ACS OMEGA

Open Access

http://pubs.acs.org/journal/acsodf

Article

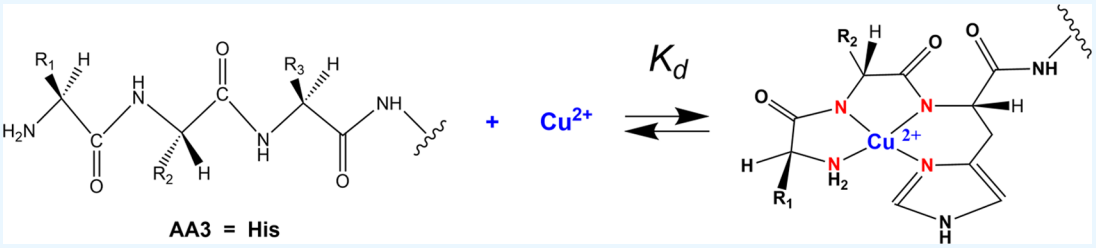
Direct Competition of ATCUN Peptides with Human Serum Albumin for Copper(II) Ions Determined by LC-ICP MS

Andra Noormägi, Tatjana Golubeva, Elina Berntsson, Sebastian K.T.S. Wärmländer, Vello Tõugu, and Peep Palumaa*

Cite This: *ACS Omega* 2023, 8, 33912−33919

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information



ABSTRACT: Copper is an indispensable biometal, primarily serving as a redox-competent cofactor in numerous proteins. Apart from preformed copper-binding sites within the protein structures, small peptide motifs exist called ATCUN, which are composed of an N-terminal tripeptide XZH, able to bind Cu(II) ions in exchangeable form. These motifs are common for serum albumin, but they are also present in a wide range of proteins and peptides. These proteins and peptides can be involved in copper metabolism, and copper ions can affect their biological role. The distribution of copper between the ATCUN peptides, including truncated amyloid-β (Aβ) peptides Aβ4−42 and Aβ11−42, which may be involved in Alzheimer's disease pathogenesis, is mainly determined by their concentrations and relative Cu(II)-binding affinities. The Cu(II)-binding affinity (log K_d) of several ATCUN peptides, determined by different methods and authors, varies by more than three orders of magnitude. This variation may be attributed to the chemical properties of peptides but can also be influenced by the differences in methods and experimental conditions used for the determination of K_d. In the current study, we performed direct competition experiments between selected ATCUN peptides and HSA by using an LC-ICP MS-based approach. We demonstrated that ATCUN and truncated Aβ peptides Aβ4−16 and Aβ11−15 bind Cu(II) ions with an affinity similar to that for HSA. Our results demonstrate that ATCUN motifs cannot compete with excess HSA for the binding of Cu(II) ions in the blood and cerebrospinal fluid.

INTRODUCTION

Copper is an essential microelement for all forms of life, but its chemical properties make it potentially toxic, which is why its metabolism is always tightly regulated in living organisms. The biological functions of copper are almost exclusively carried out in the protein-bound form. Bioinformatic analysis revealed that Cu proteome constitutes approximately 0.05−1.3% of the total proteome of an organism, considering both eukaryotes and prokaryotes.[1] It is estimated that there are 54 Cu-binding proteins in the human proteome.[2]

In addition to 3D copper-binding pockets within protein structures, Cu(II) ions can also strongly bind to an N-terminal motif called ATCUN (amino terminal Cu- and Ni-binding motif). The ATCUN motif contains a His residue in the third position (XZH) and is present in more than 400 human proteins, including human serum albumin (HSA).[3] Approximately 10% of the total copper present in human serum is bound to the ATCUN motif of HSA. This copper binding is exchangeable, and it is possible that all other extracellular ATCUN proteins can also play a role in copper metabolism or that their biological functions may depend on copper binding. To participate in copper metabolism or perform their biological functions, other extracellular ATCUN proteins must compete with HSA for copper binding. The amount of copper that these proteins can bind depends on their relative concentration and affinity for metal binding. The metal-binding affinities of peptides with an ATCUN motif have been intensively studied by different methods, and the range of log K_a values determined for ATCUN sites ranges from 11.0 to 14.6.[4] Selected values of dissociation constants for ATCUN motif-containing peptides and proteins from the literature are listed in Table 1.

ATCUN complexes have a characteristic four-nitrogen (4N) square-planar coordination mode, where the metal ion is

Received: June 29, 2023
Accepted: August 29, 2023
Published: September 7, 2023

Table 1. Dissociation Constants for Cu(II) Complexes with ATCUN Motif-Containing Peptides and Proteins

peptide/protein	method	K_d	refs
MDH-NH_2	potentiometric titration, pH 7.4	7.94×10^{-14}	5
MDHSHHMGMSYMDS-NH_2	potentiometric titration, pH 7.4	1.0×10^{-13}	6
GGH	potentiometric titration, pH 7.4	6.10×10^{-13}	7
HSA	potentiometric titration, pH 7.4	9.55×10^{-14}	7
DAH − NH_2	potentiometric titration, pH 7.4	2.00×10^{-14}	8,5
MEHFPGP (Semax)	potentiometric titration, pH 7.4	1.3×10^{-15}	9
DTHFPI-NH_2 (Hepcidin N-terminus)	potentiometric titration, pH 7.4	$2,19 \times 10^{-15}$	10
Aβ4−16-NH_2	potentiometric titration, pH 7.4	3×10^{-14}	4
Aβ11−15-NH_2	competition with Gly and His	2.5×10^{-14} (Gly) 4.2×10^{-14} (His)	11

located in the center of a square whose edges are occupied by four nitrogen atoms, provided by the N-terminal amine, two subsequent nitrogen atoms in peptide backbone, and the N-1 nitrogen of the His imidazole ring (Figure 1).

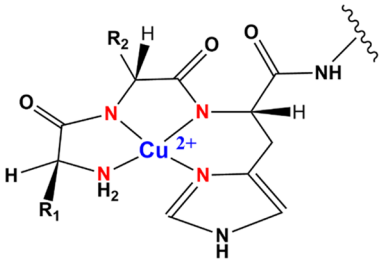


Figure 1. Schematic presentation of the Cu(II)−ATCUN complex.

