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On the ability of CuAβ$_{1-x}$ peptides to form ternary complexes: Neurotransmitter glutamate is a competitor while not a ternary partner

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ABSTRACT

In the light of conflicting reports on the ability of copper(II) complexes of amyloid beta (Aβ) peptides to form ternary complexes with small molecules co-present in the biological milieu, we performed a study of coordination equilibria in the system containing Cu(II) ions, the Aβ$_{1-16}$ peptide, glutamic acid and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid, HEPES) buffer. Using potentiometry, isothermal titration calorimetry (ITC), UV–visible spectroscopy and EPR, we concluded that glutamic acid was not able to form such a ternary complex, but can efficiently compete for the Cu(II) ion with the Aβ peptide at Glu concentrations relevant for the synaptic cleft. We also found that the literature constants for Cu(II) complexes with Glu were overestimated, but this effect was partially compensated by the formation of a ternary Cu(Glu)(HEPES) complex. Our results indicate that small molecules co-present with Cu(II) ions and Aβ peptides in the synaptic cleft are not very likely to enhance Cu(II)/Aβ interactions, but instead should be considered as a Cu(II) buffering system that may help prevent these interactions and participate in Cu(II) clearance from the synaptic cleft.

1. Introduction

The Cu(II) interactions with Aβ peptides, predominantly Aβ$_{1-42}$ and Aβ$_{1-40}$, have been implicated in the etiology of Alzheimer's Disease (AD) [1–4]. Major lines of evidence described in the literature include association of Cu(II) ions with amyloid plaques composed of Aβ peptides isolated from post-mortem AD brains, enhancement of Aβ aggregation by Cu(II) ions, and support of Cu(II)/Cu(I) redox cycling by the Aβ peptides, yielding deleterious reactive oxygen species. Monomeric Aβ peptides bind one Cu(II) ion with a conditional stability constant at pH 7.4 ($^{C}K_{7.4}$) of 10^{10} M^{-1}, and another at least 100 times weaker [5,6]. These relatively low stability constants, determined for both the short model peptide Aβ$_{1-16}$, as well as the physiologically relevant Aβ$_{1-40}$ species, may be considered to weaken the proposal associating the Aβ neurotoxicity with Cu(II) binding, because of the presence of stronger Cu(II) ligands in the brain. Human serum albumin (HSA), which binds one Cu(II) ion with $^{C}K_{7.4}$ of 10^{12} M^{-1} [7], is abundant in the cerebrospinal fluid at a micromolar level [8]. Thus, a higher conditional stability constant for Cu(II) binding, compared with Aβ$_{1-x}$ peptides, co-localization with these peptides, and a high abundance of HSA suggest that this protein could effectively compete for Cu(II) with Aβ$_{1-x}$ peptides. Indeed, this protein was shown to withdraw Cu(II) from Aβ$_{1-x}$ peptides directly and quickly, and was suggested by several research groups, including us, to be a "brain guardian" preventing Aβ-associated copper toxicity [9–12]. Recently, we combined our experimental results with previous analytical reports to show that another Aβ species, namely Aβ$_{4-x}$ (x = 16 is the model peptide, x = 42 is a dominant brain species) binds one Cu(II) ion with $^{C}K_{7.4}$ of 3.4×10^{13} M^{-1}, and withdraws Cu(II) from the Aβ$_{1-x}$ species quantitatively within the time required for sample mixing [13]. Taking into account numerous reports that Aβ$_{4-x}$ species are at least as abundant as the Aβ$_{1-x}$ species in both healthy and AD brains [14–17], and that the main Cu(II) complex of the Aβ$_{4-x}$ peptide is redox inactive, one can wonder whether the Cu(II)-mediated Aβ toxicity may ever occur *in vivo*.

One way of resolving this dilemma is by considering that the aggregated Aβ$_{1-x}$ peptides could bind Cu(II) more strongly than the monomeric peptide. This idea was tested experimentally by two research groups, but conflicting results were obtained (i.e. no enhancement *vs.* a significant and progressive enhancement) [18,19]. However, the highest enhancement of binding reported [19] still would not overcome the affinity characteristic of the Aβ$_{4-x}$ peptides [13].

