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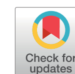
Nitration of amyloid-β peptide (1–42) as a protective mechanism for the amyloid-β peptide (1–42) against copper ion toxicity



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ABSTRACT

It is known that copper ion (Cu(II)) binds to amyloid-β peptide (Aβ), induces Aβ oligomer formation and ultimately exacerbates Aβ-aggregation neurotoxicity in Alzheimer's disease (AD). It becomes interesting to know that how this chemical modification of Aβ would affect interaction of Aβ and Cu(II) and their roles in the development of AD. In this work, we investigated the interaction of $A\beta_{1-42}$ nitration with the toxic Cu(II). It showed that Cu(II)induced $A\beta_{1-42}$ nitration in the presence of nitrite and hydrogen peroxide. Circular dichroism studies also revealed significant conformational change of $A\beta_{1-42}$ and Tyr10 nitrated amyloid-β peptide(1–42) ($A\beta_{1-42}$NT) when interacting with Cu(II). Even though nitration did not alter the binding of $A\beta_{1-42}$ to Cu(II) or the peroxidative activity of $A\beta_{1-42}$-Cu(II) complex, nitration ameliorated the aggregation and neurotoxicity of $A\beta_{1-42}$ induced by Cu(II), which was also further confirmed by the cell study. Given our previous findings that Aβ nitration dramatically inhibited its aggregation and thus reduced its toxicity, we speculated that nitration of $A\beta_{1-42}$ altered its intermolecular interaction, which protected itself against the toxicity of Cu(II). Based on this hypothesis, we propose that nitration of $A\beta_{1-42}$ may be an important protective mechanism for normal function of $A\beta_{1-42}$ and deserves more attention in AD drug development.

1. Introduction

Alzheimer's disease (AD) is one of the most prevalent neurodegenerative diseases [1]. Its pathophysiology involves oxidative stress and extensive deposition of extracellular amyloid plaques that consist of predominantly aggregated amyloid-β peptide (Aβ) [2,3]. Aβ, a peptide of 39 to 43 amino acids, is the product of the sequential cleavage of amyloid precursor protein (APP) by α- and β-secretases, and its most abundant forms are $A\beta_{1-40}$ and $A\beta_{1-42}$ [4–6]. Although $A\beta_{1-42}$ only differs two residues with $A\beta_{1-40}$, it is more inclined to aggregate and thus more pathogenic compared to $A\beta_{1-40}$ [7–9]. Soluble Aβ might aggregate into oligomers, protofibrils and mature fibrils, and even into senile plaques under pathological condition [10]. However, there is a poor correlation between the amount of senile plaques and cognitive deficits in the AD brain [11,12]. Instead, researches tend to suggest that Aβ oligomers may be the most neurotoxic to neurons and responsible for synaptic dysfunction and memory loss in AD [13,14]. It has been reported that copper ion (Cu(II)) binds strongly to Aβ, and the complex promotes the formation of Aβ oligomers and consequently elevates Aβ neurotoxicity in vitro [15–17]. Moreover, Cu(II) has been found abnormally high in senile plaques. Increasing evidence indicates that Cu (II) plays an important role in the metabolism and aggregation of Aβ in the development of AD [18–20]. Hence, it suggests a new strategy by disrupting the interaction of Aβ, which alleviates the Aβ oligomerization, for the prevention of AD development. Unfortunately, drugs designed on this assumption, as well as the aggregated-Aβ-targeted drugs, failed at clinical trials [21,22]. It generates skepticism that the view of considering Aβ, Cu(II) and the Aβ-Cu(II) complexes as the enemies tout court could be wrong [23]. In fact, synthetic monomer of $A\beta_{1-42}$ has been found to have a rescuing effect in both trophically deprived neurons and neurons undergoing excitotoxic death [24]. Accumulated evidence also suggests that Aβ may possess some important physiologic functions [25]. Therefore, there must be an unveiled mechanism to prevent Aβ from aggregating into toxic oligomers that impairs neurons.

Interestingly, our recent studies found that nitration of tyrosine (Y10) in Aβ could significantly inhibit the aggregation of Aβ and reduce its neurotoxicity by altering its intermolecular interaction. The nitration of Y10 in Aβ seemed a compensatory reaction against oxidative/

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nitrative stress and Aβ aggregation [26,27]. Moreover, Kummer et al. found that nitrated Aβ was detected in the core of Aβ plaques of APP/PS1 mice and AD brains [28]. It indicated the existence of Aβ nitration in vivo. It is already known that Aβ-Cu(II) complex is capable to catalyze peroxynitrite production in the presence of hydrogen peroxide and nitrite [29]. The production of peroxynitrite may induce nitration of tyrosine (Tyr 10) in in Aβ that is close to the binding site of Cu(II) [30–32].It explains the toxicity of Cu(II) in the Aβ-associated development of AD. As aforementioned that nitration of Y10 in Aβ exhibits significant effect on its aggregation and neurotoxicity, it becomes interesting to know that how the Aβ nitration would affect the Cu(II)-induced Aβ toxicity. In this study, we investigated the differences between synthetic $A\beta_{1-42}$NT and $A\beta_{1-42}$ in the Cu(II)-mediated neurotoxicity. Our findings provide novel insights into the relationship between Cu(II)and $A\beta_{1-42}$ in the development of AD.

2. Methods

2.1. Materials

$A\beta_{1-42}$, Tyr10 nitrated amyloid-β peptide (1–42) ($A\beta_{1-42}$NT), $A\beta_{1-16}$ and Tyr10 nitrated amyloid-β peptide (1–16) ($A\beta_{1-16}$NT) (˃95%, lyophilized powder) were purchased from China Peptides (Shanghai, China). 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), bicinchoninic acid (BCA), N-(2-Hydroxyethyl) piperazine-N′-(2-ethanesulfonicacid) (HEPES), desferrioxamine and coumarin-3-carboxylic acid (3-CCA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco (Carlsbad, CA, USA). All other reagents were analytical grade.