Numerous explanations have been proposed to account for the differences in reported K_d values for ATCUN motifs, such as differences in the pK_a values of individual peptide nitrogens,[12,13] variances in the hydrophobicity of the first two amino acid residues,[13] and variances in the secondary coordination sphere effects. Although all of these factors may influence Cu(II)-binding affinities to some extent, differences might also be caused by using many different methods for calculating metal-binding affinities. It is well known that such measurements are strongly affected by the experimental conditions, which typically vary between different studies.[14] An example depicting this is the data showing that two residues at the N-terminal side of His3 do not significantly contribute to the binding of Cu(II) ions.[12] This uncertain situation complicates the possibility of studying copper trafficking in the organisms and modeling the availability of copper ions to different peptides and proteins.

The binding of Cu(II) to an ATCUN motif substantially alters the electrochemical properties of the Cu(II)/Cu(I) redox couple. The Cu−ATCUN complex has antioxidant properties, as it shows a redox-silent component in the presence of the biological reductant ascorbate due to its high redox potential and inherent geometry problem between Cu(II) and Cu(I) complexes.[15]

Having a clear understanding of copper trafficking is crucial for understanding the possible involvement of copper ions in Alzheimer's disease (AD) pathogenesis. AD is characterized by the initial formation of amyloid plaques in the brain, followed by formation of neurofibrillary tangles inside the neurons.[16] Amyloid plaques are composed of amyloid-β peptides (Aβ) of various lengths, mainly Aβ1−40 and Aβ1−42.[16,17] However, an initial study by Masters et al. demonstrated that the plaques contained a significant amount of the truncated Aβ4−42 peptide.[18] In later mass spectrometric studies, substantial amounts of truncated Aβ4−42[19] and Aβ11−42 peptides[20,21] were found in plaques as well as in the cerebrospinal fluid (CSF).[22] Since both truncated peptides carry the ATCUN motifs, it raises the question of whether Aβ peptides can bind Cu(II) ions in vivo in the presence of HSA.

The interactions of Aβ peptides with Cu(II) ions have been extensively studied. It has been demonstrated that full-length Aβ peptides can bind Cu(II) ions with relatively strong affinity, with a K_d value up to 0.1−1 nM,[14,23,24] which is still several orders of magnitude lower than that of HSA. Truncated Aβ peptides Aβ4-X and Aβ11-X contain the FRH- and EVH-ATCUN motifs, respectively. By using potentiometric titration, it was demonstrated that Aβ4−16 binds Cu(II) with a conditional K_d value of 3×10^{-14} M at pH 7.4,[4] which characterized at that time 30 times higher affinity than that for HSA ($K_d = 1 \times 10^{-12}$ M was determined from the competition with NTA[25]). Based on these results, it was suggested that Aβ4−40 could have a physiological role in copper homeostasis,[4] which might be linked to the scavenging of Cu(II) ions in the synaptic cleft.[26] The Cu(II)-binding affinity of Aβ11−40 determined from the results of CD spectroscopic titration of the Cu(II)−Aβ11−40 complex with His and Gly is similar to that for Aβ4−16, i.e., $K_d = 3.4 \times 10^{-14}$ M.[11] At that time, this pointed toward efficient competition with HSA. Recently, the K_d value for HSA was corrected to 0.955×10^{-13} M by using potentiometric titration,[7] which reflects only three times lower affinity than those for Aβ4−16 and Aβ11−40. HSA is present in CSF at 3 μM concentration,[27] which is much higher than the nM concentration of Aβ peptides.[28] These data suggest that Aβ peptides should not be able to compete with HSA for Cu(II) binding.[11] However, direct competition experiments have not yet been conducted.

In this study, we attempted to answer the following two related questions: "Do different ATCUN motifs show different Cu(II)-binding affinities under the conditions of direct competition?" "Can truncated Aβ peptides with ATCUN motifs compete in CSF with HSA for the binding of Cu(II) ions?" To address the raised questions, we employed an innovative LC-ICP MS technique, enabling the use of size exclusion chromatography (SEC) to separate and quantify the substances with high molecular weight (HSA) and low molecular weight (ATCUN peptides) involved in metal binding within the reaction mixture.[29]

We determined the relative Cu(II)-binding affinities for several model peptides and two truncated Aβ peptides in comparison with HSA. Results demonstrate that different ATCUN-containing peptides and HSA have very similar Cu(II)-binding affinities, and truncated Aβ peptides cannot

https://doi.org/10.1021/acsomega.3c04649
ACS Omega 2023, 8, 33912−33919

compete with HSA for the binding of Cu(II) ions in blood or even in CSF.

RESULTS

First, Cu(II) competition experiments were performed with the aim of determining the incubation time required to achieve the equilibrium of Cu(II) distribution between HSA- and ATCUN-containing peptides. Figure 2 demonstrates that equilibrium is reached within 30 min.

Kinetic experiments were also conducted in conditions where Aβ(11−15) was added to the metalated HSA (Figures S1 and S2) and when HSA was added to the metalated Aβ(11−15) (data not shown). In the first case, equilibrium was reached in 30 min (Figure S3) both in the equimolar mixture and in the presence of a 2-fold excess of the competing (apo) ligand. In the second case, the equilibrium was reached very quickly within 2 min. Based on this kinetic information for subsequent experiments, Cu(II) ions were added to the mixture of HSA and ATCUN peptides, and an incubation time of 30 min was selected.

The next series of experiments involved determining the competition between HSA and ATCUN peptides for Cu(II) binding. Figure 3 shows an example of the results obtained with the Aβ(11−15) peptide. When this peptide and HSA are present in equimolar concentrations, Cu(II) ions are distributed 60% in HSA and 40% in the Aβ(11−15) peptide (Figure 3c). This suggests that both HSA and the Aβ(11−15) peptide are able to compete almost equally for Cu(II) binding. As the Aβ(11−15) concentration is increased, the distribution of Cu(II) ions shifts toward the peptide, and at an Aβ(11−15) concentration of 40 μM, the metal distribution reaches a ratio of 13:87 in favor of the Aβ(11−15) peptide (Figure 3g).

