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Properties of the copper(II)-histidine complex obtained after dialysis of human plasma with histidine

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The copper(II)-histidine complex obtained following dialysis of human plasma with histidine was investigated using various types of extraction systems and ion exchange resins. According to the results obtained, the Cu(II)-histidine complex formed under the dialysis conditions has one positive charge. Preconcentration of copper from the dialysis solution was achieved by its extraction as a dithiocarbamate complex and by sorption on cation-exchange resin or onto chelate sorbent.

Keywords: copper-histidine, human plasma dialysis, extraction, ion-exchange

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Complexes of transition metals with amino acids in proteins and peptides are utilized in numerous biological processes, such as oxygen transport, electron transfer and oxidation. In these processes, the enzymatic active site, which is very specific, often includes several histidine residues, which form complexes with divalent metal ions.

Histidine is involved in the coordination of copper ions in several copper proteins, including superoxide dismutase, ceruloplasmin, ascorbate oxidase, *etc*. In addition, histidine is thought to be involved in the transport of copper into cells *in vivo*, from albumin. Copper(II)-histidine complexes have been thoroughly investigated (1–3) and the structure and charge of the complexes have been defined (4–7). A notable review of the copper(II)-histidine complex stability constants and charge can be found (8). According to these concepts, the most probable structure of the complex is $Cu(His)_2$ (Fig. 1).

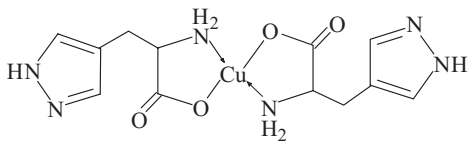

Fig. 1. Structure of the copper(II)-histidine complex.

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It is known that L-histidine can remove copper from albumin. Based on the difference in the stability constants of both Cu-albumin and Cu-histidine complexes (9), a method to extract Cu^{2+} from blood plasma based on dialysis in the presence of histidine has been devised (10–12). In our previous studies we have demonstrated the validity of this method for the determination of exchangeable copper in human plasma (10–13). However, some properties and experimental observations of the Cu(II)-histidine complex formed under dialysis conditions could not be explained. We proposed that the copper(II)-histidine complex in the dialysis solution might not be in the form of the much described $Cu(His)_2$ form, as expected, but rather as a more complex, non-stoichometric substance. Besides, the formed complex may not be neutral, but charged.

The aim of the present work was to study the properties of the copper(II)-histidine complex formed during dialysis of human plasma with histidine. For this purpose, various extraction and sorption systems were applied and evaluated.

The research was conducted under a protocol approved by the Norwick (UK) Districts Ethics Committee and written consent of the volunteers was obtained. Six healthy male subjects aged 34–57 years (non-smokers, not taking medications or nutritional supplements) were included in the study. Their biological and hematological indices fell inside the normal range.

EXPERIMENTAL

Instrumentation

Atomic absorption spectrophotometry (AAS) measurements were carried out using a Pye Unicam 1950 atomic absorption spectrometer (UK) with an air/acetylene flame. The flow rates were 5 L min^{-1} for air and 1 L min^{-1} for acetylene. The light source used was a Unicam hollow cathode lamp for Cu and the measurements were performed at 324.7 nm. The spectral bandpass was 0.2 nm. pH values were determined with a Consort Model P800 pH meter (Belgium) using a combination electrode.

