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SUMMARY of

2016 RESEARCH RESULTS REPORT
For International Collaborative Research with IPR, Osaka University

Research Title		**NMR analysis of α-synuclein and CNT**
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Summary

Carbon nanotube (CNT), a cylindrical carbon allotrope with unique electrical, chemical, mechanical, and optical properties, is a potential candidate for future cancer therapy due to its exceptional cancer targeting property. However, it is necessary to reduce the hydrophobicity of CNT for biological application because non-specific protein coagulation onto the hydrophobic surface would cause toxic consequences. Previous studies have suggested that reducing the hydrophobicity of CNT with chemically modified PEG chains or hydrophobic amino acid residues ameliorates the cell cytotoxicity. Recently, we have found out that α-synuclein is capable of dispersing CNT stably in aqueous solution, which cannot be done with other proteins. Since we expect that the complex of α-synuclein and CNT could play a significant role on the photo-thermal cancer therapy, any detailed information on the protein structure and the physical/chemical property of the complex would be valuable as they have been assessed with instrumental analyses employing NMR, DSC, and CD. For those analyses, single-walled carbon nanotube (SWNT) was used among various types of CNTs to increase accuracy and resolution of the experiments. The complex of SWNT and α-synuclein (αS-SWNT) was prepared via a forced incubation between SWNT and α-synuclein using tip-sonication in ice.

For the experiments, an optimal ratio between αS and SWNT had to be determined. When 1.0 mg of SWNT was treated with 1 ml of αS solution, at least 100 μM αS was required to disperse SWNT. The far-UV CD spectra revealed a drastic conformational change of αS from random coil to α-helix. The quantitative analysis of the spectra using BeStSel algorithm verified that the content of α-helix increased from 5.7% to 26.5% while that of random coil decreased from 49.7% to 39.1%. The result suggests that the main region of αS interacting with the hydrophobic surface of SWNT would be the N-terminus by considering the previous reports about the α-helix formation of αS upon its interaction with lipid membranes. From the ^{1}H-^{15}N HSQC spectra of αS-SWNT, it was also suggested that the C-terminus of αS might be exposed outward with relatively high flexibility, which coincides with the result of far-UV CD spectra. However, the NMR spectra of αS-SWNT was not clear enough to identify the core structure of N-terminus due to high molecular weight of αS-SWNT. In this respect, we are planning to conduct solid-state NMR to unveil more detailed information on the structure of αS bound to SWNT as our future research. The DSC analysis result showed the thermal stability of αS-SWNT near the body temperature, which ensures bio-applicability of our αS-SWNT system. In vivo evaluation of αS-SWNT for photo-thermal cancer therapy will be conducted in the near future.