The obtained results were analyzed according to eq 2, which predicts a linear relationship between X = [Cu(Pept)]([HSA]$_T$ − [Cu(HSA)]) and Y = [Cu(HSA)]([Pept]$_T$ − [Cu(Pept)]), where [Cu(HSA)] and [Cu(Pept)] are determined from the chromatograms as the copper content of high- and low-molecular-weight fractions and [HSA]$_T$ and [Pept]$_T$ are the total added concentrations of each ligand (see eqs 1−4 from the Materials and Methods). The relationship observed from three independent experiments is shown in Figure 4B, and it is linear with a slope of K_d (Aβ11−15)/K_d(HSA) equal to 1.56 ± 0.09. Using the known value of K_d for HSA, which is equal to 0.955×10^{-13} M,[7] we can calculate the K_d value for Aβ11−15 to be $1.49 \pm 0.09 \times 10^{-13}$ M.

The representative examples of primary data for other ATCUN peptides are provided in the Supporting Information. Relationships between X and Y, based on eq 2, for these peptides are shown in Figures 5 and S8.

The obtained K_d values are presented in Table 2.

DISCUSSION

The distribution of copper ions between competing ligands, such as peptides and proteins with ATCUN motifs, is determined by thermodynamic and kinetic factors. The distribution in equilibrium can be calculated from the K_d values of the ATCUN−Cu(II) complexes. However, the K_d values can be affected by several factors; for instance, they can be affected by experimental conditions and intermolecular interactions at higher concentrations of the reagents. In this paper, we have determined the relative binding affinities for different Cu(II)-binding ligands in direct competition experiments with binary mixtures. We have conducted such experiments with different Cu(II)-binding peptides and HSA as standard by using LC-ICP MS technology, which is suitable for this purpose. In these experiments, the different ATCUN-containing peptides display rather similar abilities to compete

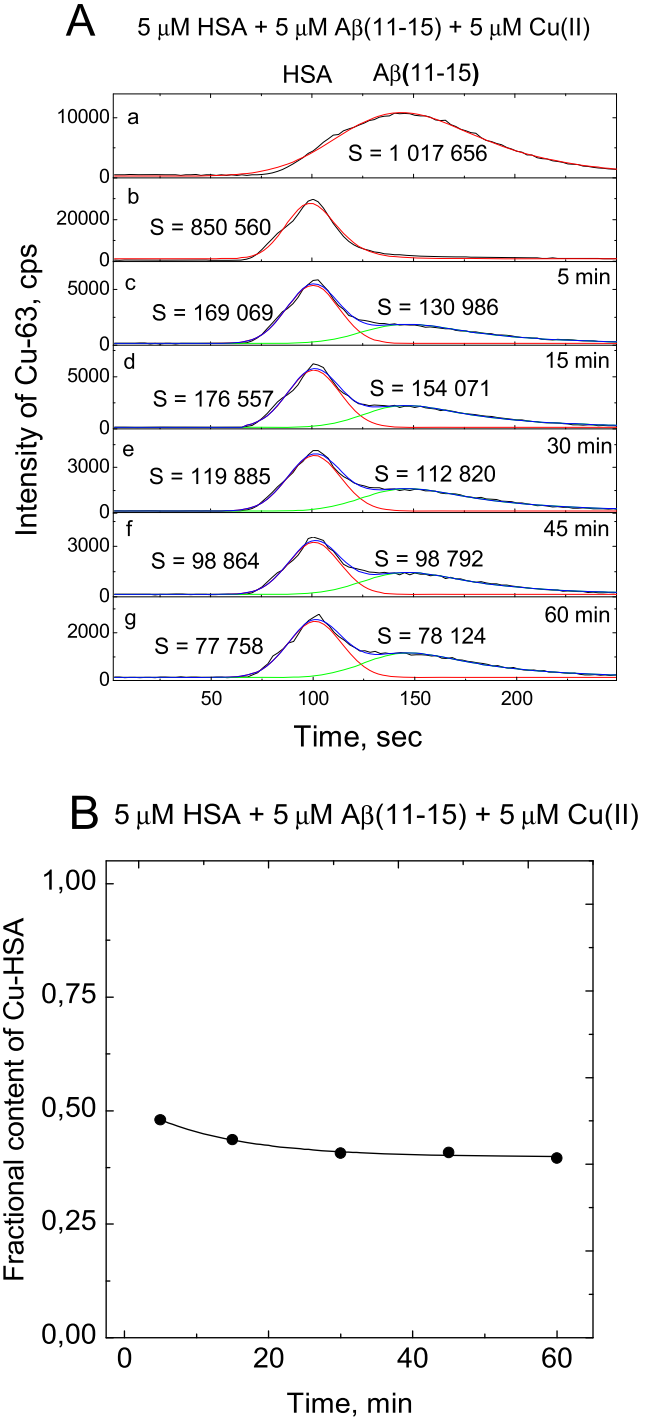


Figure 2. Competition between HSA and Aβ(11−15) peptide for Cu(II) ions. (A) SEC chromatograms of copper distribution (incubation time and integrated peak area values are shown in labels). (B) Decrease of the relative content of copper in the HSA fraction with time. Conditions: 5 μM HSA, 5 μM Aβ(11−15), and 5 μM Cu(II); incubation buffer 50 mM HEPES, 50 mM NaCl, pH 7.4; LC-ICP MS: column, 1 mL Sephadex G25 Superfine; elution buffer 200 mM NH_4NO_3, pH 7.4; flow rate 0.4 mL/min; injection volume 10 μL; Cu-63 was monitored by ICP MS.

