Interaction of Heavy Metal Ions with Glycyl-L-tryptophan in the Presence of Amyloid-β Peptides

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Metal complexes of amyloid-β peptides (Aβ), associated with Alzheimer's disease (AD), are increasingly investigated, being involved in toxic oligomer formation and fibrillogenesis. The metal ions such as copper, zinc, and iron have been shown to be involved in AD, being found in the amyloid plaques of AD brains. Here we studied the interaction of Cu^{2+}, Ni^{2+}, Ag^{+}, and Hg^{2+} ions with amyloid-β-peptide 1-40 (Aβ1-40), measuring the fluorescence of glycyl-L-tryptophan (GW) peptide added to metal-peptide complex solutions. Only some heavy metal ions proved to be quenchers of the fluorescent peptide GW, whereas Aβ peptide interfered with their fluorescence quenching alongside with the formation of metal-Aβ complexes. While copper ions decreased the fluorescence intensity of the GW peptide, nickel ions did not quench it. Silver did not influence much the GW fluorescence, while mercury had contrary effects depending on its concentration and the GW concentration. Moreover, its effect on the GW fluorescence was more influenced by the presence of Aβ peptide.

Keywords: glycyl-L-tryptophan; heavy metal ions; amyloid-β peptide; fibrillogenesis; fluorescence

There are many reports on the mechanism of interaction between the metal ions and both the monomer and the aggregated form of Aβ peptides [1]. The investigation of intrinsic fluorescence proteins has been regarded as an effective method to study protein conformations and dynamics [2]. Intrinsic tryptophan fluorescence is often used to determine conformational changes of peptides [3]. However, Aβ peptides are devoid of tryptophan. Nevertheless, the structural information is associated with the sensitivity of the tryptophan emission spectrum to the nature of its environment [4]. Fluorimetric methods are characterized by a very high sensitivity and selectivity [5].

Protein-bound metal ions, especially paramagnetic metal ions can form charge-transfer complexes, affecting markedly the lifetime [6]. Copper, zinc and iron ions are supposed to be implicated in two key steps of AD pathology: aggregation of Aβ peptides and production of reactive oxygen species (ROS) induced by Aβ. Metal ions such as zinc and copper have accumulated in the Alzheimer's brain patients [7, 8]. Indeed, Zn^{2+} and Cu^{2+} are known to bind to the histidine residues at Aâ-N-terminal chain. Now, the chelating therapy could be an alternative treatment for AD and is under clinical IIb study [9-12].

Fluorescence lifetime measurements have instead been used to infer the existence of different and unique protein conformations [13]. The sensitivity of indole fluorescence to a wide variety of environmental conditions is well recognized and is the principal factor in the diversity of fluorescence observed between different peptides and proteins, even when each of the proteins being studied contains only a single tryptophan residue.

Different functional groups (-NO_2, -Cl) or heavy metals interact with tryptophan residues and quench the fluorescence of tryptophan-containing peptides. The dynamic quenching by different intrinsic groups in a protein or metal ions depends on the structural fluctuations occurring on the same time scale as the emission process and inevitably will involve nondiffusive motions of both, the quenching agent and the indole ring of the tryptophan moiety itself [4]. There are multiple factors that can affect the fluorescence lifetime. Chemical compounds can quench tryptophan fluorescence include ionic species (e.g., histidyl, carboxyl, or arginyl residues), disulfide group, methionyl sulfur etc. [14].

