formation. Similar trends have also been observed for other simple and mixed complexes of Cu(II) [23, 24].

The formation of the two physiologically important species $[CuH_{-2}L]$ and $[CuH_{-2}L']$ in both peptide systems is exothermic in spite of the endother-

mic effect due to the ionization of the peptide nitrogens. The structures of these species have already been reported [6, 8, 9]. Our thermodynamic parameters are consistent with these structures. However, the results can neither prove nor disprove the replacement of the water molecules by the carboxyl group in the case of $[CuH_{-2}L']$ complex. In fact, owing to the weak interaction of the water molecule with the metal ion in $CuH_{-2}L'$ (the distance obtained by X-ray studies [9] is relatively great), its replacement by a carboxyl oxygen would not significantly affect the thermodynamic parameter. Work is currently underway to determine the position of the carboxyl group in the complex with the native sequence peptide of human albumin.

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## References

1 P. Z. Neumann and A. Sass-Kortsak, *J. Clin. Invest., 46,* 646 (1967).
2 B. Sarkar and Y. Wigfield, *Can. J. Biochem., 46,* 601 (1968).
3 T. Peters, Jr. and F. A. Blumenstock, *J. Biol. Chem., 242,* 1574 (1967).
4 W. T. Shearer, R. A. Bradshaw, F. R. N. Gurd and T. Peters, Jr., *J. Biol. Chem., 242,* 5451 (1967).
5 R. A. Bradshaw and T. Peters, *J. Biol. Chem., 244,* 5582 (1969).
6 K. S. N. Iyer, S. Lau, S. H. Laurie and B. Sarkar, *Biochem. J., 169,* 61 (1978).
7 B. Sarkar, H. B. F. Dixon and D. Webster, *Biochem. J., 173,* 895 (1978).
8 T. P. A. Kruck, S. Lau and B. Sarkar, *Can. J. Chem., 54,* 1300 (1976).
9 N. Camerman, A. Camerman and B. Sarkar, *Can. J. Chem., 54,* 1309 (1976).
10 B. Sarkar in 'Metal–Ligand Interaction in Organic Chemistry and Biochemistry', Part 1, B. Pullman and N. Goldblum, Eds., D. Reidel, Dordrecht–Holland.
11 G. Arena, R. Cali, E. Rizzarelli, S. Sammartano, R. Barbucci and M. J. Campbell, *J. Chem. Soc. Dalton,* 581 (1977).
12 G. Arena, R. Cali, E. Rizzarelli and S. Sammartano, *Thermochim. Acta, 16,* 315 (1976); *17,* 155 (1976).
13 C. Rigano, E. Rizzarelli and S. Sammartano, *Thermochim. Acta,* in press (1979).
14 T. P. I. and G. H. Nancollas, *Inorg. Chem., 11,* 2414 (1972).
15 A. P. Brunetti, M. C. Lim and G. H. Nancollas, *J. Am. Chem. Soc., 90,* 5120 (1968).
16 R. Cali, E. Rizzarelli, S. Sammartano and G. Siracusa, *Transition Met. Chem.,* in press (1979).
17 I. Nagypal, A. Gergely and E. Farkas, *J. Inorg. Nucl. Chem., 36,* 699 (1974).
18 J. L. Meyer and J. E. Bauman, *J. Am. Chem. Soc., 92,* 4210 (1970).
19 D. W. Appleton and B. Sarkar, *J. Biol. Chem., 246,* 5040 (1971).
20 S. Lau and B. Sarkar, *J. Biol. Chem., 246,* 5938 (1971).
21 J. J. Christensen, D. J. Eatough and R. M. Izatt, 'Handbook of Metal Ligand Heats', Marcel Dekker, New York (1975).
22 J. E. Letter and J. E. Bauman, *J. Am. Chem. Soc., 92,* 437 (1970).
23 G. Arena, R. Cali, E. Rizzarelli and S. Sammartano, *Transition Met. Chem., 3,* 147 (1978).
24 G. Arena, R. Cali, E. Rizzarelli, S. Sammartano, R. Barbucci and M. J. M. Campbell, *J. Chem. Soc. Dalton,* 1090 (1978).