Selective adhesion of single-stranded DNA-binding proteins onto hybrids of DNA and single-walled carbon nanotubes

Kazuo Umemura, Daisuke Nii

Tokyo University of Science, 1-3 Kagurazaka, Shinjuku, Tokyo, Japan
meicun2006@163.com

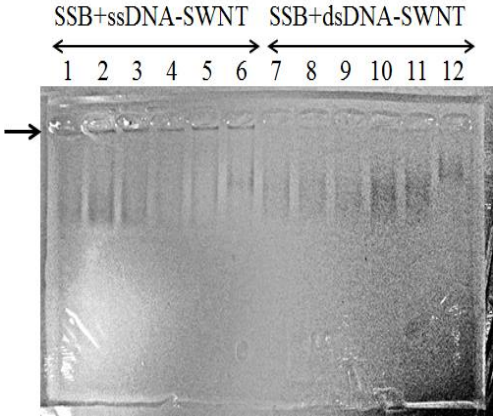
Abstract
Hybridization techniques to solubilize single-walled carbon nanotube (SWNT) molecules by wrapping their surfaces with DNA molecules have been established in 2003. [1-2] After that, any biological applications of DNA-SWNT hybrids (e.g., DNA sensors) have been proposed and many fundamental studies of DNA-SWNT interactions have been reported, including some publications by us. In one study, the unevenness of DNA-SWNT hybrid surface structures was characterized based on atomic force microscopy (AFM) using various combinations of DNA and SWNTs. [3] We reported opposite surface potentials in two types of hybrids by using a Kelvin probe microscope (KPFM), [4] and succeeded in detaching DNA molecules from SWNT surfaces by annealing. [5]

Here, we focused on the reaction between single-stranded DNA-binding (SSB) proteins and SWNT surface-bound DNA molecules. SSB molecules bind only single-stranded DNA (ssDNA) molecules, by using elaborate molecular recognition systems; they do not bind double-stranded DNA (dsDNA) molecules. However, whether selective binding is possible between ssDNA/dsDNA molecules and SWNTs is not known. Although some authors speculate that dsDNA become ssDNA on SWNT surfaces, no conclusive evidence proves this effect. [6] Study of this selective binding of SSB molecules may provide useful structural information on DNA-SWNT hybrids being developed for biological applications.

We prepared mixtures of SSB proteins and DNA-SWNT hybrids under various conditions and characterized these constructs by AFM and gel electrophoresis. In most cases, SSB molecules bound only to ssDNA-SWNT and not to dsDNA-SWNT hybrids. Thus, dsDNA molecules retain their double-stranded structure when bound to SWNTs, and that molecular recognition of SSB proteins to ssDNA occurs on ssDNA-SWNT surfaces. Some binding of SSB molecules to dsDNA-SWNT surface was observed when high relative concentrations of SSB proteins were used, but this could be attributed to non-specific adhesion. In this investigation, AFM images were not sufficient because the diameters and lengths of DNA-SWNT hybrids were not uniform; however, improved gel electrophoresis provided clear evidence of our conclusions. Our results could be useful in developing various biological applications of DNA-SWNT hybrids.

References
[1] Zheng, M., Jagota, A., Semke, E.D., Diner, B.A., Mclean, R.S., Lustig, S.R., Richardson, R.E., Tassi, N.G., Nature Mater., **2** (2003) 338.
[2] Nakashima, N., Okuzono, S., Murakami, H., Nakai, T., Yoshikawa, K., Chem. Lett., 3**2** (2003) 456.
[3] Hayashida, T., Umemura, K., Colloids Surf. B, **101** (2013) 49.
[4] Hayashida, T., Kawashima, T., Nii, D., Ozasa, K., Umemura, K., Chem. Lett., 4**2** (2013) 666.
[5] Nii, D., Hayashida, T., Umemura, K., Colloids and Surf. B, **106** (2013) 234.
[6] Cathcart, H., Quinn, S., Nicolosi, V., Kelly, J.M., Blau, W.J., Coleman, J.N., J. Phys. Chem. C, **111** (2007) 66.

Figure



Agarose gel electrophoresis
Lane 1 to 6: SSB and ssDNA-SWNT were incubated at different mixing ratios. In most cases, the samples remained at the entrance of the gel, suggesting that SSB bound with ssDNA-SWNT. Lane 7 to 12: SSB and dsDNA-SWNT were incubated at different mixing ratios.