https://doi.org/10.1021/acsomega.3c04649
ACS Omega 2023, 8, 33912−33919

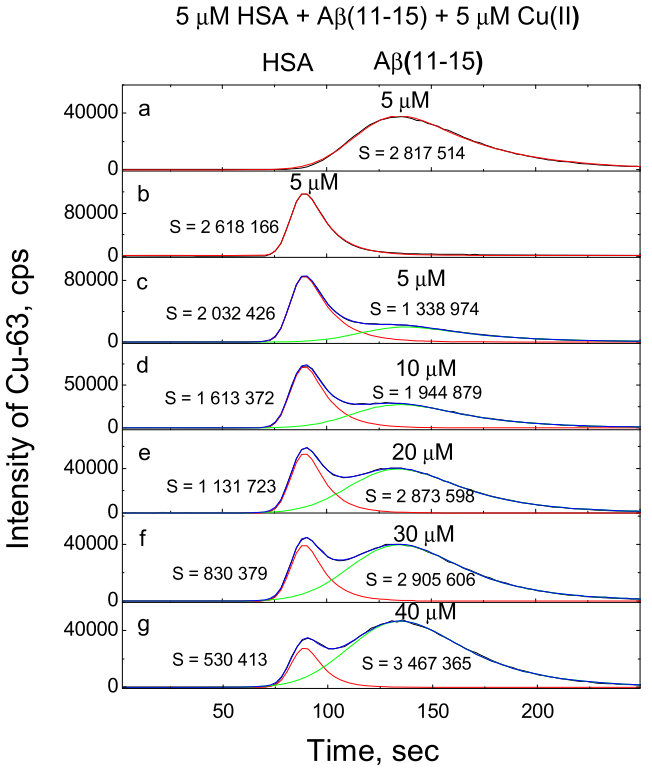


Figure 3. Competition between HSA and the Aβ(11−15) peptide for Cu(II) ions. SEC chromatograms of (a) 5 μM Cu(II)• Aβ(11−15), (b) 5 μM Cu(II)•HSA, and (c−g) 5 μM HSA and Cu(II) in the presence of increasing concentration of Aβ(11−15). The peptide concentrations and peak areas are shown in labels. Conditions; incubation buffer 50 mM HEPES, 50 mM NaCl, pH 7.4; incubation time 30 min; LC-ICP MS: column, 1 mL Sephadex G25 Superfine; elution buffer 200 mM NH_4NO_3, pH 7.4; flow rate 0.4 mL/min; injection volume 10 μL; Cu-63 was monitored by ICP MS.

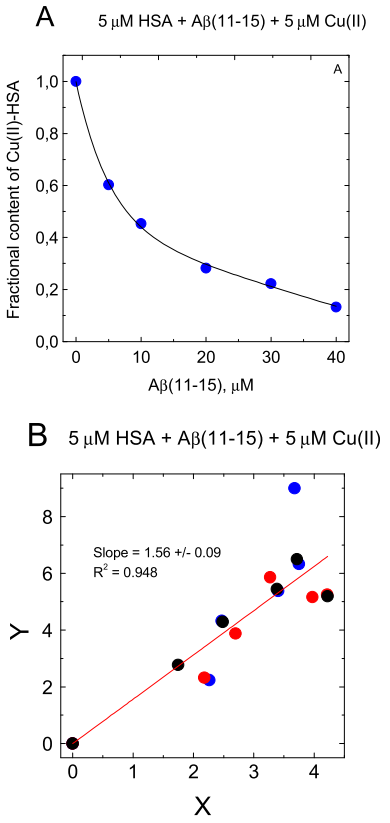


Figure 4. Calculation of the relative Cu(II)-binding affinity of Aβ(11−15) from LC-ICP MS results. (A) Decrease of the fractional content of the Cu•HSA complex, caused by the increase of Aβ(11−15) concentration. (B) Application of eq 2. Results of three separate experiments are presented with different colors—red, blue, and black.

with HSA for Cu(II) ions. Correspondingly, their binding affinities toward Cu(II) ions are also similar. These results are different from the results of potentiometric titration measurements. A comparison between DAH-NH_2 and DAH-COOH indicates that C-terminal amidated peptides have slightly higher binding affinities than peptides with a free C-terminal carboxyl group, which is in agreement with the literature.[8] At the same time, the K_d value for DAH-NH_2, a tripeptide originating from HSA, was almost similar to the K_d value for HSA, demonstrating that amidated tripeptides can be good and sufficient Cu(II)-binding models for ATCUN-containing proteins.

In the literature, the amidated hexa- and heptapeptides (DTHFPI-NH_2 and MEHFPGP) have shown binding affinities that exceed that of HSA by 44 and 73 times, respectively (Table 1). However, in the direct competition experiments, these peptides displayed rather similar K_d values to HSA and also to the simplest model peptide GGH. This result suggests that the primary coordination sphere of the ATCUN motif, composed of four nitrogen atoms provided by the N-terminal amine, two subsequent peptide nitrogen atoms, and the N-1 nitrogen of the His imidazole ring, plays a crucial role in the binding of Cu(II) ions and determines the binding affinity. It also suggests that the chemical properties of the first two amino acid side chains and the post-His amino acid residues do not contribute significantly to the metal binding.

Direct competition experiments showed that truncated Aβ peptides, i.e., Aβ4−16 and Aβ11−15, have K_d values similar to HSA for Cu(II) binding (Table 2). Aβ11−15 has a 1.3 times higher affinity toward Cu(II) ions than Aβ4−16. This small difference might be explained by the His14 residue contributing to ATCUN motif Cu(II) binding in the Aβ11−15 peptide. HSA is present in blood at 650 μM[30] and in CSF at 3 μM concentration,[27] which are much higher than the nM concentrations of Aβ peptides in CSF and blood.[28,31] Based on these numbers, we conclude that Aβ peptides cannot compete with HSA for Cu(II) binding[11] in blood or CSF.

Overall, we argue that the direct competition experiments with HSA and the K_d values determined from these experiments describe the properties of ACTUN-motif-containing peptides in biological conditions more correctly than the K_d values determined from separate potentiometric titration experiments. In the latter case, the interactions between the peptides in the solution may also affect the titration results in a way that does not occur in biological conditions where the peptide concentration is very low.