Reagents

All reagents and solvents were of analytical-reagent grade. Dowex 50-X8 (50–100 mesh, hydrogen form, Merck, Germany) and Chelex-100 (200–400 mesh, Na form, Bio-Rad, UK) resins were commercially available. Standard solutions for Cu in organic solvent were prepared from multi-element standard oil solution (*p.a.*, Merck) by dilution with *i*-butyl methyl ketone (IBMK). Aqueous standard solutions of Cu^{2+} were prepared by appropriate dilution of a stock solution (1 g L^{-1} Cu in 0.2% HNO_3, BDH (UK) standard solutions for AAS). For Ca and Mg analysis, BDH standard solutions for AAS (1 g L^{-1}) were also used. L-histidine was purchased from Sigma Scientific, UK. Beckman standard solution (120 mmol L^{-1} Na, 2 mmol L^{-1} K, Beckman Instruments, USA), ammonium pyrrolidinedithiocarbamate (APDC, *p.a.*, Merck, 1% aqueous solution), $NH_4H_2PO_4$ (*p.a.*, Merck), IBMK (*p.a.* Merck), sodium dodecylsulphonate (NaDDS, *p.a.*, Merck, 0.001 mol L^{-1} aqueous solution) and tetraoctylmethyl ammonium chloride (TOMACl, *p.a.*, Merck, 2% solution in IBMK) were used as received. Doubly distilled water was used throughout.

Dialysis procedure

Spectrapore dialysis tubing (10 mm flat with cellulose ester membrane, MWCO 5000, Spectrum Europe B.V., The Netherlands) was soaked in 50 mmol L^{-1} histidine solution, followed by at least 2 changes of water. To each of the clipped lengths of tubing, 5 mL of human plasma was added. After clipping the top of the tubes, the open end into which the sample was pipetted was rinsed to remove any residual sample. Using acid-cleaned 100 mL measuring cylinders, all the samples were dialyzed against 100 mL of 175 mmol L^{-1} ammonium phosphate buffer, pH 7.0, for 4 hours. All samples were then dialyzed against 2 × 100 mL of 175 mmol L^{-1} ammonium phosphate buffer, pH 7.0, containing 50 mmol L^{-1} of histidine, for 4 h and the dialysates were collected. The copper concentration in all acidified solutions was analyzed by AAS using standards prepared with 10% nitric acid.

Preparation of a model solution containing the Cu-histidine complex

In order to mimic the physiological levels of trace elements in human plasma, appropriate aliquots of 100 mg L^{-1} of Cu^{2+}, Ca^{2+} and Mg^{2+} were added to obtain final concentrations of copper, calcium and magnesium of 1 mg L^{-1}, 60 mg L^{-1} and 37 mg L^{-1}, respectively. Finally, $NH_4H_2PO_4$ and L-histidine were added to achieve final concentrations of 20 g L^{-1} and 7.8 g L^{-1}, respectively, in order to mimic the dialysis condition (12).

Solvent extraction

Thirty milliliters of the dialysate or of the model solution was transferred into a 30.0 mL extraction tube. The steps depended on the extraction system applied are shown in Table I.

After finishing the extraction, the organic phase was directly aspirated into the flame of the spectrometer and the copper absorption was recorded against organic standard solutions for Cu in IBMK.

Table I. Solvent extraction systems

Aqueous phase	Organic phase, volume (mL)	Contact time (min)
Cu(II)-histidine	IBMK, 2.0	5
Cu(II)-histidine + APDC (1.0 mL of 1%)	IBMK, 2.0	2
Cu(II)-histidine + NaDDS (0.01–0.05 mL of 0.001 mol L^{-1})	IBMK, 2.0	5
Cu(II)-histidine	2% TOMACl in IBMK, 2.0	5

Column solid phase extraction

Dowex 50-X8. – A column containing Dowex 50-X8 resin (0.5 g) was treated with 30 mL of 0.2 mol L^{-1} HNO_3 followed by distilled water to wash. The resin was conditioned by passing 10 mL of 0.4 mol L^{-1} HCl (flow rate 2 mL min^{-1}) followed by doubly distilled

water until the pH of the eluate reached 7.0. Then 100 mL of the model solution was passed through the column at a flow rate of 2 mL min^{-1}. Eluate fractions of 10.0 mL were collected and the concentration of Cu in these fractions was determined by flame AAS. Before elution, the resin was washed with 10 mL doubly distilled water. Copper was eluted from the resin using 4 mol L^{-1} HCl at a flow rate of 2 mL min^{-1}. The copper concentration was measured in 2.0 mL fractions of eluate.

