α-Synuclein and Neurodegeneration

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α-Synuclein is implicated in pathogenesis of various neurodegenerative diseases, but the molecular mechanisms of its action might be different. In Alzheimer's disease (AD), NAC (non-Aβ component of AD amyloid) is produced from α-synuclein, and then interacts with Aβ protein to form amyloid in senile plaques. In Parkinson's disease (PD) and Dementia with Lewy bodies (DLB), a full-length or partially truncated form of α-synuclein is a constituent of LB, that is an inclusion found in degenerating neurons. Recently, oligodendroglial cytoplasmic inclusion in multiple system atrophy was reported to be immunoreactive with anti-α-synuclein antibody. Therefore, α-synuclein is suggested to be a common mediator of neurodegenerative diseases.

Keywords : α-Synuclein / Alzheimer's disease / Parkinson's disease / Amyloid / Lewy body

I. Introduction

Amyloid deposition in the senile plaque cores is one of the major neuropathological features in the AD brain. NAC was originally identified in senile plaques as a protein other than Aβ. NAC consisting of at least 35 amino acids is supposed to be produced from a precursor protein (α-synuclein). NAC is located in the most hydrophobic portion of the α-synuclein molecule. The α-synuclein has seven incompletely repeated KTKEGV motifs and no signal peptide sequence nor N-linked glycosylation sites.

Recently, two types of mutation, Ala30Pro and Ala53Thr, in the α-synuclein gene were found in some families of PD. Furthermore, α-synuclein was found to accumulate in Lewy body (LB) that is a hallmark of idiopathic PD. These findings suggest a possible role of α-synuclein in pathogenesis of both PD and AD.

II. Physiological functions of α-synuclein

α-Synuclein has been found in association with synaptic vesicles in the rat brain by immunoelectron microscopy. In order to clarify its physiological functions, we transiently transfected α-synuclein cDNA into PC12 and COS7 cells. We found the α-synuclein to be distributed in the cytosol and neurites of differentiated PC12 cells. A confocal laser microscopic study showed colocalization of α-synuclein with a synaptic marker, synaptophysin, indicating possible role(s) of α-synuclein in synaptic function.

III. Amyloidogenicity of NAC

NAC and Aβ protein are reportedly colocalized in the core of senile plaques (Figure 1). The ratio of NAC to Aβ in senile plaques has been estimated to be less than 1:10. Structural analysis has shown that NAC has a strong ten-

BIOORGANIC CHEMISTRY — Molecular Clinical Chemistry —

Scope of research

This laboratory was founded in 1994 with the aim of linking (bio)chemical research and clinical medicine. Thus, the scope of our research encompasses the structure, function and regulation of various biomolecules, the pathophysiological significance of bioreactions in relation to human diseases, and the application of molecular techniques to clinical diagnosis and therapy. Our current interest is focused on poly(ADP-ribosyl)ation, nuclear localization of proteins in association with apoptosis, and the molecular etiology of Alzheimer's disease and other neurodegenerative disorders.