Usually, fluorimetric methods are used to determine compounds even at concentrations 1-10 μg/mL, which corresponds to 10 times higher sensitivity than several methods based on molecular absorption. The intensity of fluorescence can be decreased by a wide variety of processes. Quenching mechanism can occur by different pathways. Collision quenching appears when the excited-state fluorophore is deactivated upon contact with other molecule (quencher) in solution. Consequently, the fluorophore turns back to the ground state during a diffuse encounter with the quencher. For collisional quenching the decrease in intensity is described by the Stern-Volmer equation modified by Lehrer (1):

$$\frac{F_0}{F_0 - F} = \frac{1}{[Q]} \cdot \frac{1}{f_a} \cdot \frac{1}{K_Q} + \frac{1}{f_a} \quad (1)$$

where K_Q is the quenching constant of protein fluorescence, f_a is the fractional maximum fluorescence intensity of protein, F_0/F is the relative fluorescence of protein in absence respectively presence of quencher and $[Q]$, the quencher concentration. The quenching mechanism described above results from diffusion encounters between the fluorophore and the quencher during the lifetime of the excited species [3].

Quenching can also occur as a result of formation of a non-fluorescent ground state complex between a fluorophore and a quencher. When this complex absorbs light it immediately returns to the ground state without of a photon emission.

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Alzheimer's disease (AD) is a progressive neuropathologically disease characterized by brain deposition of the amyloid-β peptide (Aβ), which is generated by proteolytic cleavage of amyloid precursor protein (APP) by β- and γ-secretases. Aβ aggregation undergoes multiple pathways forming a variety of intermediates and oligomers [15-17]. This process seems to be stimulated by the presence of heavy metals and low *p*H environments [18-20]. Clioquinol, a drug for Alzheimer's disease specifically interfering with brain metal metabolism: structural characterization of its zinc (II) and copper (II) complexes [21].

Therefore, the present work aims at studying the interaction between glycyl-L-tryptophan peptide and heavy metals by fluorescence measurements. We also investigated the effect of GW peptide on Aβ aggregation of in the presence of heavy metals.

Experimental part

Materials

Glycyl-L-tryptophan (GW) was purchased from Serva Fenibiochemica (Germany), amyloid-β-peptide 1-40 (Aβ1-40) from GenicBio (BioTech Co., Shanghai, China), whereas $CuSO_4$, $NiCl_2$, $AgNO_3$, and $HgCl_2$ from Sigma-Aldrich.

Sample preparation

Samples were prepared in deionization MilliQ grade water (18.2 MΩ·cm). Stock solution of glycyl-L-tryptophan (GW) 10^{-4} M was freshly prepared, and diluted to a concentration of $5 \cdot 10^{-6}$ M, before measurements. The final salts concentrations of Cu^{2+}, Ni^{2+}, Hg^{2+}, and Ag^{+} ions was chosen in order to obtain various salt: peptide molar ratios. Thus, in this report a $1.5 \cdot 10^{-4}$ M stock solution metal ions was prepared. Aβ solution was made by dissolving a few crystals (0.13 mg) in 2 mL of 1:1 hexafluoretanol : H_2O (V:V) solution. The concentration was checked spectrophotometrically using a molar extinction coefficient, $\varepsilon = 1490\ M^{-1}cm^{-1}$. The solution was evaporated to dryness on a glass plate in fresh air, to remove the organic solvent and to remain under monomer state.

Fluorescence measurements

Fluorescence spectra were recorded using a SFM-25 Kontron spectrofluorimeter (Kontron Instruments, Eching, Germany), equipped with 1 cm quartz cuvette (3 mL). Initially, 3 mL of glycyl-L-tryptophan was placed in the quartz cuvette and the emission spectra were recorded. Subsequently, 0.1 mL of solution containing Cu^{2+}, Ni^{2+}, Ag^{+}, or Hg^{2+} ions was added and the mixture was homogenized. Emission spectra were recorded in the range 300-800 nm. The corections of spectra were done according with dillution.

Results and discussions

Amyloid peptides have pathological implications in neurodegenerative diseases such as Alzheimer's disease. These peptides are non fluorescent because they are devoid of tryptophan fluorophore. However, Aβ contains a tyrosine (Tyr^{10}) residue, which absorbs at 276 nm, and whose emission around 300 nm does not interfere with the tryptophan fluorescence. Thus, the study of their conformation by spectrofluorimetric techniques is achieved by adding to the solution of fluorophores such as thioflavin [22]. In this study, we introduced a fluorescent dipeptide, GW, which interacts both with amyloid peptides and heavy metal ions.

