PROBING OF Cu^{2+} IONS BINDING TO A$\beta_{(5-16)}$ PEPTIDE USING ITC MEASUREMENTS AND MD SIMULATIONS

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(received: 15 September 2016; revised: 12 October 2016;
accepted: 21 October 2016; published online: 31 October 2016)

Abstract: It is shown that probably three residues: His6, His14 and His16 in the original sequence A$\beta_{(1-42)}$ serve as metal-binding sites for Cu^{2+} ions. On the other hand, there is a possibility that only one of them plays a crucial role in the formation of the {A$\beta_{(1-42)}$–Cu^{2+}} complex. The isothermal titration calorimetry (ITC) measurements supported by molecular dynamic simulation (MD) with the NMR-derived restrains were used to investigate the interactions of Cu^{2+} with A$\beta_{(5-16)}$, a fragment of the A$\beta_{(1-42)}$ protein, with the following sequence: Ac–Arg–His–Asp–Ser–Gly–Tyr–Glu–Val–His–His–Gln–Lys–NH_2, termed HZ1. The conditional thermodynamic parameters suggest that the formation of the Cu^{2+}–HZ1 complex is both an enthalpy and entropy driven process under the experimental conditions. The studies presented here (after comparison with our previous results) show that the affinity of peptides to copper metal ions depends on two factors: the primary structure (amino acid composition) and the shape of the peptide conformation adopted.

Keywords: A$\beta_{(5-16)}$ fragments; metal-peptide binding; isothermal titration calorimetry; molecular dynamics simulations

DOI: https://doi.org/10.17466/tq2016/20.4/p

1. Introduction

The major components of neuritic plaques found in the Alzheimer disease (AD) are peptides known as amyloid beta-peptides (Aβ), which derive from

the proteolitic cleavage of the amyloid precursor proteins [1]. It has been already known that the A$\beta_{(1-42)}$ polypeptide adopts helical conformations in aqueous solutions: two helical regions encompassing residues 8–25 and 28–38, connected by a regular type I beta-turn. According to the earlier proposed mechanism, nicotine [2], cotinine [3] or trigonelline [4] bind the segment of Aβ between amino acids 1–28 when folded in an alfa-helical conformation. Therefore, nicotine, cotinine as well as trigonelline probably inhibit the conformational change from alfa helix to the beta-sheet conformation (inhibit the Aβ aggregation process) known as the amyloidogenic conformation and the neurotoxic form. On the other hand, it has been proven that high concentrations (in the millimolar range) of several transition-metal ions, mainly Cu^{2+}, Zn^{2+}, and Fe^{3+}, may induce a formation of the amyloid plaques (the Cu^{2+} and Zn^{2+} being bound to the Aβ peptide) [5–7]. Moreover, it is known that, A$\beta_{(1-42)}$ aggregates, unlike their monomeric form, can seize copper from the human serum albumin, an abundant copper-containing protein in the brain and cerebrospinal fluid [8]. However, the role of metal ions in the AD etiology is still unclear. Hence, the understanding of the relationship between the influences of systems mentioned above on the amyloid formation is limited and the results are not conclusive, showing the need for further studies on the binding mechanism of peptides. It has been shown that probably three residues: His6, His14 and His16 [9, 10] in the original sequence A$\beta_{(1-42)}$ serve as metal-binding sites for Cu^{2+} ions. On the other hand, there is a possibility that only one of them plays a crucial role in the formation of the {A$\beta_{(1-42)}$–Cu^{2+}} complex [9]. However, it should be noted that binding metals may not only determine the activity of the peptides but also act as linkers between peptides, especially when metal binding residues act as connectors to fix a functional group or conjugate another binding motif through one or more metal ions. The type of the metal binding site and its position in the peptide not only determine the structure of the assemblies but also have a significant influence on the properties of the assemblies.

In our previous studies we have demonstrated the affinity of Cu^{2+} ions to the peptides which are very mobile and have a tendency to be bent [11]. In this study we have taken into consideration a peptide which adopts a more rigid structure in aqueous solutions where most of the side chains in the central part of the sequence are inaccessible for other systems. We wanted to show (in comparison to our earlier investigations) that the affinity of peptides to metal ions depends not only on the primary structure (amino acid composition) but also – to a large extent – on the shape of the peptide conformation adopted. We report herein the type of interactions between A$\beta_{(5-16)}$, the peptide fragment of the A$\beta_{(1-42)}$ polypeptide: Ac–Arg–His–Asp–Ser–Gly-Tyr–Glu–Val–His–His–Gln–Lys–NH_2 hereafter referred to as HZ1, and Cu^{2+} ions estimated by using isothermal titration calorimetry and MD calculations.