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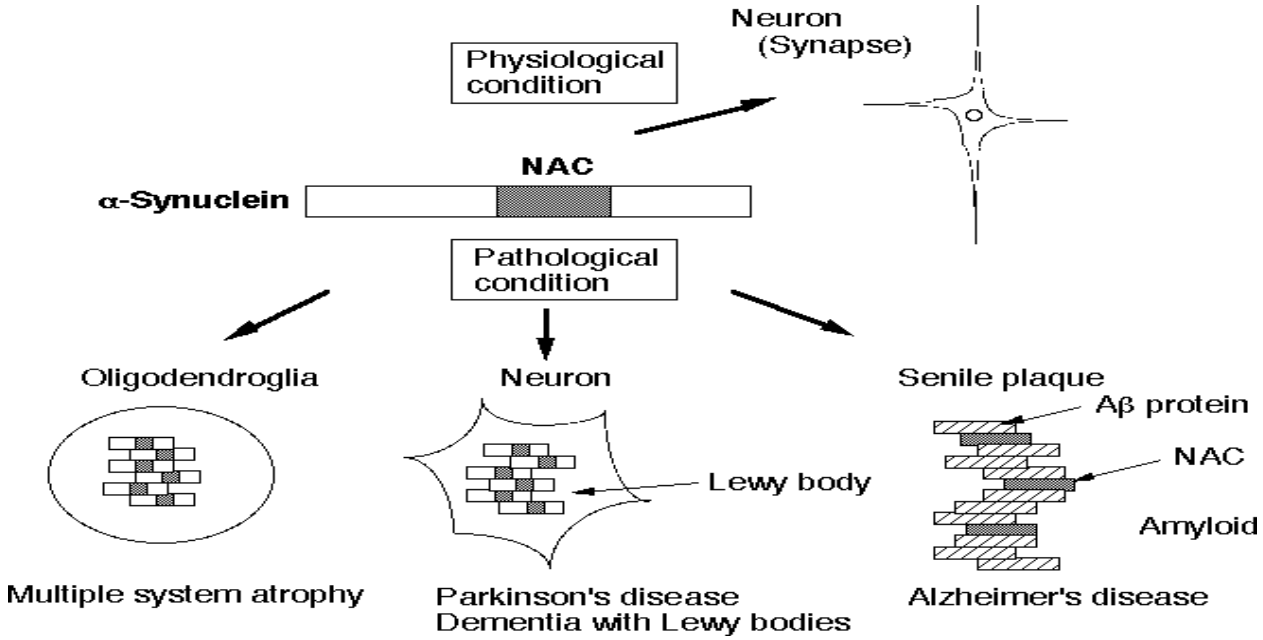

Figure 1. α-Synuclein and neurodegenerative diseases.

dency to form a β-sheet structure. We confirmed that fibrils are easily formed from synthetic NAC peptides, and stained with Congo red to give green images suggestive of amyloid under polarized light. We then analyzed kinetics of amyloid fibril formation by monitering thioflavine T fluorescence. Formation of $A\beta_{1\text{-}40}$ fibrils was facilitated, with no nucleation phase, by the addition of NAC fibrils preformed, suggeting that NAC fibrils could serve as a nucleus for the amyloid formation. In order to analyze the process of NAC production from α-synuclein, we labeled α-synuclein metabolically with [^{35}S]methionine in transfected COS7 cells. The half-life of ^{35}S-labeled α-synuclein in COS7 cells was longer than 24 hours. We could not detect any proteolytic products in the cell lysate nor in the culture medium. Although exact steps of α-synuclein processing remains to be clarified, it is possible that α-synuclein is released from damaged neurites and extracellularly proteolysed to produce NAC.

IV. Neurotoxicity of NAC amyloid

We found NAC to induce mitochondrial dysfunction in neuronally differentiated PC12 cells at 100 nM, which is comparable to the cytotoxic effect of $A\beta_{25\text{-}35}$. This finding was confirmed by nuclear stainings with Hoechst 33258 and propidium iodide (PI); the former staining all cells and the latter, dead cells. Some nuclei were condensed and others were swollen, indicating that the cytotoxicity was a mixture of apoptotic and necrotic effects. In order to clarify the molecular mechanism by which NAC exerted the toxic effects, we screened several chemicals for protective effects against NAC toxicity. We found that two antioxidants, propylgallate and N-*t*-butylphenylnitrone, effectively reduce the NAC cytotoxicity, suggesting a role of reactive oxygen species in the toxic event.

V. α-Synuclein and Lewy body

DLB is considered to be the second commonest form of degenerative dementia in old ages after AD. The antibodies against N- and C-terminal portions of α-synuclein stained positively Lewy bodies (LBs), indicating that full-length α-synuclein is a constituent of LB (see Figure 1). Full-length as well as C-terminal truncated forms of α-synuclein have been biochemically demonstrated in LBs. Recombinant α-synuclein was shown to form amyloid-like fibrils *in vitro*, and the fibril formation to be accelerated by mutations found in familial PD (Ala30Pro and Ala53Thr). Cytochrome c, a component of the mitochondrial electron transport chain, was suggested to contribute to the oxidative stress-induced aggregation of α-synuclein. Since cytochrome c is a mediator of apoptotic signals, the formation of LB might be facilitated by cytochrome c released in apoptotic cells in neurodegenerative diseases.

References

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