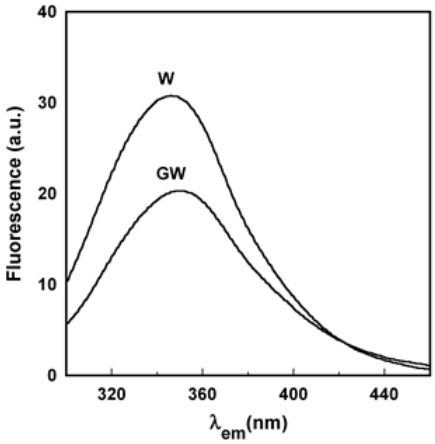

Fig.1. The fluorescence spectra of $5 \cdot 10^{-6}$M glycyl-L-tryptophan (GW) peptide and the amino-acid tryptophan (W), respectively

Fluorescence quench of GW peptide by heavy metals

The fluorescence spectrum of glycyl-L-tryptophan (GW) dipeptide obtained in the 3 mL aqueous solution of 5μM peptide was compared with that of tryptophan. An excitation wavelength of 295 nm was used, and a maximum at 346 nm was measured (the maximum of the amino acid tryptophan) (fig.1). Glycyl residue probably decrease the dipole moment of GW molecule or shadow the indole moiety, which determines a decrease in the fluorescence intensity of this system. Moreover, a 5 nm red shift was observed for GW peptide. Analougsly, FW and AW display emission maxima red shifted by 6 nm and 57 nm [23].

Different volumes of heavy metal ion solution (Cu^{2+}, Ni^{2+}, Hg^{2+}, and Ag^{+}) were added under stirring into the GW solution and peptide fluorescence was varied, depending on the type of the heavy metal ion used and its concentration. Copper and nickel ions proved to have high affinity for the N-terminal chain of Aβ peptides, especially for histidine residues in the positions 6, 13 and 14. Mercury causes atrophy of the brain nerve cells and of different portions of the cerebral cortex. High concentrations of this metal interferes with mitochondrial activity and induces oxidative stress by triggering the formation of reactive oxygen species (ROS).

Copper-induced fluorescence quenching of GW

The fluorescence intensity of the GW peptide decreased with the increasing amounts of copper ions added (fig. 2). Thus, upon adding stoechiometric amounts of copper ions, the fluorescence intensity decreased by ~20%. When an excess of copper ions was used, the relative intensity dropped down to 44% (1:5) or 52 % (1:10). Stern-Volmer diagram for GW interaction with copper ions indicated their strong binding to peptide (fig.2, detail insert). The calculated quenching constant, K_Q, was 726 M^{-1}.

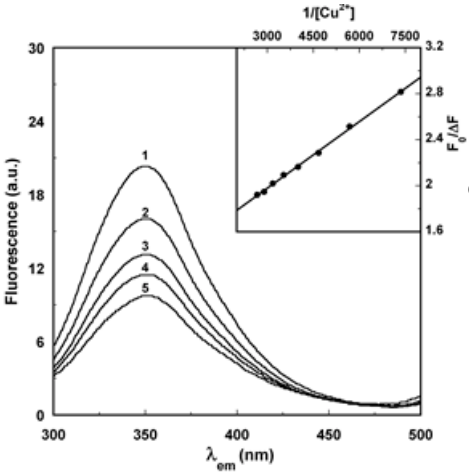

Fig. 2. The emission spectra of GW ($5 \cdot 10^{-6}$ M) peptide in the absence (1) or in the presence of copper ions (lines 2-4 :1,3,5 equivalents; line 5: 10 equivalents). Insert- modified Stern Volmer diagram