2.2. Preparation of Aβ monomer solution

$A\beta_{1-42}$ and $A\beta_{1-42}$NT were solubilized in HFIP overnight to remove pre-existing aggregates. Then HFIP was evaporated in vacuum, and the obtained peptide film was stored at −20 °C. The peptide film was dissolved to 5 mM in 10 mM NaOH solution and further diluted to desired concentration with 20 mM phosphate buffer solution (PBS), pH 7.4, prior to use. $A\beta_{1-16}$ and $A\beta_{1-16}$NT were dissolved to 1 mM in deionized water without pretreatment. Then the solution was diluted to required concentration with 20 mM PBS, pH 7.4.

2.3. Dot blot immunoassay

To study the nitration of $A\beta_{1-42}$ by Cu(II), 50 μM $A\beta_{1-42}$ was incubated with 50 μM Cu(II), 1 mM nitrite and 1 mM hydrogen peroxide in 20 mM PBS, pH 7.4, at 37 °C for 6 h. After 6 h incubation, 3 μl sample was transferred to nitrocellulose membrane. A rabbit polyclonal antibody against 3-nitrotyrosine was used to detect the nitrated Aβ.

2.4. Electrospray ionization mass spectrometry

Electrospray ionization mass spectrometry (ESI-MS) spectra of the Aβ and Aβ-Cu(II) complex were recorded on a time-of-flight high-resolution mass spectrometer (micrOTOF-II BRUKER), equipped with a conventional ESI source. The instrumental parameters were as follows: dry gas, nitrogen; temperature, 180 °C; scan range, *m/z* 400–3000, and capillary voltage, 4500 V. The scan range was m/z 400–3000. For ESI-MS experiment, stock solution of Aβ peptide was diluted to 100 μM in deionized water with 80 μM Cu(II) added.

2.5. Electron paramagnetic resonance spectroscopy

The X-band electron paramagnetic resonance (EPR) spectrum was recorded on a JEOL JES-FA200 X-band ESR spectrometer (JEOL, Tokyo, Japan). Experimental conditions were follows: microwave frequency, 9.15 GHz; microwave power, 20 mW; modulation frequency, 100 kHz; temperature, 120 K; sweep time, 32 s; time constant, 100 ms; 3 averages. For EPR experiment, stock solution of Aβ peptide was diluted to 1 mM in deionized water with 0.8 mM Cu(II) added. The samples were rapidly transferred to EPR tubes and frozen immediately in liquid nitrogen. EPR data was processed and analyzed using the method described by Peisach and Blumberg [33].

2.6. Isothermal titration calorimetry

Isothermal titration calorimetry (ITC) was carried out on a MicorCal iTC-200 microcalorimeter (Northampton, MA) at 25 °C. The reaction cell contained 60 μM peptide in 20 mM HEPES buffer at pH 7.4 with the ionic strength adjusted to 160 mM with NaCl. The buffer was chosen because of its negligible interference with Cu(II). The injection syringe contained 700 μM Cu(II) with 4 equivalent glycine in a matching buffer solution same to the reaction cell. All solutions were degassed under vacuum for 15 min prior to use. For the experiment, 3 μl 700 μM Cu(II) were titrated into 200 μl 60 μM Aβ peptide over 4 s with a 150 s interval between each injection. Thirteen injections were made in total. In addition, the stir speed was kept constant at 1000 rpm to achieve homogeneous mixture in the cell. ITC data analysis was performed by using one-site binding model in Origin 7.0 (Microcal). A non-linear least squares method was used to obtain the apparent association constant K, the number of binding sites n, and the change of enthalpy ΔH.

2.7. Gel electrophoresis analysis of Aβ

Aβ peptide (50 μM) were incubated in the presence or absence of Cu (II) (50 μM) at 37 °C for 3 days. Then the samples were mixed with loading buffer and resolved in Nu-PAGE 4%–12% Bis-Tris Protein Gels (invitrogen) with 2-(Nmorpholino) ethanesulfonic acid running buffer. Finally, the peptide was visualized by silver staining [34].

2.8. Circular Dichroism spectroscopy (CD)

Aβ samples were prepared by incubating with or without equimolar Cu(II) in 5 mM PBS, pH 7.4, at 37 °C for 12 h. The spectral region was recorded from 200 to 260 nm, with a 1 nm bandwidth, on a JASCO circular dichroism spectrometer at room temperature. A cuvette cell with 1 mm path length was used. The scanning speed was 100 nm/min and the spectra represented the average of three scans. The relevant baseline was subtracted by running PBS alone or PBS containing Cu(II) as a blank.

2.9. Measurements of hydroxyl radical

Hydroxyl radical generation was detected by 3-CCA assay. Nonfluorescent 3-CCA can be converted to highly fluorescent 7-OH-CCA by hydroxyl radical [35,36]. The reaction was started by the addition of ascorbate (300 μM) to the samples containing Cu(II) (10 μM) and peptide (10 μM). All measurements were performed in 20 mM PBS, pH 7.4, containing 1 mM 3-CCA. In this experiment, desferrioxamine was supplemented to remove trace metals in PBS. After 1 h incubation at 37 °C, samples were measured using a fluorescence spectrometer with excitation at 395 nm and emission at 450 nm.

2.10. Cell culture

SH-SY5Y cells were grown in DMEM supplemented with 10% fetal bovine serum (FBS), 4 mM L-glutamine, 100 U/ml penicilin and 100 μg/ml streptomycin at 37 °C in a humidified atmosphere with 5% CO_2.

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