Journal of Inorganic Biochemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

Complicated function of dopamine in Aβ-related neurotoxicity: Dual interactions with Tyr^{10} and SNK(26–28) of Aβ

Mengmeng Liu [1], Lu Kou [1], Yannan Bin [1], Liping Wan, Juan Xiang *

College of Chemistry and Chemical Engineering, Central South University, Changsha 410083, PR China

ARTICLE INFO

Article history:
Received 19 April 2016
Received in revised form 31 August 2016
Accepted 13 September 2016
Available online xxxx

Keywords:
Amyloid β peptide
Dopamine
Binding
Fibril
Cytotoxicity

ABSTRACT

With the capability to inhibit the formation of amyloid β peptides (Aβ) fibril, dopamine (DA) and other catechol derivatives have been considered for the potential treatment of Alzheimer's disease (AD). Such treatment, however, remains debatable because of the diverse functions of Aβ and DA in AD pathology. Moreover, the complicated oxidation accompanying DA has caused the majority of the previous research to focus on the binding of DA oxides onto Aβ. The molecular mechanism by which Aβ interacts with the reduction state of DA, which is correlative with the brain function, should be urgently explored. By controlling rigorous anaerobic experimental conditions, this work investigated the molecular mechanism of the Aβ/DA interaction, and two binding sites were revealed. For the binding of DA, Tyrosine (Tyr^{10}) was identified as the strong binding site, and serine-asparagine-lysing (SNK(26–28)) segment was the weak binding segment. Furthermore, the Thioflavin T (THT) fluorescence confirmed DA's positive function of inhibiting Aβ aggregation through its weakly binding with SNK(26–28) segment. Meanwhile, 7-OHCCA fluorescence exhibited DA's negative function of enhancing •OH generation through inhibiting the Aβ/Cu^{2+} coordination. The viability tests of the neuroblastoma SH-SY5Y cells displayed that the coexistence of DA, Cu^{2+}, and Aβ induced lower cell viability than free Cu^{2+}, indicating the significant negative effect of excessive DA on AD progression. This research revealed the potential DA-induced damage in AD brain, which is significant for understanding the function of DA in AD neuropathology and for designing a DA-related therapeutic strategy for AD.

1. Introduction

Despite the continuous debates on their function in Alzheimer's disease (AD) [1], amyloid β peptide (Aβ) aggregates identified in the plaques of the hippocampus and neocortical regions of the human brain are considered the neuropathology hallmarks of neurodegenerative AD [2,3]. The amphipathic nature of Aβ aggregates facilitates their own interaction with anionic and hydrophobic components of the cell membranes [4,5] and induces cytotoxicity [6]. Meanwhile, hydrophilic Aβ segment can bind Cu^{2+}, thereby reducing the amount of free Cu^{2+} and protecting the neurons from Cu^{2+}-induced reactive oxygen species (ROS) damage [7,8]. Given that the oligomer intermediates are toxic to neuronal cells [9–11], researchers focus on discovering complexes that can inhibit Aβ aggregation (oligomers or fibrils). Several studies have reported that dopamine (DA) can disaggregate the Aβ fibril and delay Aβ fibril formation in vitro [12,13]. Based on these observations, DA and other catechol derivatives are indicated as potential inhibitors of Aβ aggregation and can serve as effective treatment of AD [13–16]. However, due to insufficient findings on the effects of DA on Aβ/Cu^{2+} interaction and subsequent oxidative stress, the strategy for AD therapy is still debatable. In this event, the molecular mechanism of the Aβ/DA interaction (including the affinity and binding sites) and its effect on Aβ/Cu^{2+} interaction should be urgently revealed.

Unlike exogenous anti-aggregation agents, DA is a neurotransmitter. DA plays an important role in reward, addiction and the control of movement and balance. Considerable evidence suggests that the dysfunction of DA in specific brain regions is important in AD [14,17]. Mattson et al. reported that DA, at high concentrations, increases Aβ neurotoxicity (oxidative stress and mitochondrial dysfunction) and induces neuronal cell death [18]. Moreover, a study has posited that the synaptic release of DA is reduced by Aβ in at least three different brain areas (nucleus accumbens, striatum, and hippocampus), resulting in early neuropsychiatric disturbances in AD [19]. Meanwhile, the protective effect of DA against the toxicity of Aβ for PC12 cells was observed in vitro [20]. DA was also reported to inhibit iron/ascorbate stimulated lipid peroxidation with its ability to chelate iron or scavenge free radicals [21,22]. Given the controversial physiological functions of DA, an overall evaluation in vitro, at both the molecular and cellular levels, should be performed prior to applying DA as a means of treating AD.