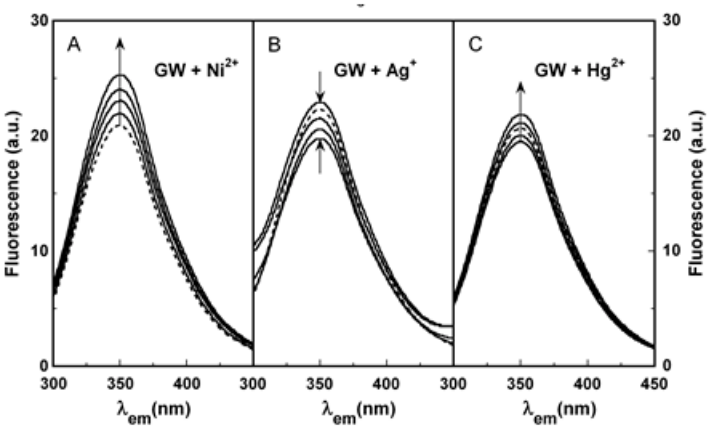

Fig. 3. The emission spectra of GW peptide ($5 \cdot 10^{-6}$M) - dotted line, in the presence of nickel ions (Panel A), silver ions (Panel B) and mercury ions (Panel C). The equivalents calculated for nickel ions were 2, 5, 8 and 10; for silver ions were 1, 2, 6 and 7, or 1, 3, 7 and 10 for mercury ions, respectively

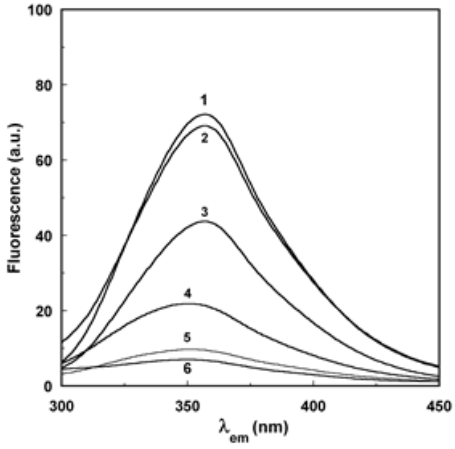

Fig. 4. The fluorescence spectra of the $5 \cdot 10^{-5}$ M GW peptide solution in the presence of two heavy metals (copper and mercury) and Aβ peptide. line 1: GW, line 2: GW+Aβ, line 3: GW+Aβ+Hg, line 4: GW+Hg, line 5: GW+Cu, line 6: GW+Aβ+Cu

The influence of nickel, silver and mercury ions on GW fluorescence

Similarly, the influence of nickel, silver and mercury ions to GW peptide was studied (fig. 3). Contrary to copper ions, nickel did not quench the GW fluorescence. In all fluorescence experiments, intensity of the peptide was similar. On adding 1-3 equivalents of metal ions, the fluorescence intensity slightly increased (Panel A). At higher concentration of nickel ions, a 20% fluorescence intensity increase (10 equivalents of Ni^{2+}) was observed. We explained this phenomenon by nickel addition to peptide whose molecule was stabilized (the structure became more planar and rigid).

Therefore, while copper ions reduced drastically the fluorescence intensity of GW, the nickel ions increased it. Another explanation for this phenomenon may be related to the different behavior of the two metals in the presence of peptide as follows: copper ions have a higher affinity for N (-NH_2) and O (-COOH) atoms, producing peptide oligomerization, while the nickel ions are closely linked to the peptide, increasing its dipole moment.

The fluorescence intensity of GW was fluctuated during silver ion titration of peptide (Panel B). In fact, silver did not influence much the GW fluorescence, and therefore, the small increase or decrease in the fluorescence intensity are under the experimental errors. Moreover, we suspected that the binding process could be accompanyed by the formation of nanoparticles and therefore the fluorescence intensity of GW would be practical the same or slightly decreased.

