QUANTITATIVE PROTEOMICS STUDY OF CARBON NANOTUBES BINDING PROTEINS AND CARBON NANOTUBES BIOCOMPATIBILITY INVESTIGATION

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Quantitative Proteomics study of Carbon Nanotubes binding proteins and Carbon nanotubes biocompatibility investigation

利用定量蛋白組學技術研究碳納米管結合蛋白以及碳納米管的生物適應性檢測

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Abstract

Carbon nanotubes (CNTs) are the most important and most studied nanomaterials which are allotropes of carbon with nano-sized fullerene-related structures. CNTs have shown promise for much applications as molecular electronics, biomedical materials, ultrasensitive biosensors.

The absorption of various proteins on the sidewalls of CNTs has been reported. some proteins or peptides were also observed to interact with CNTs with high selectivity, and these interactions can apparently modulate protein structures and functions.

In this study, I have confirmed and extended the study of the protein-binding characteristics of CNTs. I showed that MWNTs (Multi-Walled Carbon Nanotubes) could bind to specific subsets of proteins from a wide diversity of organisms, including human cell line, zebrafish, mouse liver, drosophila and algae. I have then employed SILAC (Stable Isotope Labeling by Amino Acids in Cell Culture)-based mass spectrometry (MS), a powerful quantitative proteomic technique to systematically identify proteins from a total human cell lysate that bound to MWNTs. The relative CNTs-binding efficiency of each identified proteins was also measured. In parallel, I investigated by SILAC-based MS the protein binding properties of carbon black (CB), another allotrope of carbon , Of the 1477 proteins identified in one proteomics detection, I found that MWNTs specifically bound to 485 proteins, whereas, only 50 proteins were found to bind to CB. Furthermore, when the CNT- and CB-binding proteins were examined, I concluded that these two allotropes of carbon bound to a distinct group of cellular proteins under the same conditions. Statistical analysis of cell component, sequence length, PI, amino acid composition was performed to clarify the properties of the MWNTs specific binding proteins.

To investigate the interactions between CNTs and proteins, a comparison of the amount of binding proteins were performed in CNTs with different size. It was found that MWNTs with a diameter of 20-40 nm or more could bind a significant amount of proteins, whereas those with a diameter below 10nm bound to virtually none. The interactions, however, were independent on the length of the CNTs used. Moreover, the interactions between MWNTs and proteins were not affected by washing with gradient NaCl and Trition-X-100 and that the bound proteins could not be eluted by various organic solvents such as acetonitrile, Chloroform/Methanol or DMSO. Taken together, the interactions between protein and MWNTs were not only protein-specific but also highly stable.

The study of MWNTs binding proteins provides information on the MWNTs potential target proteins of MWNTs in organisms, which will facilitate a comprehensive evaluation of the risks of CNTs. Moreover, it provides new insights into handling and manipulating CNTs, such as non-covalent functionalized CNTs with specific proteins.This will ensure that the functionalized CNTs are more biocompatible with the target organism and more effective to be as drug delivery or biosensors.

To this end, the biocompatibility of MWNTs was tested both *in vitro* and *in vivo*, it showed that the cell proliferation of HeLa, HEK293T, 3T3 and HepG2 were not significantly inhibited by MWNTs. In addition, after injecting mice with MWNTs, I revealed that the levels of various members of the heat shock protein family remained unchanged in mouse liver, suggesting that no physiological stress occurred even through the liver was filled with MWNTs after the injection. Although MWNTs seems to have presented no significant toxicity to the cell line and mouse in this study,

an integrated study of the potential risks of CNTs still needs to be carried out before they can be applied in the biomedical field.

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List of Abbreviations

2-DE	Two-Dimension gel electrophoreis
AFM	Atomic Force Microscope
BE	Binding efficiency
BR	Bacteriorhodopsin
BSA	Bovine serum albumin
CB	Carbon Black
CVD	Chemical vapour deposition
CD	Circular dichroism spectroscopies
CNTs	Carbon Naotubes
CRIg	Complement receptor of the immunoglobulin family
CT	R-chymotrypsin
cyt-c	Cytochrome C
DAPI	4',6-diamidino-2-phenylindole
DMEM medium	Dulbecco's Modified Eagle's Medium
DMF	N,N-dimethylformamide
DWNTs	Double-walled carbon nanotubes
ESI	Electrospray ionization source
FBS	Fetal Bovine Serum
FMDV	Foot and mouth disease virus
f-MWNTs	Functionalized MWNTs
FT	Fourier transform ion cyclotron
FT-IR	Fourier-transform infrared
GIT	Strointestinal tract

HCAI	Human carbonic anhydrase I
HEK293T	Human Embryonic Kidney 293T cells
HiPco	High-pressure carbon monoxide method
HPLC	High performance liquid chromatography
Hsp	Heat shock proteins
IEF	Isoelectric focusing
IL-8	Interleukin 8
IPG	pH gradient strip
LDS	Lithium Dodecyl Sulfate
L(S)-MWNTs-2040	Long (Short) Multi-Walled Carbon Nanotubes with diameter of 20-40nm
L(S)-MWNTs-60100	Long (Short) Multi-Walled Carbon Nanotubes with diameter of 60-100nm
L(S)-MWNTs-10	Long (Short) Multi-Walled Carbon Nanotubes with diameter of <10nm
MALDI	Matrix-assisted laser desorption/ionization
MS	Mass spectrometry
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	Molecular weight
MWNTs	Multi-Walled Carbon Nanotubes
NF-κB	Nuclear factor κB
NMR	Nuclear Magnetic Resonance
PBS	Phosphate Buffered Saline
PCR	Polymerase chain reaction
PDB	Protein Data Bank

PI	Isoelectric point
PMF	Peptide Mass Fingerprint
PNTs	Purified nanotubes
RNase A	Ribonuclease A
SBP	Soybean peroxidase
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel
SILAC	Stable isotope labeling by amino acids in cell cuture
SWNT	Single-Walled Carbon Nanotube
TEM	Transmission electron microscopy
TOF	Time-of-flight

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