* Corresponding author.
E-mail address: xiangj@csu.edu.cn (J. Xiang).
[1] Mengmeng Liu, Lu Kou, and Yannan Bin contributed equally to this work.

http://dx.doi.org/10.1016/j.jinorgbio.2016.09.007
0162-0134/

Thus far, only a few studies have explored the mechanism of Aβ/DA interactions. And lysine side chain [12] and aromatic group amino acids [23] were suggested as the binding site candidates on Aβ. However, due to the susceptibility of DA to oxygen [24–26], all previous research focused on the binding of Aβ with DA quinone. In brain, DA is tightly controlled as its reduction state, whether it is stored in DA vesicles or released upon exocytosis [27–30]. Even under oxidative stress, the initial DA autoxidation rate is slow (only $0.61 \pm 0.05\ nM^{-1}\ s^{-1}$) [31]. Based on the above conditions, analyzing the molecular mechanism of Aβ interacting with DA, but not its oxidation forms, is remarkably important. In this research, the interaction of DA with Aβ in vitro, at both molecular and cellular levels, was comprehensively evaluated by controlling rigorous anaerobic experimental conditions. In particular, the molecular mechanism of the interaction, including the binding sites and dissociation constant, was explored. Moreover, the physiological significance of DA on Aβ aggregation and Aβ/Cu^{2+}-induced redox stress was elucidated. The cytotoxicity of DA/Aβ/Cu^{2+} mixture was also evaluated. This research presented a relatively different outline on DA in Aβ-related AD neuropathology that is deemed significant for the DA-related AD therapeutic strategy.

2. Materials and methods

2.1. Material and reagents

$Aβ_{1-42}$ was purchased from American Peptide Company (Sunnyvale, CA, USA). All other peptides including $Aβ_{1-16}$, $Aβ_{12-25}$, $Aβ_{1-16}$(Y10A) (Tyrosin (Tyr^{10}) replaced by alanine), $Aβ_{12-28}$(Δ26), $Aβ_{12-28}$(Δ27), $Aβ_{12-28}$(Δ28), $Aβ_{1-42}$(Y10A), $Aβ_{1-42}$(Δ26–28), and $Aβ_{1-42}$(Y10A, Δ26–28) were provided by Shanghai Apeptide Co., Ltd. (Shanghai, China). *N*-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), 11-Mercaptoundecanoic acid (MUA), ethanolamine hydrochloride (AE), NaOH, KH_2PO_4, and K_2HPO_4 were acquired from Sigma (St. Louis, MO). SH-SY5Y cell (human neuroblastoma) was obtained from American Type Culture Collection (Manassas, VA, USA), and fetal bovine serum (FBS) was acquired from Hyclone (Logan, UT, USA). The Dulbecco's modified Eagle's medium (DMEM) and Ham's F12 medium were both procured from Mediatech Hyclone (Logan, UT, USA). The mixture of penicillin and streptomycin for cell culture and cytotoxicity study was purchased from Millipore (Billerica, MA, USA). Other regents were all of analytical grade and were used as received. All stock solutions were prepared daily with deionized water treated with a water purification system (Simplicity 185, Millipore Corp, Billerica, MA).

2.2. Solution preparation

The samples of $Aβ_{1-42}$ were diluted with phosphate-buffered saline (PBS). The Aβ stock solutions (0.5 mM) were prepared daily according the procedures conducted in our previous studies [8]. Briefly, lyophilized Aβ samples were dissolved in 10 mM NaOH solution, in which the aggregation of Aβ is considered effectively inhibited. Upon sonication for 1 min, the solution was centrifuged at 13,000 rpm for 30 min to remove any insoluble particles, and the supernatants were pipetted out and were diluted with 10 mM PBS buffer (pH 7.4, 0.1 M Na_2SO_4) for the experiments. The stock Aβ concentration was determined from the UV–vis spectra by setting the extinction coefficient at 276 nm ($\varepsilon = 1410\ M^{-1}\ cm^{-1}$ for tyrosine). All other Aβ stock solutions (200 μM) were prepared by directly dissolving the lyophilized samples in 10 mM NaOH at 4 °C. They were then diluted with PBS to desired concentrations. $Aβ_{1-42}$-Cu solution was prepared by mixing $Aβ_{1-42}$ and Cu^{2+} at equal molar ratio. All DA-related solutions were prepared in a Mikrouna Ipure glove box (Mikrouna Co., Ltd., Shanghai, China) in which attainable O_2 concentration was below 1 ppm. The DA stock solutions were prepared daily by dissolving 10 mM DA in 10 mM PBS buffer (pH 7.4, 0.1 M Na_2SO_4). The purity of DA solution was confirmed from the UV–vis spectra with a single absorption at 280 nm [32]. The Cu^{2+} stock solution was prepared by dissolving 1 mM $CuCl_2$ in a 1 mM H_2SO_4 solution.

