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Spectroscopic study on the interaction of $A\beta_{42}$ with di(picolyl)amine derivatives and the toxicity to SH-S5Y5 cells

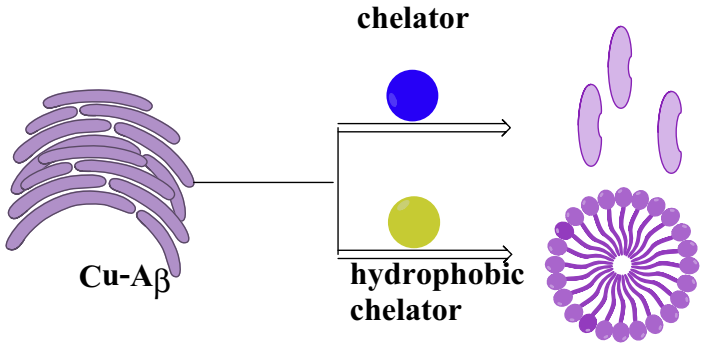
Meng-Yun Kong, Qiu-Yun Chen *, Ling Yao, Yin-Bing Wang

School of Chemistry and Chemical Engineering, Jiangsu University, Zhenjiang 212013, PR China

HIGHLIGHTS

- The interaction of bifunctional chelators with $A\beta_{42}$ was reported.
- The compound with hydrophobic group (BDA) shows high affinity to $A\beta_{42}$.
- The chelator–Cu(II)–$A\beta_{42}$ neurotoxic nanospheres were revealed.
- The change of $A\beta_{42}$ secondary structure induced by chelators was reported.
- The toxicity of chelators was reported.

GRAPHICAL ABSTRACT



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ABSTRACT

In order to confirm the neurotoxicity of bifunctional chelators containing hydrophobic groups and metal chelating moiety, the interaction of di(picolyl)amine (dpa) derivatives toward $A\beta_{42}$ peptide was investigated. Fluorescence titration reveals that a hydrophobic chelator (such as BODIPY) shows high binding affinity to amyloid $A\beta_{42}$. Circular dichroism (CD) spectra confirm that the hydrophobic bifunctional chelator can decrease α-helix fraction and increase the β-sheet fraction of amyloid $A\beta_{42}$. In particular, experimental results indicate that a bifunctional chelator can assemble with Cu(II)–$A\beta_{42}$ forming chelator–Cu(II)–$A\beta_{42}$ nanospheres, which are toxic to SH-S5Y5 cells. The hydrophobic interaction between the chelator and the amyloid peptide ($A\beta_{42}$) has great contribution to the formation of neurotoxic chelator–Cu(II)–$A\beta_{42}$ nanospheres. This work gives a general guide to the development of low cytotoxic inhibitors of $A\beta_{42}$ aggregation.

Introduction

Alzheimer's disease (AD) is a degenerative disease of central nervous system, whose main neuropathological hallmarks are the deposition of Aβ peptides into plaques and formation of neurofibrillary tangles, while the main clinical symptoms are memory loss and cognitive impairment [1–3]. The pathogenesis of AD is very complex and involves some variance in the cellulars as well as physiological processes [4]. Although the mechanism of AD pathogenesis remains controversial due to its complexity, recent studies have demonstrated that the formation of Aβ fibrils is a critical step in the pathogenesis [5,6]. Notably, about 90% of Aβ peptides are $A\beta_{40}$ while the rest mainly is $A\beta_{42}$. Compared with $A\beta_{40}$, a soluble species, $A\beta_{42}$ has a higher tendency to aggregate and cause more neurotoxicity [7,8]. Moreover, metal ions such as Cu(II) and Zn(II)

Abbreviations: HFIP, hexafluoroisopropanol; CQ, clioquinol; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Dpa, di(picolyl)amine; DMSO, dimethyl sulfoxide; PBS, phosphate buffer sacolumn; BODIPY, 4,4-difluoro-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-s-indacene; BDA, (8-[di(2-picolyl)amine-3-benzyl]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene.