2. Materials and methods

2.1. Materials

The HZ1 peptide was synthesized using the procedure described in our previous work [12]. The structure of the A$\beta_{(1-42)}$ polypeptide (PDB id code 1IYT) [1] and the sequence of the HZ1 peptide derived from A$\beta_{(1-42)}$ are shown in Figure 1. The purity of the peptide was confirmed by the analytical HPLC and MALDI-TOF analysis. Copper (II) nitrate was obtained from Sigma-Aldrich. The stock solution of Cu^{2+} (1 mM) was prepared by dissolving an appropriate amount of $Cu(NO_3)_2$ in 20 mM of a MES buffer (pH 6). All stock solutions were stored at 4°C.

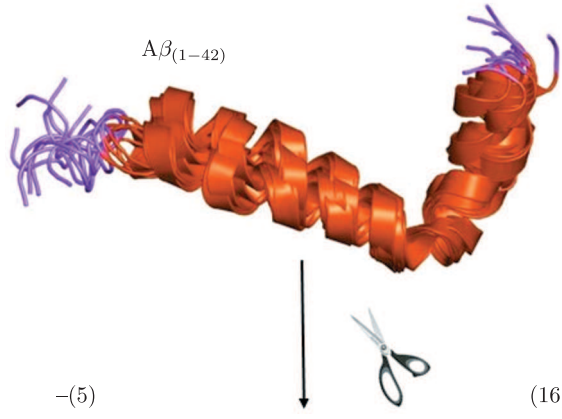

Figure 1. The structure of A$\beta_{(1-42)}$ polypeptide (PDB id code: 1IYT) [1] and the sequence of the peptide derived from A$\beta_{(1-42)}$ which was selected to be synthesized and analyzed (HZ1)

2.2. Methods

2.2.1. Isothermal titration calorimetry

The ITC experiment was performed at 298.15 K using the AutoITC isothermal titration calorimeter (MicroCal Inc Northampton, USA) with the 1.4491 mL sample and reference cells. The reference cell contained distilled water. All the details of the measuring devices and the experimental setup have been described previously [13, 14]. The reagent was dissolved directly in the 20 mM buffer solution of MES. The pH of the buffer solution was adjusted to 6.0 with 100 mM $HClO_4$. The experiment consisted of injecting 10.02 μL (29 injections, 2 μL for the first injection only) of the 1 mM buffered solution of HZ1 into the reaction cell that initially contained 0.1 mM of the buffered solution of Cu^{2+} ions. A background titration, consisting of an identical titrant solution but with the buffer solution in the reaction cell only, was removed from each experimental titration to account for the heat of dilution. All solutions were degassed prior to the titration. The titrant was injected at 5-min intervals to ensure that the titration peak

returned to the baseline before the next injection. Each injection lasted 20 s. For the sake of homogeneous mixing in the cell, the stirrer speed was kept constant at 300 rpm.

2.2.2. Molecular dynamics simulations (MD) with NMR-derived restrains

The NMR experiments of the HZ1 peptide were measured on the Bruker AVANCE 700 MHz spectrometer in the NMR Laboratory at the Faculty of Chemistry (University of Gdansk). The following $2D^1H^{-1}H$ NMR spectra were recorded: DQF-COSY, TOCSY (80 ms) and NOESY (250 ms) at 283 K. The samples were dissolved in $H_2O/^2H_2O$ (9:1 by vol.). The concentration of the sample was about 5 mM. The spectra were processed by using the Sparky program [15].

Molecular dynamics calculations (MD) were carried out by using the AMBER 11 [16] program with the AMBER ff99SB force field [17] at a constant volume and temperature (the NVT scheme). All simulations were performed in a periodic box of TIP3P water with the particle-mesh Ewald procedure for long-range electrostatic interactions at $T = 283$ K. At first, we estimated the shape of the dominant conformation in the solution for the HZ1 peptide. Next, we carried out MD simulations with the Cu^{2+} ion added to HZ1. In simulations for HZ1, both the distance restraints resulting from NOEs (175) and restraints on dihedral angles (32) resulting from the coupling constants were included. Additionally, "anti-NOE" restraints were included that prevented the protons of the pairs with no NOEs observed from being close to each other. This approach should minimize any bias from the AMBER ff99SB force field which seems to favor turn and α-helical conformations.