Chelex-100. – A column containing 1 g Chelex-100 resin (styrene divinyl benzene copolymer with iminodiacetate functional groups) was washed with 20 mL 2.5 mol L^{-1} nitric acid and 30 mL of doubly distilled water. The resin was conditioned by passing 20 mL of 1 mol L^{-1} ammonium acetate (pH 5.5). Then 100 mL of model solution of Cu(II)-histidine was passed through the column at a flow rate of 2 mL min^{-1}. Eluate fractions of 10.0 mL were collected and Cu concentration was determined by flame AAS. The column was washed with 20 mL doubly distilled water to remove any remaining matrix components. Copper was eluted from the resin using 2.5 mol L^{-1} HNO_3 at a flow rate of 2 mL min^{-1}. The concentration of Cu was measured in each 2 mL of eluate.

RESULTS AND DISCUSSION

Investigation of the extraction properties of the copper(II)-histidine complex gives information about (*i*) the charge of the Cu(II)-histidine complex formed during the dialysis process, and (*ii*) the potential for preconcentration of copper from the dialysis solution.

In the case the investigated complex was neutral, a quantitative extraction of $Cu(His)_2$ into organic solvent as IBMK was expected. The results presented in Table II show that less than 1% of the complex was extracted, and hence it was not neutral. A negative charge of the complex would predict high extraction recovery when a long chain quaternary ammonium salt as TOMACl, dissolved in IBMK, is used as positively charged counter ion. It is obvious from the results presented in Table II that no formation of an ion associate complex took place and that it was therefore not negatively charged. Quantitative extraction of Cu(II)-histidine complex into IBMK was achieved only with a big organic dodecylbenzenesulphonate anion as counter-ion and the formation of an extractable ion associate complex. This shows that the complex was positively charged. This conclusion was further confirmed by investigations of the sorption properties of the Cu(II)-histidine complex using the cation exchange resin Dowex 50-X8 (Fig. 2).

Cation exchange was observed for the model solution and dialysate when using the resin prepared in the H^+ form (dynamic capacity 160 μg g^{-1} Cu^{2+}) and in the Na^+ form (dynamic capacity 180 μg g^{-1} Cu^{2+}). No ion exchange was observed when the resin Dowex 50-X8 was in Ca^{2+} form. It may be deduced from this information that the complex bears a positive charge of +1. It could be also deduced that one mol of the copper(II)--histidine complex interacts with one mol dodecylsulfonate (DDS) anion to form an ion associate. To prove this assumption, the equilibrium shift method was applied to determination of the composition of the extracted ion associate. The extraction equilibria can be presented as:

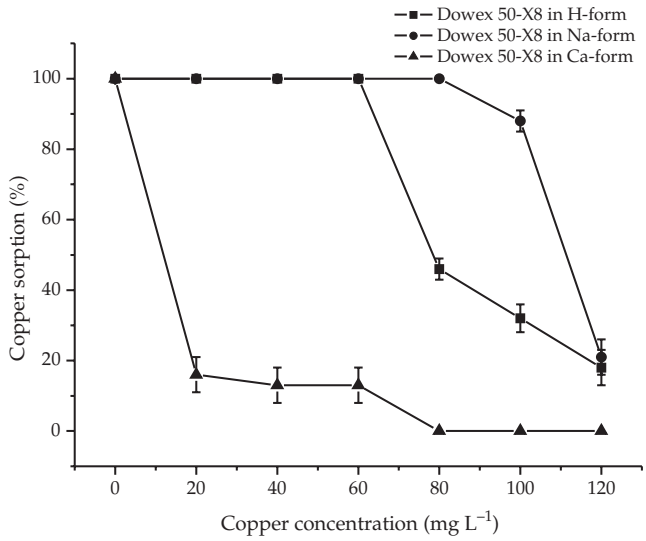

Fig. 2. Adsorption profiles of the Cu(II)-histidine standard solution on Dowex 50-X8 resin in different forms (mean ± SD, n = 3).