* Corresponding author. Tel.: +86 0511 8879800; fax: +86 0511 88791602.
E-mail address: chenqy@ujs.edu.cn (Q.-Y. Chen).

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ions play an important role in the Aβ aggregation. In addition, the reaction between Cu(II) and Aβ peptide can lead to the formation of reactive oxygen species (ROS) with oxidative stress [9,10]. Given the recognized interaction of Aβ with metal ions, numerous studies have demonstrated that metal-ion chelators such as clioquinol (CQ) and desferroxiamine (DFO) are potential therapies for AD because they can inhibit metal-induced Aβ aggregation and block ROS formation as well as reduce neurotoxicity by removing the coordinated metal ions from Aβ plaques. However, these inhibitors have no selectivity to biomolecules and will cause some side effects. For example, it may disturb the normal physiological environment due to the remove of other essential biometals [11]. Recently, bifunctional chelators which both can interact with Aβ peptide and bind the metal ions from Aβ-metal have aroused people's attention. Wang et al. has produced two macrocyclic platiniferous chelators (PC1, PC2) with cyclen as the metal-chelating unit and Pt(bipyridine)Cl_2 as the Aβ-binding unit [12]. In addition to the chelation effect, these chelators also interfere with Aβ aggregation *via* specific coordination of Pt(II) with the residues in Aβ peptide. Moreover, Mirica et al. have reported two bifunctional compounds, containing both amyloid-binding and metal-chelating molecular motifs, which are able to inhibit the metal-mediated Aβ aggregation and disassemble preformed Aβ fibrils [13]. Thus, combination of metal chelators and amyloid-binding moiety is expected to be a good attenuator of Cu(II) ions induced aggregation [14]. In the previous report, we found that 8-[di(2-picolyl)amine-3-benzyl]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene (BDA), which contains both a metal-binding moiety (di(2-picolyl)amine) and an $Aβ_{42}$-binding moiety (hydrophobic section) can distinguish the copper(II) ions and target mitochondria in cells [15,16]. Hydrophobic interaction between chelators and amyloids can enhance their affinity to amyloids [17,18]. Therefore, it is necessary to investigate on interactions of hydrophobic chelators and amyloids, and their possible neurotoxicity in order to develop low toxicity therapeutic inhibitors of amyloids. In this content, the interaction and neurotoxicity of bifunctional chelator were investigated. It is confirmed that di(picolyl)amine derivatives (BDA and dpa) have the ability to chelate Cu(II) and bind $Aβ_{42}$, while they can induce the formation of soluble chelator–Cu–$Aβ_{42}$ nanospheres by hydrophobic interactions. This is the first time to reveal the existence of chelator–Cu–$Aβ_{42}$ nanospheres. At the same time, we found that chelator–Cu–$Aβ_{42}$ nanospheres have some toxicity to nerve cells.

Experimental

Material

$Aβ_{42}$ was purchased from Shanghai peptide Co., Ltd. hexafluoroisopropanol (HFIP) and clioquinol (CQ) were purchased from Adamas. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Amresco. Di(picolyl) amine (dpa), dimethyl sulfoxide (DMSO), ethylenediaminetetraacetic acid, and phosphomolybdic acid n-hydrate were purchased from Sinopharm. PBS (phosphate buffer sacolumn), NaCl (137 mmol L^{-1}), KCl (2.7 mmol L^{-1}), Na_2HPO_4 (10 mmol L^{-1}) and KH_2PO_4 (2 mmol L^{-1}) were prepared with double-distilled water. BODIPY (4,4-difluoro-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-s-indacene) and BDA (8-[di(2-picolyl)amine-3-benzyl]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene) were synthesized according to the reported method [15]. $Aβ_{42}$ monomeric films were prepared by dissolving commercial $Aβ_{42}$ in HFIP (hexafluoroisopropanol) at a concentration of 2 mg mL^{-1}, and cultivated at room temperature for about 1 h until $Aβ_{42}$ was totally dissolved. HFIP was removed by a flow of nitrogen to give a clear film and further dried by vacuum. HFIP-treated $Aβ_{42}$ was then dissolved in 0.2 mL DMSO to a final concentration of 1.1 mM and stored at −80 °C as a stock solution. For Cu-containing $Aβ_{42}$ peptides, Cu^{2+} was added before the initiation of the fibrillation conditions. Compounds were dissolved in DMSO and diluted with PBS. For disaggregation studies, the Cu-containing $Aβ_{42}$ peptides were treated with compounds and further incubated for 24 h at 37 °C (see Scheme 1).