2.3. Surface plasmon resonance (SPR) measurements

The measurements for flow-injection SPR were conducted with a BI-SPR 3000 system (Biosensing Instrument Inc., Tempe, AZ). 10 mM PBS buffer (pH 7.4, 0.1 M Na_2SO_4) was degassed via vacuum pumping for 30 min and was used as the carrier solution. The samples were preloaded into a 200 μL sample loop on a six-port valve and were then delivered to the flow cell with an internal volume of ca. 1.0 μL with a syringe pump (Model KDS260, KD Scientific, Holliston, MA). The instrument can cut off the dispersed front and tail ends of injected sample plugs prior to introducing the samples into the SPR sensing areas [33].

The Au films coated onto BK7 glass slides were purchased from Biosensing Instrument Inc. and were annealed in a hydrogen flame to eliminate surface contaminants. The MUA-covered SPR chip was formed by immersing the cleaned substrate in a 4.0 mM MUA ethanol solution for 18 h [8,34]. Upon the formation of the MUA self-assembled monolayers (SAMs), the chips were removed from the solution, rinsed with absolute ethanol and deionized water, and were then dried with nitrogen.

The immobilization of DA (or $Aβ_{1-42}$-Cu) was performed by cross-linking the molecules from 10 mM DA (or 100 μM $Aβ_{1-42}$-Cu) solution onto a MUA SAM via the standard amine coupling reaction [35]. The MUA chip was mounted onto the SPR instrument. After a stable baseline was obtained, 200 μL aliquots of the EDC/NHS mixture were injected into the fluidic channel. EDC/NHS solution was prepared by mixing 0.4 M EDC and 0.1 M NHS in water right before the MUA film was activated. Subsequently, 200 μL aliquots of the DA or $Aβ_{1-42}$-Cu solution were injected into the fluidic channel. This proceeding was followed by injecting 1.0 M ethanolamine onto the chips to block the unreacted sites. The chips were then washed with carrier solution for 10 min.

In all SPR experiments, the carrier solution was 10 mM PBS buffer (pH 7.4, 0.1 M Na_2SO_4), and the flow rate was 30 μL/min. The kinetics analysis of the SPR data was discussed in our previous paper [8]. After each measurement, the surface was regenerated via one or more injections of 10 mM NaOH. The oxidation of DA was eliminated by applying nitrogen-saturated solutions and performing in inert-atmosphere glove box.

2.4. Atomic force microscopy

Atomic force microscopy (AFM) images were obtained on a Veeco Dimension V AFM (Veeco Instruments, UK) in tapping mode. For AFM imaging, the aliquots of 10 μL of the samples were dropped on a freshly cleaved mica substrate. After incubation for 15 min, the substrate was rinsed with deionized water to remove salt and loosely bound protein; the substrate was then blown dry with N_2. The imaging was performed at a scan rate of 1–2 line/s with 512 data points per line.

2.5. Electrochemical measurements

Differential pulse voltammetry measurements were performed to detect the contents of free Cu^{2+} using a Gamry Reference 600 electrochemical workstation (Gamry Instruments, USA). A glassy carbon disk electrode and a platinum wire were used as the working and counter electrodes, respectively. An Ag/AgCl was used as reference electrode. The $Aβ_{1-42}$(25 μM)/Cu^{2+}(2.5 μM) mixture was obtained by mixing freshly prepared solution of $Aβ_{1-42}$ (0.5 mM) with $CuCl_2$ (1 mM) in 50 mM HEPES (pH 7.4, 0.1 M $NaNO_3$), and the final $Aβ_{1-42}$ and Cu^{2+} concentrations were 25 μM and 2.5 μM, respectively. The $Aβ_{1-42}$(25 μM)/Cu^{2+}(2.5 μM)/DA(50 μM) mixture was obtained by adding freshly prepared DA solution (1 mM) into the $Aβ_{1-42}$(25 μM)/Cu^{2+}(2.5 μM) mixture, and the final DA concentrations was 50 μM. Before electrochemical measurements, each Cu^{2+}-containing solution

Please cite this article as: M. Liu, et al., Complicated function of dopamine in Aβ-related neurotoxicity: Dual interactions with Tyr^{10} and SNK(26–28) of Aβ, J. Inorg. Biochem. (2016), http://dx.doi.org/10.1016/j.jinorgbio.2016.09.007