The formation of ternary complexes could be another way of enhancing Cu(II) binding to Aβ$_{1-x}$ peptides. The susceptibility of model

Abbreviations: Aβ, amyloid beta peptides; Aβ$_{1-16}$, DAEFRHDSGYEVHHQK-amide; AD, Alzheimer's Disease; B, fully deprotonated HEPES buffer molecule; $^{C}K_{7.4}$, conditional affinity constant at pH 7.4; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid or 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid; ITC, Isothermal Titration Calorimetry; L, fully deprotonated glutamic acid molecule; PBT2, 5,7-dichloro-2-[(dimethylamino)methyl]quinolin-8-ol.

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peptide $A\beta_{1-16}$ to formation of ternary complexes with small molecules and common buffers was postulated in our early paper [20]. Further research did not yield evidence for such abilities of HEPES buffer, but a recent report indicated the formation of a ternary Tris complex (although the reaction was studied only at pH 4.0) [21]. In our recent paper we demonstrated that $A\beta_{1-16}$ was able to form a ternary Cu(II) complex with 2-[(dimethylamino)methyl]-8-hydroxyquinoline, an analog of the experimental drug PBT2 (5,7-dichloro-2-[(dimethylamino)methyl]quinolin-8-ol) which was designed to compete Cu(II) from the Aβ complex [22]. Consideration of the most sui candidates for a ternary ligand in vivo led us to propose glutamic acid (Glu), since AD pathology initially affects glutamatergic synapses and the concentrations of Glu in these synapses during neurotransmission may exceed 1 mM [23]. We therefore sought to identify ternary complexes in the Cu(II)/$A\beta_{1-16}$/Glu system.

2. Experimental

2.1. Potentiometry

Potentiometric titrations of the Cu(II)/Glu and Cu(II)/Glu/HEPES systems were performed on a Titrando 907 automatic titrator (Metrohm), using a combined glass-Ag/AgCl electrode (InLab®Micro, Mettler Toledo, Switzerland), which was calibrated daily by nitric acid titrations [24]. 0.1 M NaOH (carbon dioxide free) was used as titrant. Sample volumes of 1.0–1.5 mL were used. For the binary Cu(II)/Glu system the samples contained 0.5–1.0 mM Glu free acid, dissolved in 4 mM HNO_3/96 mM KNO_3. The Cu(II) complex formation was studied for 1:2, 1:2.5, and 1:3 metal-to-ligand stoichiometries, using Cu(II) added as $Cu(NO_3)_2$. For the ternary system, the following sets of millimolar Cu(II)/Glu/HEPES concentrations were used: 1/2/2, 1/1/4 and 0.5/1/4. All experiments were performed under argon at 25 °C, in the pH range 2.3 to 12.2. The collected data were analyzed using the SUPERQUAD and HYPERQUAD programs [25,26]. Three to five titrations were included simultaneously into calculations, separately for Glu and HEPES protonation, binary Cu(II) complexes and the ternary system.

2.2. UV–visible (UV–vis) spectroscopy

UV–vis spectroscopy was used to characterize the formation of Cu(II) complexes with Glu, HEPES, and $A\beta_{1-16}$ (synthesized according to the Fmoc strategy and purified in our laboratory as described [13]). The UV–vis spectra were recorded at 25 °C on a LAMBDA 950 UV/vis/NIR spectrophotometer (PerkinElmer), over the spectral range of 250–850 nm. The optical path for all experiments was 1 cm. The concentration of $A\beta_{1-16}$ was determined using an extinction coefficient of 1400 M^{-1} cm^{-1} at 275 nm [13].

In acid/base titrations of Cu(II)/Glu and Cu(II)/Glu/HEPES systems, 0.5 mM Cu(II), 0.45:1 Cu(II)-to-Glu molar ratio, and 0.45:1:1 Cu(II)-to-Glu-to-HEPES molar ratio were used. The samples were titrated with NaOH by careful manual additions of very small amounts of the concentrated base solution, in the pH range of 2.9–11.0.

In separate experiments Cu(II)/$A\beta_{1-16}$ (0.9:1 ratio) or Cu(II)/Glu (0.9:1 ratio) in 50 mM HEPES (pH 7.4) was titrated with 40 mM Glu in the same buffer, to the final excess of Glu over Cu(II) of 77 for each sample. The initial concentrations of Cu(II) were equal to 0.5 mM.

