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Membrane-bound conformation of the non-amyloid-β component of α-synuclein characterized by vacuum-ultraviolet circular dichroism and molecular-dynamics simulation

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α-synuclein (αS) interacts with synaptic vesicle membranes in neurons to forms amyloid fibrils, which are involved in the pathogenesis of neurodegenerative diseases [1]. αS is composed of 140 amino acid residues and is divided into three regions: N-terminal region (residues 1-60), non-amyloid β-component (NAC) region (residues 61-95), and C-terminal region (residues 96-140) [2]. Among them, the NAC region has been proposed as a core region of amyloid fibril formations on the membrane in vivo [3]. In this study, synchrotron radiation vacuum-ultraviolet circular dichroism (VUVCD) [4] and molecular dynamics (MD) simulation were applied to characterize the membrane-bound structures of the peptides (αS_{57-102}) including the NAC region.

The VUVCD spectrum of αS_{57-102} was measured in the absence of membrane (native state) and in the presence of liposome membrane composed of DMPG lipid molecules. The native state exhibited the characteristic peaks of the random structure, while the spectrum in the presence of liposome showed the characteristic ones of α-helix structure as the L/P (DMPG lipid/αS_{57-102} peptide) ratio increased (L/P = 0 ~ 100), giving the two isoelliptic points at 201 and 202 nm and the saturated CD intensity around L/P=100. From the plots of CD values at 222 nm, we found that αS_{57-102} formed an intermediate structure around L/P =50~60 and formed the membrane-bound state around L/P=100.

To characterize the interaction mechanism between αS_{57-102} and membrane, MD simulation was conducted for the system composed of αS_{57-102} and DMPG membrane. During the simulation, some parts of αS_{57-102} were inserted into the membrane, disclosing that the V70-V95 region of αS_{57-102}, where several hydrophobic residues are localized, formed the hydrophobic interactions with the membrane interior, and the K96 and K97 residues formed the electrostatic interactions with negatively charged lipid head groups on the membrane surface. These unique hydrophobic and electrostatic interactions would induce the intermediate state of αS_{57-102} peptide.

The intermediate structure of αS_{57-102}, which would be partially folded in the membrane, is expected to influence the amyloid fibril formation. The peptide with L/P =50~60 was incubated for about 12 hours (37°C, shaking at 2000 rpm) and it was found that the β-strand structure increased only these L/P ratios, suggesting an intermediate structure of αS_{57-102} in the membrane is important factor for the aggregation or amyloid fibrils formation process.

REFERENCES

1. D. J. Selkoe, *Nature,* vol. 426, pp. 900-904, 2003.
2. Aaron D. Gitler, Brooke J. Bevis, James Shorter, Katherine E. Strathearn, Shusei Hamamichi, Linhui Julie Su, Kim A. Caldwell, Guy A. Caldwell, Jean-Christophe Rochet, J. Michael McCaffery, Charles Barlowe, Susan Lindquist, *Proceedings of the National Academy of Sciences,* vol. 105, no. 1, pp. 145-150, 2008
3. Ueda, K., Fukushima, H., Masliah, E., Xia, Y.U., Iwai, A., Yoshimoto, M., Otero, D.A., Kondo, J., Ihara, Y. and Saitoh, T., *Proceedings of the National Academy of Sciences*, vol.*90*, no. 23, pp.11282-11286, 1993.
4. Koichi Matsuo, Kunihiko Gekko, *Methods in Molecular Biology,* vol. 974, pp. 253-279, 2013.5.