CONCLUSIONS

Peptides with His in the third position are known as ATCUN peptides, which are able to bind Cu(II) ions with a high affinity. In the literature, the reported dissociation constants for different ATCUN peptides vary widely. We performed direct competition experiments with selected ATCUN motifs, including truncated Aβ peptides, in relation to HSA using an LC-ICP MS-based approach. We demonstrated that Cu(II)-

https://doi.org/10.1021/acsomega.3c04649
ACS Omega 2023, 8, 33912−33919

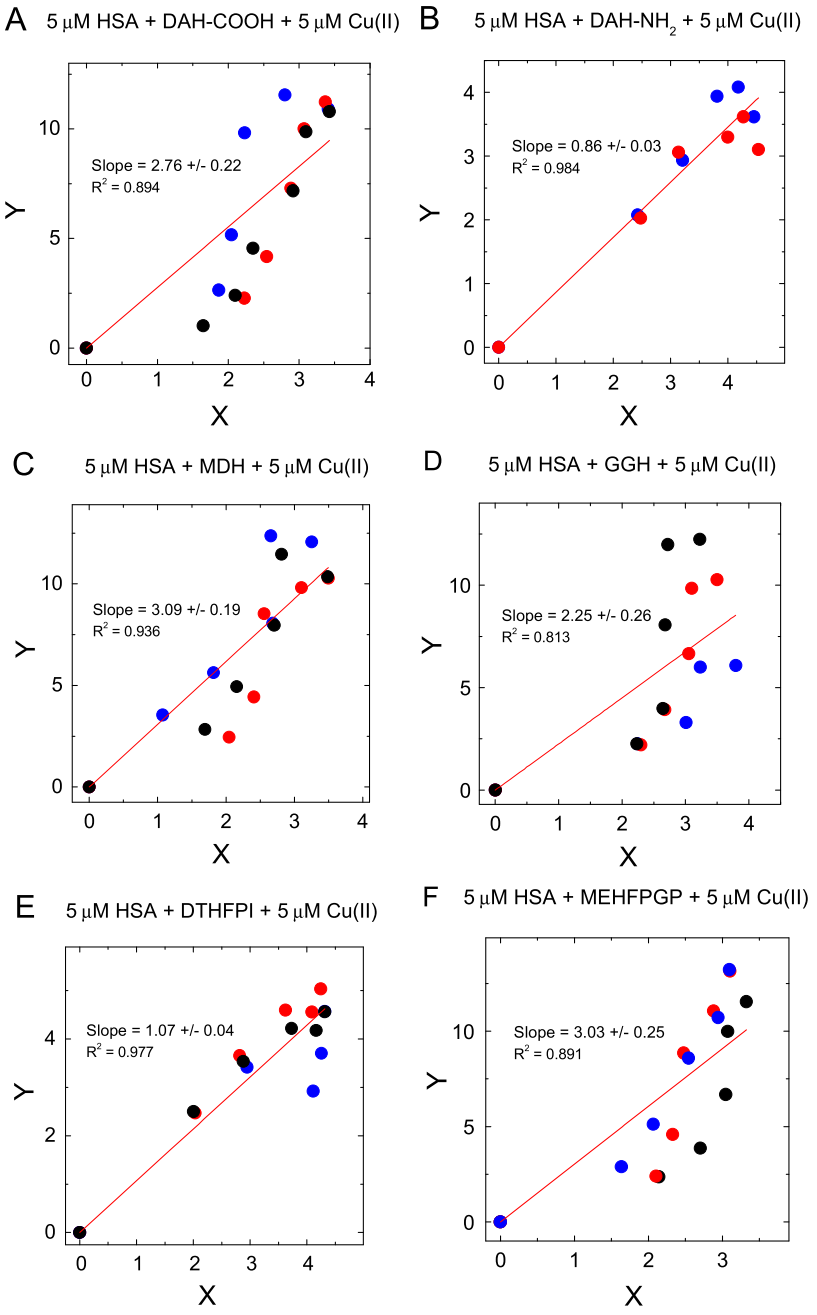


Figure 5. Calculation of the relative Cu(II)-binding affinity of ATCUN peptides from LC-ICP MS results according to eq 2. (A) DAH-COOH, (B) DAH-NH_2, (C) MDH-NH_2, (D) GGH-NH_2, (E) DTHFPI-NH_2, and (F) MEHFPGP-NH_2. Results of three separate experiments are presented with different colors: red, blue, and black.

binding affinities of ATCUN and truncated Aβ peptides are similar to those for HSA and vary in a narrow range. Our results demonstrate that ATCUN motifs cannot remove substantial amounts of Cu(II) ions from excess HSA in blood and CSF environments.

MATERIALS AND METHODS

Materials. The following lyophilized protein and peptides were used: human serum albumin (HSA) from Sigma/Merck (Darmstadt, Germany), Aβ(4−16) and Aβ(11−15) peptides from rPeptide (Watkinsville, GA), and DAH-COOH, DAH-NH_2, MDH-NH_2, GGH-NH_2, DTHFPI-NH_2, and MEHFPGP-NH_2 were synthesized at the Institute of Technology of the University of Tartu. The peptides were synthesized on an automated peptide synthesizer (Biotage Initiator+ Alstra, Sweden) using a fluorenylmethyloxycarbonyl (Fmoc) solid-phase peptide synthesis strategy and purified by reverse-phase liquid chromatography on a C4 column (Phenomenex Jupiter C4, 5 μm, 300 A, 250 × 10 mm) using a gradient of acetonitrile/water containing 0.1% TFA. The molecular weight of the peptides was determined by a MALDI-TOF mass spectrometer (Bruker Microflex LT/SH). 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP), sodium chloride, sodium hydroxide, and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes) were purchased from Sigma-Aldrich (St. Louis, MO). Cu(II)acetate was purchased from Sigma (Sigma/Merck KGaA, Darmstadt, Germany). Nitric acid (HNO_3, trace metal grade) was purchased from Fisher

https://doi.org/10.1021/acsomega.3c04649
ACS Omega 2023, 8, 33912−33919

Table 2. Dissociation Constants for Cu(II) Complexes of ATCUN Motif-Containing Peptides and HSA

peptide	K_d, M
GGH-NH_2	$(2.15 \pm 0.25) \times 10^{-13}$
MDH-NH_2	$(2.95 \pm 0.18) \times 10^{-13}$
DAH-COOH	$(2.64 \pm 0.21) \times 10^{-13}$
DAH-NH_2	$(8.21 \pm 0.29) \times 10^{-14}$
DTHFPI-NH_2	$(1.02 \pm 0.04) \times 10^{-13}$
MEHFPGP-NH_2	$(2.89 \pm 0.24) \times 10^{-13}$
Aβ(11−15)	$(1.49 \pm 0.09) \times 10^{-13}$
Aβ(4−16)	$(2.00 \pm 0.11) \times 10^{-13}$
HSA*	9.55×10^{-14}