2.3. Isothermal titration calorimetry (ITC)

Calorimetric titrations were carried out on the Nano ITC Standard Volume calorimeter (TA Instruments). The sample cell (950 μL) was filled with a Cu(II) and $A\beta_{1-16}$ solution (with metal to peptide ratio of 0.9:1) in 50 mM HEPES buffer at pH 7.4. Concentrations of the resulting Cu(II)/$A\beta_{1-16}$ complex were within the range of 48–600 μM. The syringe (250 μL) was loaded with 10 mM Glu in 50 mM HEPES at pH 7.4. Typically, 8 μL of the Glu solution was added to the Cu(II)/$A\beta_{1-16}$ complex solution at 1000 s intervals using a stirring speed of 200 rpm. The measurements were performed at 25 °C.

2.4. EPR spectroscopy

Samples containing Cu(II)/$A\beta_{1-16}$/Glu in the ratio 1:1:n ($n = 0$–100) were prepared in 20 mM HEPES pH 6.9. Copper was added from a 10 mM $^{65}CuCl_2$ stock to a final concentration of 200 μM, with 10% v/v glycerol was included as a cryoprotectant as described previously [22,27]. Reference spectra corresponding to $CuA\beta_{1-16}$ and $CuGlu_2$ were obtained using Cu(II)/$A\beta_{1-16}$ 0.9:1 and Cu(II)/Glu 1:50 mixtures, respectively.

X-band (9.45 GHz) continuous-wave EPR spectra were obtained at 77 K using a Bruker Elexsys E500 spectrometer fitted with a Bruker super-high-Q probehead (ER 4122SHQE) and a quartz cold finger insert (Wilmad, WG-816-B-Q). The following experimental settings were used: microwave frequency, 9.45 GHz; microwave power, 10 mW; magnetic field modulation amplitude, 5 G; field modulation frequency, 100 kHz; receiver time constant, 164 ms; receiver gain, 56 dB; sweep rate, 6.67 gauss s^{-1}. Baseline correction was performed by subtraction of a spectrum of buffer only.

To deconvolute the Cu(II)/$A\beta_{1-16}$/Glu 1:1:n spectra, all of the experimental first-harmonic spectra were first normalized by double-integration. A reconstruction of the Cu(II)/$A\beta_{1-16}$/Glu 1:1:n spectra was then made by weighted addition of normalized $CuA\beta_{1-16}$ and $Cu(Glu)_2$ spectra, with the weightings adjusted in order to minimize the mean-squared deviation between the reconstruction and the Cu/$A\beta_{1-16}$/Glu 1:1:n spectrum. The speciation of the CuGlu spectral component was at most only a few percent for all conditions and was therefore not explicitly included in the fits.

2.5. Computational methods

Calculations were performed with the use of a custom-made software implemented in the Wolfram Mathematica 9 environment, which serves the purpose of determination of binding constants in an investigated system, along with the deconvolution of spectroscopic data in the course of the fitting procedure.

The binding constants for Cu(II)/Glu and Cu(II)/Glu/HEPES systems were obtained from potentiometric experiments. The species concentration curves were calculated from these binding constants and deconvolution of the spectra was performed by the linear least square fitting to the aforementioned curves. The UV–vis spectra of $CuHL^+$, CuL and CuL_2^{2-} complex species (L denotes fully deprotonated glutamic acid molecule) were obtained by global deconvolution of acid/base titrations and Glu titrations of the Cu(II)/HEPES samples. The spectrum of the $CuLB^-$ complex (B denotes fully deprotonated HEPES buffer molecule) was obtained from the deconvolution of pH titration spectra in the presence of HEPES, with all other spectra fixed. Subsequently, the results of the deconvolution were applied to the experiment in which the Cu(II)/$A\beta_{1-16}$ samples were titrated with Glu. The full set of UV–vis spectra was fully explained by the competition model where the Cu(II) ion was largely transferred from the $CuA\beta_{1-16}$ complex to the CuL_2^{2-} complexes. To account for the dilution of sample constituents, actual total concentrations of Cu(II), $A\beta_{1-16}$, Glu, and HEPES were considered in calculations for every corresponding raw spectrum.

In the calorimetric experiments satisfactory fits were obtained for the competition reaction model where Cu(II) ions were extracted from the $CuA\beta_{1-16}$ complex to CuL and subsequently CuL_2^{2-} complexes, with traces of the $CuLB^-$ complex. The assumption of a model including the formation of a ternary $CuA\beta_{1-16}$L complex had no significant impact on the quality of the fits.