$$\text{Cu-His}^{n+}_{aq} + n\ \text{DDS}^{-}_{aq} \rightleftarrows [(\text{Cu-His})(\text{DDS})_n]_{org}$$

The equilibrium constant, K_{ex}:

$$K_{ex} = [(\text{Cu-His})(\text{DDS})_n]_{org}/[\ \text{Cu-His}^{n+}]_{aq}\ [\text{DDS}^{-}]^n_{aq}$$

The distribution coefficient, D:

$$D = [(\text{Cu-His})(\text{DDS})_n]_{org}/[\text{Cu-His}^{n+}]_{aq}$$

or

$$\log D = \log K_{ex} + n \log [\text{DDS}^{-}]_{aq}$$

The concentration of DDS was varied in the range $1.5 \times 10^{-5} - 3 \times 10^{-6}$ mol L^{-1} and the log D was calculated measuring the copper concentration in the organic and in the aqueous phase. The results are graphically presented in Fig. 3.

It can be seen that one mole Cu-histidine forms an ion associate with one mole DDS (n = 1); hence the charge of the Cu-histidine complex is +1.

Extraction preconcentration of copper from dialysis solutions is possible after transformation of the Cu(II)-histidine complex into a neutral copper pyrrolidinedithiocarbamate chelate complex $Cu(PDC)_2$. This transformation is possible due to the higher stability of $Cu(PDC)_2$ ($\beta = 10^{28}$) compared to the Cu(II)-histidine complex ($\beta = 10^{17}$). The extraction of Cu is quantitative (recovery > 99%) for a broad range of pH values (6 to 10,

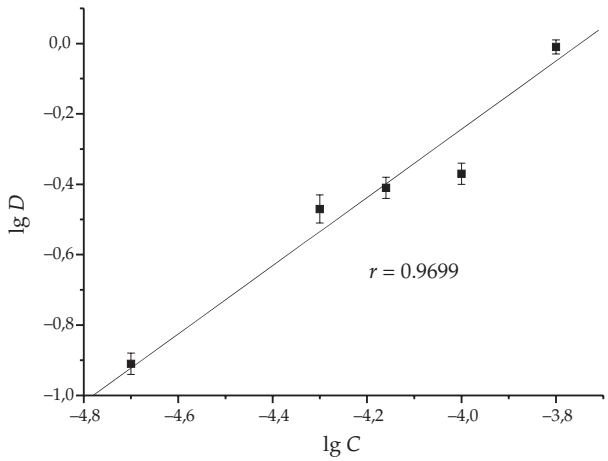

Fig. 3. Equilibrium shift method for determination of the composition of the extracted ion associate (Cu-His) $(DDS)_n$ ($c_{Cu} = 3\times 10^{-6}$ mol L^{-1}, $c_{NaDDS} = 1.5\times 10^{-5} - 3\times 10^{-6}$ mol L^{-1}); n – moles of DDS^- (mean ± SD, n = 4).

Table II) and for a ratio of aqueous to organic phase of up to 50 : 3. This equates to a 17-fold extraction preconcentration. This preconcentration procedure showed higher precision and ruggedness. RSD for the recovery obtained on different days by different operators, in different laboratories, with different batches of reagents varied between 0.2 and 1%) than extraction of copper as Cu-histidine-DDS complex (RSD under reproducibility conditions as previously mentioned varied between 3 and 8%).

Dowex 50-X8 resin (H^+-form, 0.5 g resin) can be used for quantitative sorption of up to 80 μg copper as the Cu(II)-histidine complex (Fig. 2). The large excess of Ca^{2+} and Mg^{2+} in the dialysis solution reduces the amount of copper absorbed, due to limited dynamic ion exchange capacity. For quantitative elution, 14 mL of 4 mol L^{-1} HCl are necessary (Fig. 4).