The total simulation time was 10 ns for each trajectory, and the integration time step was 2 fs. For such a short peptide (12 amino acid residues) the MD simulations with the NMR-derived restrains with time 10 ns is enough to reach satisfactory, stabilized systems (this is the standard time for such short systems especially when the MD is based on the experimental data). In considering peptide structures, it should always be kept in mind that, differently from proteins, peptides can be too small to assume a "globular" structure. In fact, peptides seldom adopt a single, well defined structure in a solution, which makes the interpretation of structural data more contentious for peptides than it is for proteins. However, we assessed the convergence of simulations by monitoring the histograms of distances for the last stage of MD simulations of: the Arg1(N)−Lys1(C) and His2(C)−Gln11(C) for the HZ1 peptide over time windows. HZ1 after 10 ns of MD became quite stable (data not shown). The set of final conformations was clustered by using the MOLMOL program [18]. MOLMOL uses a hierarchical minimal spanning tree method [19–22]. The rms-deviation cut-off value of ~ 1.7 Å for peptide over the Asp3−His10 residues was used for the clustering. That relatively small rmsd cut-off value was achievable because of the small ability to move the HZ1 system.

3. Results

3.1. Isothermal titration calorimetry

The stoichiometry (N) and the binding enthalpy (ΔH) of the Cu^{2+}–HZ1 complex were obtained directly from the ITC experiments by fitting the binding isotherms, using nonlinear least-squares procedures, to a model that assumes a single set of identical binding sites. These parameters depend on the condition under which the ITC experiments were performed (*i.e.* temperature, pH as well as the kind of the buffer solution). Thus, the obtained data are considered as condition-dependent parameters [23]. Then, the entropy change ($\Delta_{\mathrm{ITC}}S$) was calculated using the standard thermodynamic relationship: $\Delta_{\mathrm{ITC}}G = -RT\ln K_S = \Delta_{\mathrm{ITC}}H - T\Delta_{\mathrm{ITC}}S$. They are collected in Table 1 [11].

Table 1. The thermodynamic parameters of Cu^{2+} binding to HZ1 in 20 mM a MES buffer (pH 6, 298.15 K) [11]

Parameter	HZ1
N(Cu^{2+}:peptide molar ratio)	1.25
$\Delta_{\mathrm{ITC}}H$ (kcal/mol)	−2.28
$T\Delta_{\mathrm{ITC}}S$ (kcal/mol)	3.72

The stoichiometry of the resulting Cu^{2+}–HZ1 complex equals 1:1. The negative binding enthalpy ($\Delta_{\mathrm{ITC}}H$) and the favorable entropy factor ($T\Delta_{\mathrm{ITC}}S$) suggest an important contribution of hydrogen bonding as well as hydrophobic interactions to the Cu^{2+}–HZ1 affinity. Furthermore, based on the $\Delta_{\mathrm{ITC}}H$ and $T\Delta_{\mathrm{ITC}}S$ values (Table 1) it can be concluded that the formation of the complex is both an enthalpy and entropy driven process. The negative value of the enthalpy indicates that the endothermic effects connected with the dehydration of both the Cu^{2+} ion and the peptide are overcompensated by an exothermic effect due to the formation of new Cu^{2+}-peptide bonds (Table 1). The entropic gain may arise from the desolvation of Cu^{2+} and HZ1 upon the interaction.

3.2. MD calculations

The main family of conformations of the HZ1 peptide, obtained by MD simulations with time-averaged restraints derived from NMR measurements and clustered by using the MOLMOL [18] program is shown in Figures 2 A and B. The dominant conformation of HZ1 forms a bent shape structure which is very stable in section Ser4–His10 (Ser8–His14 in the original $A\beta_{(1-42)}$ polypeptide) and only the ends are flexible while the central part seems to have a well-defined bent structure. It has been shown that some short protein fragments can fold in an aqueous solution into conformations with a similar shape to that assumed in the parent protein [24]. If local interactions are important in the protein stability, it should be expected that isolated secondary structure elements will tend to populate native-like structures in the absence of tertiary contacts. The average conformation of the main family of HZ1 superposes quite well on the corresponding section of the

native A$\beta_{(1-42)}$, especially when we compare the orientation of residues presented in the central part of the sequence with the most expanded side chains: Asp7, Tyr10, His14 (see Figure 2 (a) and (b)).