*From ref 4.

Scientific (UK Limited Leicestershire, UK), and ammonium hydroxide 25% solution was from Honeywell Fluka (Seelze, Germany). Ultrapure Milli-Q water with a resistivity of 18.2 MΩ/cm, produced by a Merck Millipore Direct-Q & Direct−Q UV water purification system (Merck KGaA, Darmstadt, Germany), was used for all applications.

Competition for Cu(II) Ions Determined by LC-ICP MS Analysis. *Sample Preparation.* All lyophilized peptides were dissolved in HFIP at a concentration of 100 μM to disassemble the preformed aggregates. The solution was divided into aliquots; HFIP was evaporated in vacuum, and the tubes containing the peptide film were stored at −80 °C until used.

A stock solution of HSA was prepared in Milli-Q water and further diluted into a reaction buffer solution composed of 50 mM Hepes and 50 mM NaCl, pH 7.4. HFIP-treated peptide aliquots were dissolved in 50 mM HEPES and 50 mM NaCl, pH 7.4, at a concentration of 50 μM and further diluted to the final concentration with reaction buffer. Cu(II)acetate was used as a Cu(II) source for proteins and peptides. For a competition experiment, an appropriate concentration of competing peptides (Aβ(11−15), Aβ(4−16), DAH-COOH, DAH-NH_2, MDH-NH_2, GGH-NH_2, DTHFPI-NH_2, or MEHFPGP-NH_2) and a 5 μM solution of HSA were mixed with 5 μM Cu(II), and the sample was incubated 30 min at room temperature. All reagent solutions were prepared daily before experiments.

LC-ICP MS Measurements. For LC-ICP MS analyses, an Agilent Technologies (Santa Clara) Infinity HPLC system, which consisted of a 1260 series μ-degasser, a 1200 series capillary pump, a Micro WPS autosampler, and a 1200 series MWD VL detector, was connected to an Agilent 7800 series ICP MS instrument. For instrument control and data acquisition, ICP MS MassHunter 4.4 software, version C 01.04, from Agilent was used. ICP MS was operated under the following conditions: RF power: 1550 W, nebulizer gas flow: 1.03 L/min, auxiliary gas flow: 0.90 L/min, plasma gas flow: 15 L/min, nebulizer type: MicroMist, and isotope monitored: Cu-63. To perform the separation of HMW and LMW pools, a 1 mL gel filtration column, self-packed with HiTrap Desalting resin Sephadex G25 Superfine (Amersham/GE Healthcare, Buckinghamshire, UK), was used. An injection volume of 10 μL for each sample was used. The mobile phase for gel filtration was 200 mM NH_4NO_3 at pH 7.5, prepared from TraceMetal grade nitric acid and ammonium hydroxide 25% solution, which is compatible with ICP MS. An ICP MS compatible flow rate of 0.4 mL/min was used in all separations. To get rid of contaminating metal ions in the buffer, the mobile phase was eluted through the Chelex 100 Chelating Ion Exchange resin (Sigma, Merck KGaA, Darmstadt, Germany) prior to liquid chromatographic separation. The demetalation of the SEC columns before each experiment was conducted by injecting 1 mM EDTA into the columns (injection volumes were the same for all experiments).

Separation of the HMW and LMW peak areas was conducted with Origin Pro 8.5 software. An exponentially modified Gaussian peak function (GaussMod) was used to obtain peak areas that were further used to calculate a decrease in the fractional content of the metalated protein. All experiments were repeated three times.

Determination of Relative Dissociation Constants. Experimental results for competition for Cu(II) ions between HSA- and ATCUN-containing peptides were used for the determination of the relative dissociation constant for the ATCUN-containing peptide and HSA copper complexes. The ratio of the dissociation constants for the peptide and HSA can be expressed as follows[7]

$$\frac{K^{Cu}_{d(Pept)}}{K^{Cu}_{d(HSA)}} = \frac{[Cu(HSA)]([Pept]_T - [Cu(Pept)])}{[Cu(Pept)]([HSA]_T - [Cu(HSA)])} \quad (1)$$

or

$$[Cu(HSA)]([Pept]_T - [Cu(Pept)]) = \frac{K^{Cu}_{d(Peptide)}}{K^{Cu}_{d(HSA)}}[Cu(Pept)]([HSA]_T - [Cu(HSA)]) \quad (2)$$

$$X = [Cu(Pept)]([HSA]_T - [Cu(HSA)]) \quad (3)$$

$$Y = [Cu(HSA)]([Pept]_T - [Cu(Pept)]) \quad (4)$$

where [Cu(HSA)] and [Cu(Pept)] are determined from the chromatograms as the copper content of high- and low-molecular-weight fractions, and $[HSA]_T$ and $[Pept]_T$ are the total added concentrations of each ligand.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c04649.