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3. Results

3.1. Preliminary studies

Our initial strategy of determination of coordination equilibria in the Cu(II)/Aβ$_{1-16}$/Glu system was based on three independent experimental methods. ITC titrations of equimolar Cu(II)/Aβ$_{1-16}$ samples with a glutamic acid solution were followed by deconvolution of titration curves with the affinity constants of both component binary systems (Cu(II)/Aβ$_{1-16}$ and Cu(II)/Glu) taken from previous studies. UV–vis studies were aimed at providing independent support for these deconvolutions, and EPR experiments were anticipated to provide us with parameters of the ternary complex(es) that would help us in deciphering its(their) structure.

The ITC experiments were performed in a 50 mM HEPES buffer at pH 7.4. We used the $^{C}K_{7.4}$ value for the CuAβ$_{1-16}$ complex, established by spectroscopic titrations and the full set of logarithmic values of cumulative stability constants (log β) for the Cu(II)/Glu system presented in the literature [5]. The calculations performed using only the binary systems indicated a need to include additional species, which we initially interpreted as evidence for a ternary complex. The fits were, however, unsatisfactory (Fig. S1). We did not include HEPES in our initial considerations, because it would not interact with the Cu(II)/Aβ$_{1-16}$ system under these experimental conditions [5,28], but then we realized that HEPES, present at a high molar excess over Cu(II) and Glu might interfere with the Cu(II)/Glu system, by forming a ternary complex [29]. Therefore we decided to re-evaluate the Cu(II)/Glu/HEPES system by potentiometry and UV–vis spectroscopy and reanalyze the ITC results using the newly established stability constants.

3.2. Potentiometric and UV–vis studies of the Cu(II)/Glu/HEPES system

These experiments started with the potentiometric and UV–vis studies of the Cu(II)/Glu system. The stability constants obtained by potentiometry are presented in Table 1. The potentiometric results were validated by spectrophotometric pH titrations.

Examples of UV–vis acid/base titrations are shown in Fig. 1. In addition, we performed spectrophotometric titrations of Cu(II)/Glu (0.9:1) with Glu in 50 mM HEPES at pH 7.4. The spectra from all titrations were pooled together in calculations, and deconvoluted according to the coordination model using the stability constants presented in Table 1. The spectral parameters obtained are presented in Table 2.

Table 1
Protonation constants and stability constants of Cu(II) complexes (log β values) of glutamic acid (L) and HEPES (B) at I = 0.1 M (KNO_3) and 25 °C.

Species	log β (this work)[a]	log β (reference study)[b]
Glutamic acid		
H_3L^+	15.92(2)	16.27
H_2L	13.811(9)	13.99
HL^-	9.603(6)	9.746
$CuHL^+$	12.82(2)	12.73
CuL	8.24(1)	8.545
CuH_2L_2	n.d.[c]	25.18
$CuHL_2^-$	n.d.	20.57
CuL_2^{2-}	14.32(2)	15.22
HEPES		
H_2B^+	10.53(1)	10.397
HB	7.377(4)	7.409
CuB^+	3.22[d]	3.22
Ternary		
$CuLB^-$	10.71(7)	n.d.

[a] Standard deviations on the least significant digits, provided by HYPERQUAD [24], are given in parentheses.
[b] Ref. [30] for Glu, ref. [28] for HEPES.
[c] n.d.; not detected.
[d] value not fitted, but found to be correct in the course of potentiometric calculations.

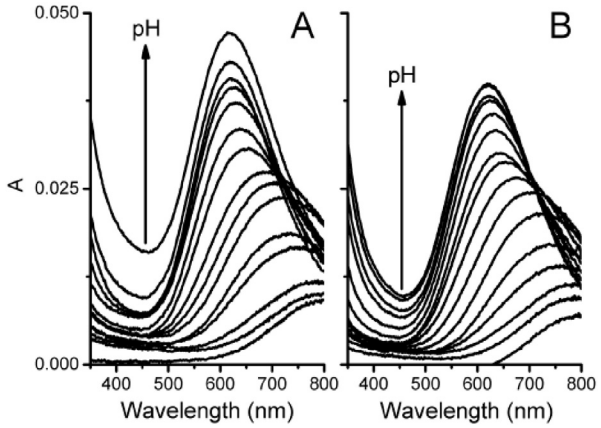

Fig. 1. UV–vis spectra of Cu(II) complexes with Glu (A) and with Glu/HEPES (B) at different pH values. The spectra were recorded at 25 °C for samples containing 0.5 mM $Cu(NO_3)_2$ and 1.2 mM Glu or/and 1.2 mM HEPES.