Upon adding mercury ions, the relative fluorescence intensity of GW changed, depending on the concentrations of Hg^{2+} (Panel C). Thus, by adding 1-5 equivalents of mercury ions the fluorescence intensity decreased. At higher concentrations of mercury (6-10 equivalents), an increase in the fluorescence intensity was noticed. We consider that GW peptide underwent a changing in the conformation in the presence of low concentrations of mercury, which resulted in a decrease in the fluorescence intensity. Contrary, at higher concentrations, the mercury ions strongly bind to GW, changing the conformation with an increase in the dipole moment of the molecule, which induce a higher fluorescence intensity of peptide.

Aβ interaction with the system GW-heavy metals ions

The Aβ peptide reduced moderately the fluorescence intensity of GW peptide, probably by their binding to each other (fig. 4). On adding copper ions, the maximum of fluorescence intensity at 356 nm drastically decreased, reaching less than 10%. Indeed, copper ions are electrochemical active and generate reactive oxygen species (ROS) in the presence of H_2O_2 or other reducing agents. Therefore, they have a neurotoxic effect especially in the presence of Aβ peptide. Copper ions bind Aβ and modulate its aggregation. However, our results suggest that copper quench the GW fluorescence both in the presence or absence of Aβ peptide. Further experiments are needed to show the time-dependent influence of copper ions on Aβ aggregation.

Mercury ions may decrease the relative fluorescence intensity of $5 \cdot 10^{-5}$ M GW peptide in a different manner from that of $5 \cdot 10^{-6}$ M GW. Moreover, they also interact with Aβ and therefore will increase the GW fluorescence. Thus, Aβ may bind mercury ions to aggregate, and these aggregates may influence the emission properties of GW peptide in the ternary solutions. We found thus different patterns for Aβ interaction with heavy metals that are unknown in the literature of AD pathology.

The human brain produces normally Aβ peptides. In AD, the production of Aβ is higher and the aggregation occur more rapidly forming insoluble plaques. The aggregates are toxic and the producing of ROS is inevitable. There is a controversy regarding the binding of metals to Aβ peptides and the neurotoxicity of their system. Some authors claim that copper ions increase the toxicity of brain by increasing the production of Aβ aggregates, while zinc ions have a neuroprotective effect. Mercury ions contribute to neuronal damage in different pathologies. Our results confirm that heavy metals associate with amyloid peptides and influence also by electronic interactions between the metal ions and fluorescent amino acids such as tryptophan and tyrosine. Copper and iron ions, metals with redox activity, induced formation of free radicals due to the complex Aβ-ion formation. Their interaction should be clarified also within further fluorescence experiments.

Conclusions

Fluorescence studies on the interaction of some metal ions such as Cu^{2+}, Ni^{2+}, Hg^{2+}, and Ag^{+}, with the fluorescent glycyl-tryptophan (GW) peptide in the presence of amyloid-β 1-40 peptide (Aβ) revealed different patterns of quenching or stimulating fluorescence depending on the type of metal and the concentrations of each component in the solutions used. These data could be of interest for

understanding the role of transition metal ions in AD pathology. Some heavy metal ions proved to be quenchers of the fluorescent peptide GW, whereas the other stimulate it. Aβ peptide interfered with copper and mercury fluorescence quenching alongside with the formation of metal-Aβ complexes. While copper ions decreased the fluorescence intensity of the GW peptide, nickel ions did not quench it. Silver did not influence significantly the GW fluorescence, while mercury had contrary effects, depending on its concentration and the GW concentration. Mercury effect on the GW fluorescence was much influenced by the presence of Aβ peptide.

Taken together these results might be of paramount importance for understanding the interaction of heavy metal ions with Aβ peptides associated with Alzheimer disease. However, further experiments based on fluorescence measurements of Aβ, fluorescent peptides, and heavy metal ions are needed to clarify metal binding to Aβ peptides.

Acknowledgments: Financial support by Romanian Government (UEFISCDI IDEI 313/2011) is grateful acknowledged. Olga Pintilie gratefully acknowledges the financial support by the strategic grant POSDRU/159/1.5/S/133652 from EU.

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Manuscript recceived: 15.12.2015