Table II. Extractability of Cu(II) from dialysis solution in dependence on the extraction system [copper as Cu(II)-histidine complex]

Extraction system	Extraction recovery (%)
IBMK	< 1 %
TOMACl/IBMK	< 1 %
NaDDS/IBMK	97.0 ± 3.2[a]
APDC/IBMK	
pH 6	99.8 ± 0.3[a]
pH 7	99.9 ± 0.2[a]
pH 8	99.5 ± 0.4[a]
pH 9	99.4 ± 0.4[a]
pH 10	99.2 ± 0.5[a]

[a] Mean ± SD, n = 3.

Another proposed method for preconcentrating copper from the dialysis solutions utilizes the chelate sorbent Chelex-100. Quantitative sorption of up to 100 μg copper is achieved from dialysis solutions with pH 5–7. Quantitative elution follows with 14 mL of 2.5 mol L^{-1} HNO_3 (Fig. 4).

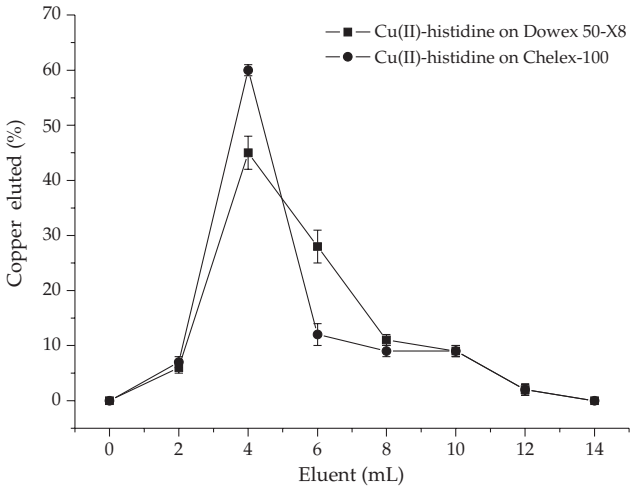

Fig. 4. Elution profiles of the Cu(II)-histidine complex on Dowex 50-X8 and Chelex-100 resins (mean ± SD, n = 3). Eluent: -■- 2.5 mol L^{-1} HNO_3, -●- 4 mol L^{-1} HCl.

CONCLUSIONS

The study of the extraction and sorption of the Cu(II)-histidine complex onto ion exchange resins leads to the conclusion that the copper(II)-histidine complex formed during the dialysis of human plasma is positively charged and bears a single positive charge. The extraction of ion associates, and of neutral Cu(II)-pyrrolidinedithiocarbamate chelate into IBMK as well as sorption onto cation exchange resin or onto chelate sorbent can be used for preconcentration of copper from the dialysis solution.

The investigations performed also help to study the speciation of copper binding to proteins in human plasma, to study the kinetics of copper turnover in exchangeable and non-exchangeable plasma copper fractions. It leads to a better understanding of the human copper metabolism, to a more precise estimation of dietary requirements and of the risk of copper intoxication.

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S A Ž E T A K

Svojstva bakar(II)-histidin kompleksa izoliranog iz dijalizirane humane plazme s histidinom

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U radu su ispitivani različiti sustavi za ekstrakciju i ionske-izmjenjivačke smole za izolaciju bakar(II)-histidin kompleksa iz dijalizirane humane plazme s histidinom. Prema dobivenim rezultatima, bakar(II)-histidin kompleks ima jedan pozitivan naboj. Prekoncentracija bakra iz otopine za dijalizu postignuta je ekstrakcijom ditiokarbamatnog kompleksa i sorpcijom na kation-izmjenjivačku smolu ili stvaranjem kelata.

Ključne riječi: bakar-histidin, dijaliza humane plazme, ekstrakcija, ionska izmjena

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