Fluorescence measurements

Fluorescence measurements were performed on a fluorescence spectrofluorometer Model CARY Eclipse (VARIAN, USA), a 1.0 cm quartz cell (λ_{ex} = 460 nm, slit width = 5 nm). For Cu(II) chelation studies, inhibitors (3 mL, 2 μM) and $CuCl_2$ (60 μL, 20 μM) were added into the cells, the change of fluorescence intensity was recorded. For $Aβ_{42}$ fibrils binding experiments, the inhibitor (3 mL, 2 μM) and $Aβ_{42}$ (10 μL, 110 μM) were added into the cells, the change of fluorescence intensity was recorded. The dissociation constant (Kd) was calculated using one-site binding equation from GraphPad Prism 5.0.

Transmission electron microscopy (TEM)

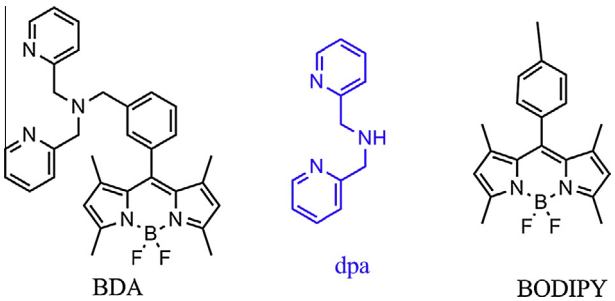
$Aβ_{42}$ peptides (20 μM) together with $CuCl_2$ (20 μM) were cultivated for 24 h at room temperature, and then the inhibitor (20 μM) (BDA, BODIPY, dpa, EDTA, CQ) was added, the solutions were stirred for 24 h at 37 °C. Carbon-coated Cu grids were treated with $Aβ_{42}$ samples (20 μM, 20 μL) for 2–3 min at room temperature. Excess solution was removed; grids were washed with H_2O and negatively stained with 2% phosphomolydic acid solution for 1 min and dried in air before detected by transmission electron microscope (JEOL JEM-2100).

Circular dichroism (CD) spectroscopy

The CD spectra were collected with Jasco J-815 spectropolarimeter (Easton, MD) using a 0.1 mm path length suprasil quartz cell at pH 7.4. Each test was performed in triplicate.

MTT assay

The cytotoxicity of Cu–$Aβ_{42}$ complexes in the absence and presence of chelators toward SH-S5Y5 cells were investigated by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. SH-S5Y5 cells were cultured in RPMI 1640 medium containing 4.8 g L^{-1} of hepes, 2.2 g L^{-1} $NaHCO_3$ and supplemented with penicillin/streptomycin (1000 units ml^{-1}) and 10% calf serum. Cells were seeded at a density of 4×10^4 cells ml^{-1} into sterile 96 well plates and grown in 5% CO_2 at 37 °C. MTT assay was determined by measuring the absorbance at 570 nm with an ELISA reader. Each test was performed in triplicate. Comparisons were made by one-way analysis of variance. Differences were considered to be significant when $p < 0.05$ [13].



Scheme 1. The structures of BDA, dpa and BODIPY.