The deconvolution confirmed the presence of the ternary complex formed by Cu(II) with Glu and HEPES, with a $CuLB^-$ stoichiometry (Figs. 2, S2-S4).

3.3. UV–vis and EPR studies of the Cu(II)/Aβ$_{1-16}$/Glu/HEPES system

The next step was to perform UV–vis monitored titrations of the Cu(II)/Aβ$_{1-16}$ system in HEPES at pH 7.4 with a concentrated (40 mM) Glu solution. These experiments allowed us to test the working hypothesis assuming the presence of ternary Cu(II)/Aβ$_{1-16}$/Glu complexes. The model without a ternary complex fully explained changes of absorbance during titrations. The values of absorbance for selected wavelengths along with the best fit to the final coordination model are presented in Fig. 3. In line with the UV–vis observations, changes in the EPR spectra during titration of Glu into 1:1 Cu(II)/Aβ$_{1-16}$ could be accounted for by the presence of only binary CuAβ$_{1-16}$ and $CuGlu_2$ complexes (Fig. 4).

3.4. ITC studies of the Cu(II)/Aβ$_{1-16}$/Glu/HEPES system

We also performed ITC experiments analogous to UV–vis titrations. First, from a titration of Aβ$_{1-16}$ in HEPES at pH 7.4 with an aqueous Cu(II) solution we determined the ΔH value for Aβ$_{1-16}$ (−34.7 ± 1.6 kJ/mol), which we applied in the calculations of parameters of the four-component system where the Glu solution in HEPES was titrated to Cu(II)/Aβ$_{1-16}$ samples in HEPES. Control experiments showed that Glu did not interact with HEPES and Aβ$_{1-16}$ in the absence of Cu(II) ions (Fig. S6 in the Supplementary Information file). For the Cu(II)/Aβ$_{1-16}$/Glu/HEPES system we used a range of concentrations of Cu(II)/Aβ$_{1-16}$ samples from 0.048 to 0.600 mM, but maintained the concentrations of HEPES and added (syringe) Glu constant at 50 and 10 mM, respectively. Thus, the results were obtained for a range of ratios of HEPES and Glu to other reagents. This allowed us to test the existence of all potential ternary complexes. The global fit of the model assuming the following complexes: CuAβ$_{1-16}$, CuL, CuL_2^{2-} and $CuLB^-$ is shown in Fig. 5. After taking into consideration the existence of $CuLB^-$ we obtained a better fit than the one mentioned in the Section 3.1. The

Table 2
Parameters of deconvoluted UV–vis spectra (the least square fitting parameters were $\chi^2 = 2.42 \times 10^{-5}$ and $R^2 = 0.999994$).

Species	λ_{max} (nm)	ε (M^{-1} cm^{-1})
$CuHL^+$	734	41
CuL	706	47
CuL_2^{2-}	617	72
$CuLB^-$	643	48

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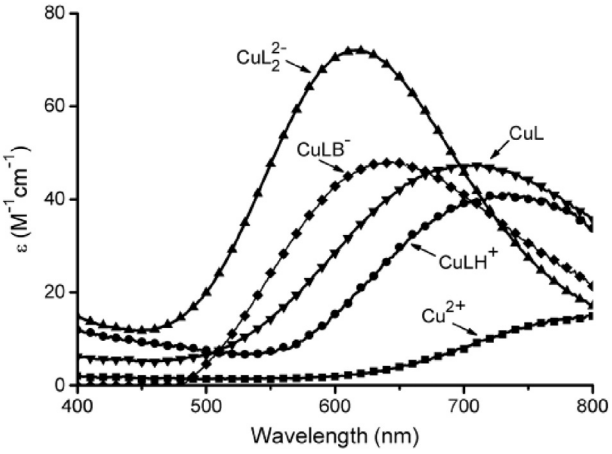

Fig. 2. The spectra of Cu(II) complexes with glutamic acid and HEPES, calculated on the basis of potentiometric data shown in Table 1, and UV–vis titration data. L denotes Glu, B denotes HEPES.