Kinetics of Cu(II) exchange between HSA and ATCUN peptides (Figures S1−S3); competitions between HSA and ATCUN peptides for Cu(II) ions (Figures S4−S10); and calculation of relative Cu(II)-binding affinity of Aβ(4−16) (Figure S11) (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Peep Palumaa − *Department of Chemistry and Biotechnology, Tallinn University of Technology, 12618 Tallinn, Estonia;* orcid.org/0000-0002-3505-3466; Phone: +372 6204410; Email: peep.palumaa@taltech.ee

Authors

Andra Noormägi − *Department of Chemistry and Biotechnology, Tallinn University of Technology, 12618 Tallinn, Estonia*

Tatjana Golubeva − *Department of Chemistry and Biotechnology, Tallinn University of Technology, 12618 Tallinn, Estonia*

Elina Berntsson − *Department of Chemistry and Biotechnology, Tallinn University of Technology, 12618*

https://doi.org/10.1021/acsomega.3c04649
ACS Omega 2023, 8, 33912−33919

Tallinn, Estonia; Chemistry Section, Stockholm University, 10691 Stockholm, Sweden; orcid.org/0000-0002-7544-092X

Sebastian K.T.S. Wärmländer − *Chemistry Section, Stockholm University, 10691 Stockholm, Sweden*

Vello Tõugu − *Department of Chemistry and Biotechnology, Tallinn University of Technology, 12618 Tallinn, Estonia*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c04649

Author Contributions

P.P., V.T., and A.N. conceived and designed the experiments. A.N., T.G., and E.B. carried out the experiments. All authors contributed to the analysis and interpretation of the results. P.P. and V.T. wrote the manuscript with support from S.W. and A.N. Review and editing were carried out by P.P., V.T., and S.W. All authors discussed the results and contributed to the final manuscript. P.P. supervised the project.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the Estonian Research Council grant (PRG 1289) to P.P. and by a grant from the Magnus Bergvall Foundation to S.W. The authors thank Heleri Heike Härk for peptide synthesis and Astrid Gräslund and Jüri Jarvet for helpful discussion.

■ REFERENCES

(1) Andreini, C.; Banci, L.; Bertini, I.; Rosato, A. Occurrence of copper proteins through the three domains of life: A bioinformatic approach. *J. Proteome Res.* **2008**, *7* (1), 209−216.

(2) Blockhuys, S.; Celauro, E.; Hildesjo, C.; Feizi, A.; Stal, O.; Fierro-Gonzalez, J. C.; Wittung-Stafshede, P. Defining the human copper proteome and analysis of its expression variation in cancers. *Metallomics* **2017**, *9* (2), 112−123.

(3) Frączyk, T. Cu(II)-Binding N-Terminal Sequences of Human Proteins. *Chem. Biodiversity* **2021**, *18* (4), No. e2100043.

(4) Mital, M.; Wezynfeld, N. E.; Fraczyk, T.; Wiloch, M. Z.; Wawrzyniak, U. E.; Bonna, A.; Tumpach, C.; Barnham, K. J.; Haigh, C. L.; Bal, W.; et al. A Functional Role for Abeta in Metal Homeostasis? N-Truncation and High-Affinity Copper Binding. *Angew. Chem., Int. Ed. Engl.* **2015**, *54* (36), 10460−10464. Research Support, Non-U.S. Gov't

(5) Bossak, K.; Drew, S. C.; Stefaniak, E.; Plonka, D.; Bonna, A.; Bal, W. The Cu(II) affinity of the N-terminus of human copper transporter CTR1: Comparison of human and mouse sequences. *J. Inorg. Biochem.* **2018**, *182*, 230−237.

(6) Stefaniak, E.; Plonka, D.; Drew, S. C.; Bossak-Ahmad, K.; Haas, K. L.; Pushie, M. J.; Faller, P.; Wezynfeld, N. E.; Bal, W. The N-terminal 14-mer model peptide of human Ctr1 can collect Cu(ii) from albumin. Implications for copper uptake by Ctr1. *Metallomics* **2018**, *10* (12), 1723−1727.

(7) Bossak-Ahmad, K.; Fraczyk, T.; Bal, W.; Drew, S. C. The Sub-picomolar Cu(2+) Dissociation Constant of Human Serum Albumin. *Chembiochem* **2020**, *21* (3), 331−334. From NLM Medline.

(8) Mlynarz, P.; Valensin, D.; Kociolek, K.; Zabrocki, J.; Olejnik, J.; Kozlowski, H. Impact of the peptide sequence on the coordination abilities of albumin-like tripeptides towards Cu2+, Ni2+ and Zn2+ ions. Potential albumin-like peptide chelators. *New J. Chem.* **2002**, *26* (2), 264−268.

(9) Tabbì, G.; Magri, A.; Giuffrida, A.; Lanza, V.; Pappalardo, G.; Naletova, I.; Nicoletti, V. G.; Attanasio, F.; Rizzarelli, E. Semax, an ACTH4−10 peptide analog with high affinity for copper(II) ion and protective ability against metal induced cell toxicity. *J. Inorg. Biochem.* **2015**, *142*, 39−46. From NLM Medline.

(10) Płonka, D.; Bal, W. The N-terminus of hepcidin is a strong and potentially biologically relevant Cu(II) chelator. *Inorg. Chim. Acta* **2018**, *472*, 76−81.

(11) Barritt, J. D.; Viles, J. H. Truncated Amyloid-beta(11−40/42) from Alzheimer Disease Binds Cu2+ with a Femtomolar Affinity and Influences Fiber Assembly. *J. Biol. Chem.* **2015**, *290* (46), 27791−27802. Research Support, Non-U.S. Gov't

(12) Mlynarz, P.; Bal, W.; Kowalik-Jankowska, T.; Stasiak, M.; Leplawy, M. T.; Kozlowski, H. Introduction of alpha-hydroxymethylserine residues in a peptide sequence results in the strongest peptidic, albumin-like, copper(II) chelator known to date. *J. Chem. Soc. Dalton Trans.* **1999**, No. 2, 109−110.

(13) Miyamoto, T.; Fukino, Y.; Kamino, S.; Ueda, M.; Enomoto, S. Enhanced stability of Cu2+-ATCUN complexes under physiologically relevant conditions by insertion of structurally bulky and hydrophobic amino acid residues into the ATCUN motif. *Dalton Trans.* **2016**, *45* (23), 9436−9445.

