D203

Catechol derivatives inhibit the fibril formation of amyloid-beta peptides

(Grad. Sch. of Eng. Sci., Osaka Univ.) ○Huong Thi Vu, Toshinori Shimanouchi, Naoya Shimauchi, Hiroshi Umakoshi, Ryoichi Kuboi*

The progressive deposition of amyloid-β protein (Aβ) in Alzheimer's disease is generally considered to be fundamental to the development of neurodegenerative pathology [1]. Many researchers have demonstrated that Aβ fibrils promote the neurodegeneration in cell culture systems [2]. The soluble monomeric Aβ is found to be non-toxic although its physiological function is not known in detail. The deposition of amyloid Aβ fibrils is believed to be causally linked to Alzheimer's disease (AD) [3]. The aggregation of the soluble Aβ monomer into toxic oligomeric or fibrillar species is considered to be a crucial step in the pathology of the disease [4]. It was reported that the most neurotoxic species are oligomers acting as intermediates during the formation of fibrils [5]. Currently, there is no way to cure Alzheimer's disease or stop its progression. Therefore, preventing the formation of Aβ oligomers and fibrils are promising therapeutic strategies against AD.

In this study, we examined the inhibitory effect of catechol derivatives on the fibril formation of Aβ(1-40) not only in the bulk phase but also on the fatty acid and cholesterol-containing domain-like liposomes mimicking biomembranes.

1. Experiments

10 μM Aβ (1-40) was incubated for 48 h with various catechol derivatives (**Fig.1(a)**) in the presence of fatty acid- and cholesterol-containing domain-like liposomes as a model biomembrane. Direct observation of fibrils. A total internal reflection fluorescence microscopy (TIRFM) combining with a fluorescence probe, thioflavin T (ThT), and a transmittance electron microscopy (TEM) were used to observe the fibrils according to the previous reports[6].

2. Results and Discussion

The direct observation of Aβ fibrils was performed **(Fig.1b)** to confirm inhibitory effect of catecholamines on Aβ fibril formation. In the absence of catechol derivatives, the fibrillar aggregate painted by ThT was observed (**Fig.1(b1)**). The microscopic structure was typical in amyloid fibrils with a TEM observation (**Fig.1(b2)**). In the presence of dopamine (DA), the ThT fluorescence could not be observed in TIRFM observation (**Fig.1(b3)**), suggesting the inhibitory effect of DA against the fibril formation of Aβ(1-40). In the microscopic observation with a TEM, no fibril structure was observed (**Fig.1(b4)**). We thus considered that DA could inhibit the fibril formation of Aβ. Other catechol derivatives could also inhibit the amyloid fibril formation except for tyrosine (Tyr).

In the presence of liposomes including fatty acid or cholesterol, the similar inhibitory effect by catechol derivatives was observed even under fibrils strongly interact with the liposome membranes (data not shown).

In order to clarify the mechanism of inhibitory effect by catechol derivatives, the kinetic analysis of Aβ fibril formation was performed. The lag time, corresponding to the nucleation step, was significantly prolonged by DA. Meanwhile, the growth from seeds of Aβ fibrils could not be effectively inhibited, suggesting that the catechol derivatives affected the nucleation step rather than the elongation step.

In conclusion, catechol derivatives would be useful for inhibiting the formation of Aβ fibrils in its early stages.

References

1) M. P. Mattson, *Nature*, **430**, 631-639 (2004), 2) L.L.Iversen *et al*., *Biochem. J*., **311**, 1-16 (1995), 3) B.A.Yankner, *Neuron*, **16**, 921-932 (1996), 4) R. Resende *et al*., *Neuroscience*, **155**, 725-737 (2008), 5) R. Kayed *et al*., *Science*, **300**, 486-489 (2003), 6) H.T. Vu, T. Shimanouchi, N. Shimauchi, H. Yagi, H. Umakoshi, Y.Goto, R. Kuboi, *J.Biosci.Bioeng.*, in press (2010)

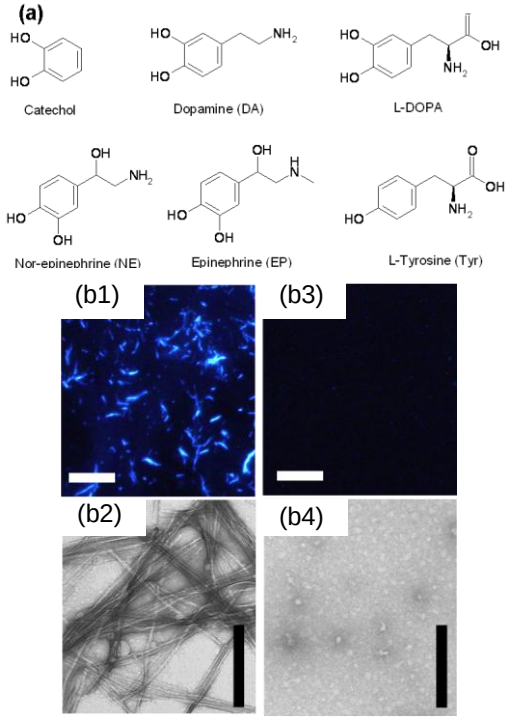

Fig. 1. (a) Chemical structure of the compounds used in this study. (b) Direct observation of fibrils with TIRFM images (scale: 10 μm) and TEM images (scale: 200 nm) of Aβ(1-40) fibrils formed in the absence of DA (b1, b2) and in the presence of 100 μM DA (b3, b4), respectively.

E-mail: msb@cheng.es.osaka-u.ac.jp
TEL/FAX: +81-(0)6-6850-628