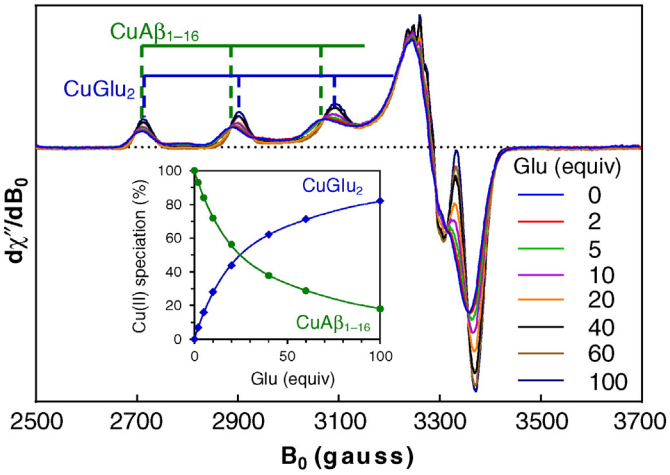

Fig. 4. X-band (9.45 GHz) EPR spectra (77 K) monitoring of the titration of Glu into a solution containing 0.2 mM Cu/Aβ$_{1-16}$ 1:1 in HEPES pH 6.9 with 10% *v*/v glycerol. Dashed vertical lines show resolved hyperfine lines corresponding to $A_{||}(^{65}Cu)$ for CuAβ$_{1-16}$ and $CuGlu_2$. The speciation curves (inset) were obtained by deconvolution of the spectra (shown in Fig. S5 in the Supplementary Information file).

comparison of these fits is presented in Fig. S7 in the Supplementary Information file. The addition of hypothetical ternary complexes (other than $CuLB^-$) containing Cu(II), Aβ$_{1-16}$ and Glu did not improve the fit, because the predicted amounts of such complexes were far below the error of the estimate. This result was also consistent with UV–vis and EPR results.

4. Discussion

4.1. Ternary Cu(II) complexes of Aβ$_{1-16}$

Using potentiometry, ITC, UV–vis and EPR we obtained a comprehensive description of Cu(II) coordination equilibria in the presence of Aβ$_{1-16}$, Glu and HEPES. Contrary to our initial expectations, we proved the absence of ternary Cu(II) complexes with Aβ$_{1-16}$ and Glu. This finding has important consequences. Previously we determined the structure of a ternary complex formed by Cu(II) ion with Aβ$_{1-16}$ and 2-[(dimethylamino)methyl]-8-hydroxyquinoline [22]. In that complex the main chelating agent was the terdentate quinoline, and the

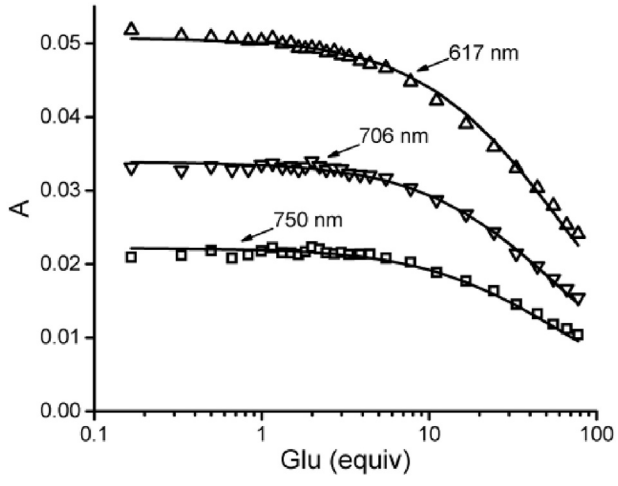

Fig. 3. Absorbance values in the Cu(II)/Aβ$_{1-16}$/Glu/HEPES system in a function of a molar ratio of Glu to Cu(II). A Glu solution (40 mM) in 50 mM HEPES (pH 7.4) was added to Cu(II)/Aβ$_{1-16}$ in 50 mM HEPES (pH 7.4). The best fit for the coordination model considering the presence of Cu(II)/Aβ$_{1-16}$, Cu(II)/HEPES, Cu(II)/Glu/HEPES, Cu(II)/Glu, and Cu(II)/Glu_2 is represented by solid lines, at various wavelengths. The following wavelength values were selected for illustration of whole spectra fits: 617 nm (λ_{max} for CuL_2), 706 nm (λ_{max} for CuL), and 750 nm (chosen as a wavelength at which all complex species present in the equilibrium absorb significantly).