(14) Alies, B.; Renaglia, E.; Rozga, M.; Bal, W.; Faller, P.; Hureau, C. Cu(II) affinity for the Alzheimer's peptide: tyrosine fluorescence studies revisited. *Anal. Chem.* **2013**, *85* (3), 1501−1508.

(15) Gonzalez, P.; Bossak, K.; Stefaniak, E.; Hureau, C.; Raibaut, L.; Bal, W.; Faller, P. N-Terminal Cu-Binding Motifs (Xxx-Zzz-His, Xxx-His) and Their Derivatives: Chemistry, Biology and Medicinal Applications. *Chem. − Eur. J.* **2018**, *24* (32), 8029−8041.

(16) Selkoe, D. J.; Hardy, J. The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol. Med.* **2016**, *8* (6), 595−608.

(17) Glenner, G. G.; Wong, C. W. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem. Biophys. Res. Commun.* **1984**, *120* (3), 885−890.

(18) Masters, C. L.; Simms, G.; Weinman, N. A.; Multhaup, G.; McDonald, B. L.; Beyreuther, K. Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82* (12), 4245−4249. Research Support, Non-U.S. Gov't

(19) Portelius, E.; Bogdanovic, N.; Gustavsson, M. K.; Volkmann, I.; Brinkmalm, G.; Zetterberg, H.; Winblad, B.; Blennow, K. Mass spectrometric characterization of brain amyloid beta isoform signatures in familial and sporadic Alzheimer's disease. *Acta Neuropathol.* **2010**, *120* (2), 185−193. Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't

(20) Näslund, J.; Schierhorn, A.; Hellman, U.; Lannfelt, L.; Roses, A. D.; Tjernberg, L. O.; Silberring, J.; Gandy, S. E.; Winblad, B.; Greengard, P.; et al. Relative abundance of Alzheimer A beta amyloid peptide variants in Alzheimer disease and normal aging. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91* (18), 8378−8382. Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S

(21) Liu, K.; Solano, I.; Mann, D.; Lemere, C.; Mercken, M.; Trojanowski, J. Q.; Lee, V. M. Characterization of Abeta11−40/42 peptide deposition in Alzheimer's disease and young Down's syndrome brains: implication of N-terminally truncated Abeta species in the pathogenesis of Alzheimer's disease. *Acta Neuropathol.* **2006**, *112* (2), 163−174. Research Support, N.I.H., Extramural

(22) Seubert, P.; Vigo-Pelfrey, C.; Esch, F.; Lee, M.; Dovey, H.; Davis, D.; Sinha, S.; Schlossmacher, M.; Whaley, J.; Swindlehurst, C.; et al. Isolation and quantification of soluble Alzheimer's beta-peptide from biological fluids. *Nature* **1992**, *359* (6393), 325−327. From NLM Medline.

(23) Young, T. R.; Kirchner, A.; Wedd, A. G.; Xiao, Z. An integrated study of the affinities of the Abeta16 peptide for Cu(I) and Cu(II): implications for the catalytic production of reactive oxygen species. *Metallomics* **2014**, *6* (3), 505−517. Research Support, Non-U.S. Gov't

(24) Conte-Daban, A.; Borghesani, V.; Sayen, S.; Guillon, E.; Journaux, Y.; Gontard, G.; Lisnard, L.; Hureau, C. Link between Affinity and Cu(II) Binding Sites to Amyloid-beta Peptides Evaluated by a New Water-Soluble UV-Visible Ratiometric Dye with a Moderate Cu(II) Affinity. *Anal. Chem.* **2017**, *89* (3), 2155−2162.

(25) Rózga, M.; Sokolowska, M.; Protas, A. M.; Bal, W. Human serum albumin coordinates Cu(II) at its N-terminal binding site with 1 pM affinity. *J. Biol. Inorg. Chem.* **2007**, *12* (6), 913−918.
(26) Wezynfeld, N. E.; Stefaniak, E.; Stachucy, K.; Drozd, A.; Plonka, D.; Drew, S. C.; Krezel, A.; Bal, W. Resistance of Cu(Abeta4−16) to Copper Capture by Metallothionein-3 Supports a Function for the Abeta4−42 Peptide as a Synaptic Cu(II) Scavenger. *Angew. Chem., Int. Ed.* **2016**, *55* (29), 8235−8238. Research Support, Non-U.S. Gov't
(27) Stevens, R. W.; Elmendorf, D.; Gourlay, M.; Stroebel, E.; Gaafar, H. A. Application of fluoroimmunoassay to cerebrospinal fluid immunoglobulin G and albumin. *J. Clin. Microbiol.* **1979**, *10* (3), 346−350. From NLM Medline.
(28) van Steenoven, I.; van der Flier, W. M.; Scheltens, P.; Teunissen, C. E.; Lemstra, A. W. Amyloid-beta peptides in cerebrospinal fluid of patients with dementia with Lewy bodies. *Alzheimers Res. Ther.* **2019**, *11* (1), 83. From NLM Medline.
(29) Kirsipuu, T.; Zadorožnaja, A.; Smirnova, J.; Friedemann, M.; Plitz, T.; Tõugu, V.; Palumaa, P. Copper(II)-binding equilibria in human blood. *Sci. Rep.* **2020**, *10*, No. 5686.
(30) Jun, J. E.; Lee, S. E.; Lee, Y. B.; Jee, J. H.; Bae, J. C.; Jin, S. M.; Hur, K. Y.; Lee, M. K.; Kim, J. H. Increase in serum albumin concentration is associated with prediabetes development and progression to overt diabetes independently of metabolic syndrome. *PLoS One* **2017**, *12* (4), No. e0176209.
(31) Wallin, C.; Luo, J.; Jarvet, J.; Wärmländer, S. K. T. S.; Gräslund, G. The Amyloid-b Peptide in Amyloid Formation Processes: Interactions with Blood Proteins and Naturally Occurring Metal Ions. *Isr. J. Chem.* **2017**, *57*, 674−685.

https://doi.org/10.1021/acsomega.3c04649
ACS Omega 2023, 8, 33912−33919