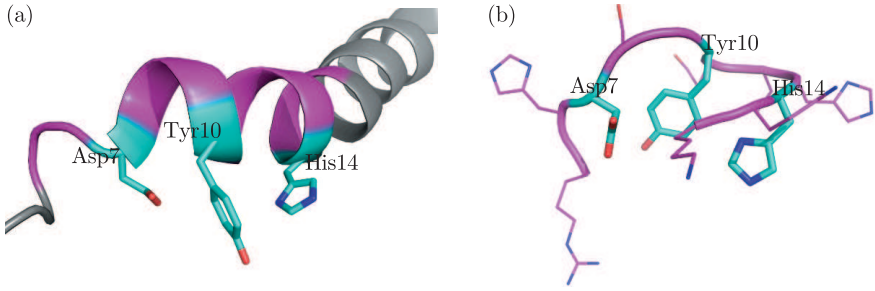

Figure 2. The {6–15} fragment of A$\beta_{(1-42)}$ polypeptide structure (a) and the most representative conformation ofthe HZ1 peptide (b) with marked side chains of Asp7, Tyr10, His14 residues

However, as it has been already mentioned three residues (His6, His14 and His16) [9, 10] in the original A$\beta_{(1-42)}$ sequence serve as metal-binding sites for Cu^{2+} ions or only one of them. Our investigation reported in this paper showed that for the A$\beta_{(1-42)}$ part named here as HZ1, the most plausible whereabouts of the Cu^{2+} were nearby the terminal parts (see Figure 3) of the sequence. Our investigation showed that Cu(II) ion was localized probably close to the flanked residues of HZ1 and this explains the small changes in the distance between

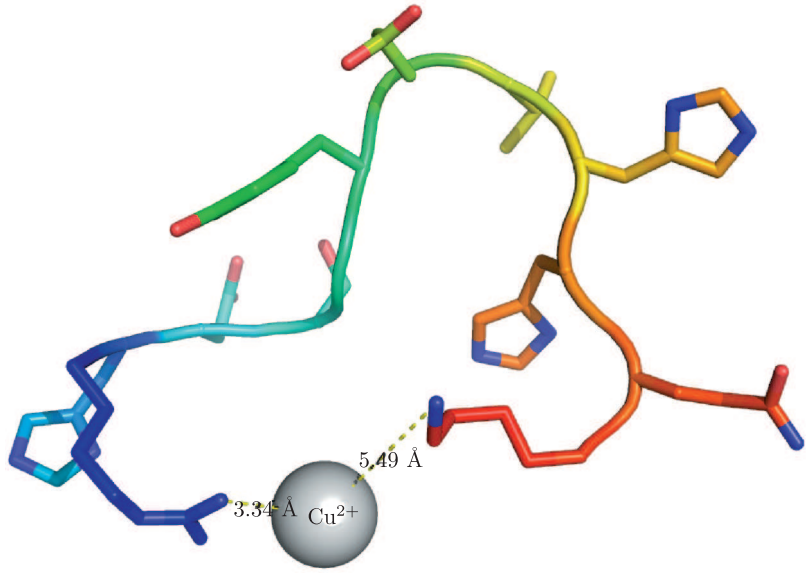

Figure 3. Probable location of the Cu^{2+} ion in the HZ1 peptide region (distances between Cu^{2+} and N atoms of the side chains of Arg and Lys residues were defined by the center of mass)

residues Arg 1 and Lys12 in the final stage of the molecular dynamics. However, it should be noted that that the distance between the metal ion and the system HZ1 was only estimated. During the MD simulations, the Cu(II) ion was most often located close to the terminal region of HZ1 system but it was permanently mobile in this region. To obtain more accurate results a more polarizable force field, which can better describe the polarization effect to simulate a system such as {HZ1/Cu(II)} should be used.

During the molecular dynamics calculations of HZ1 the system was very stable and interestingly quite stiff. The central part of the peptide chain seems to be unavailable for other systems because of the occurrence of expanded side chains in this region. The most available parts of the peptide chain for the Cu(II) ion are terminal segments.

4. Conclusions

The stoichiometry of the Cu^{2+}–HZ1 complex (1:1) and the thermodynamic parameters ($\Delta_{ITC}H$, $\Delta_{ITC}S$) were determined with the use of isothermal titration calorimetry. The conditional thermodynamic parameters suggest that the formation of the Cu^{2+}–HZ1 complex is both an enthalpy and entropy driven process under the experimental conditions. The MD calculations allowed us to explain the difference in the values of ΔH for HZ1 and other systems which had been previously determined experimentally [11]. It is supposed that the difference in both the donor-acceptor bond lengths and donor-acceptor properties of hypothetical atoms involved in the interaction constitutes the most important factor responsible for the release of a larger amount of energy during the formation of the Cu^{2+}–HZ1 complex. Furthermore, it can be concluded that the affinity of the peptides to copper metal ions depends on two factors: the primary structure (amino acid composition) and the shape of the peptide conformation adopted.

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