contribution of Aβ$_{1-16}$ to the 1st coordination sphere was via a single His residue. These two facts suggest a general rule, according to which Aβ$_{1-16}$, and more generally, Aβ$_{1-x}$ peptides, form ternary complexes preferentially by providing auxiliary His ligands to complete the coordination sphere of the Cu(II) ion pre-formed by terdentate chelating ligands. This in turn is possible when the stability constant of the binary complex with the terdentate ligand is higher than that of the CuAβ$_{1-x}$ complex. Glutamic acid, and generally amino acids except perhaps His, do not meet this criterion. Incidentally, this finding provides an additional argument for the validity of the method of determination of binding constants of Cu(II) complexes with Aβ peptides based on Gly as competitive ligand [5].

4.2. Re-evaluated stability constants for the Cu(II)/Glu system and Cu(II) binding in the synaptic cleft

The full characterization of the Cu(II)/Aβ$_{1-16}$/Glu system in terms of stability constants empowered us to investigate the potential impact of the neurotransmitter (Glu) on the likelihood of formation of Cu(II) complexes of Aβ$_{1-x}$ peptides in the synaptic cleft, the most probable localization of neurotoxic reactivity of these Aβ$_{1-x}$ peptides [1]. We calculated the distribution of Cu(II) ions between Aβ$_{1-16}$ and Glu for several concentrations of reagents possibly relevant for the conditions in the synaptic cleft (Fig. 6). Glu is a neurotransmitter, with peak concentrations higher than 1 mM shortly after its release from the presynaptic neuron, but falling to low micromolar values prior to the next burst [23]. To simplify these model calculations, we fixed Glu concentration at 2 mM, to represent this burst. We set Cu(II) and Aβ to be equimolar and varied their concentrations between 1 μM and 300 μM. The lower limit corresponds to just a few atoms/molecules in the synaptic cleft, the upper one is similar to the highest Cu(II) concentration observed during neurotransmission [31,32], and the average concentration of Cu(II) in the synaptic cleft, derived from measurements done in synaptosomes, of 14 μM, is within this range [33]. Interestingly, our calculations show that Glu could significantly reduce the amount of Aβ bound Cu(II) ions, especially at a low abundance of CuAβ. Therefore, Glu and similar small molecules could have an additional function in clearing Cu(II) ions from the synaptic cleft, and thus preventing the formation of deleterious CuAβ$_{1-x}$ complexes. Notably, under the conditions of our calculations the abundance of Glu bound Cu(II) ions never exceeded 1% of the total neurotransmitter, and thus this hypothetical interaction would not likely interfere with neurotransmission.

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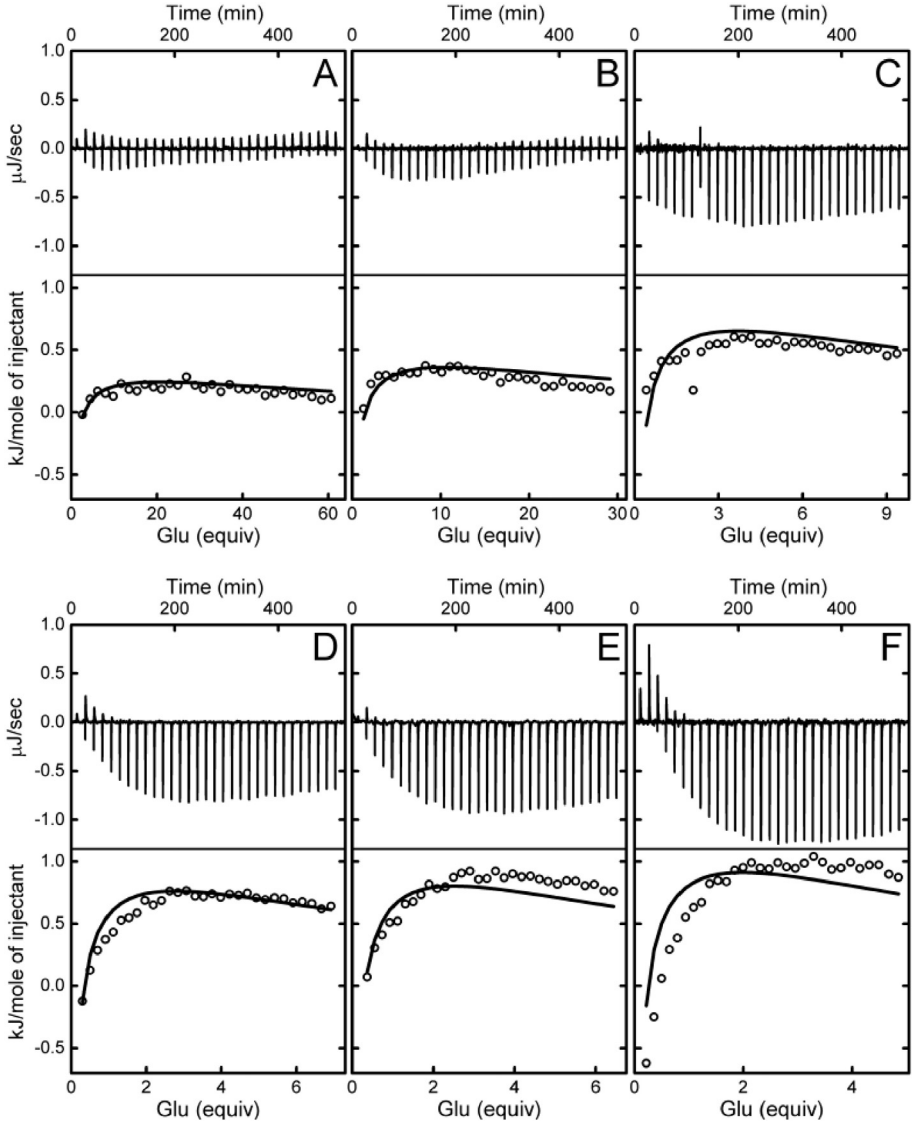

Fig. 5. ITC titrations of Cu(II)/Aβ_{1-16} (0.9/1) in 50 mM HEPES at pH 7.4 and 25 °C. The initial concentrations of Cu(II) were 0.048 (A), 0.100 (B), 0.311 (C), 0.421 (D) 0.466 (E), and 0.600 mM (F). The upper plots show raw data from the experiments; the lower plots show the heat in each injection (open dots) after subtraction of heat of Glu dilution (data from Fig. S6) in a function of molar ratio of Glu to Cu(II), with the fitting of the model assuming the presence of the following set of species: Cu(II)/Aβ_{1-16}, Cu(II)/HEPES, Cu(II)/Glu/HEPES, Cu(II)/Glu, and Cu(II)/Glu_2. Values of log β were derived from ref. [5] for Cu(II)/Aβ_{1-16}, ref. [28] for Cu(II)/HEPES, and from potentiometric experiments (this paper) for all other complexes.

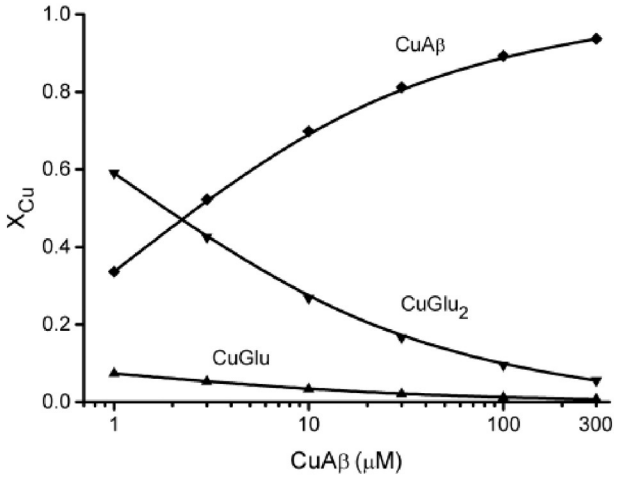

Fig. 6. Cu(II) species distribution simulated for a range of equimolar concentrations of Cu(II) and Aβ (from 1 to 300 µM) and 2 mM Glu at pH 7.4.

5. Conclusions

Our study provided three significant results. The nonexistence of ternary complexes with Glu (and, more broadly, with amino acids) in a system containing equimolar Cu(II) and Aβ provides a significant benchmark for studies of chemistry of these complexes. A tentative rule on formation of such ternary complexes will assist studies on Cu(II) chelating agents as potential AD drugs. Finally, our quantitative results indicate that Glu released in the process of neurotransmission may be part of the synaptic clearance system for Cu(II) ions preventing the formation of neurotoxic complexes of Aβ peptides.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jinorgbio.2016.02.035.

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