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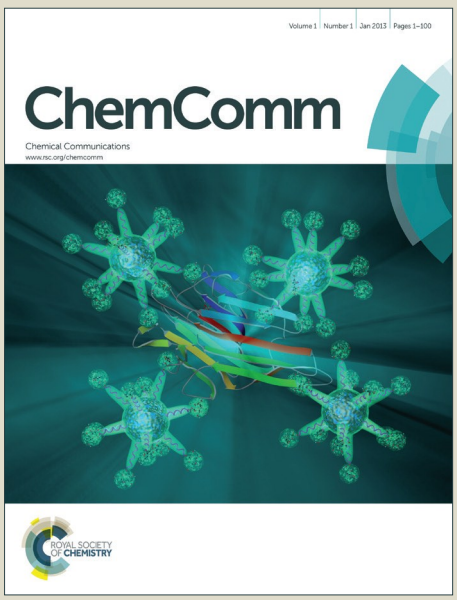

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DNA Aptamer Release from DNA-SWNT Hybrid by Protein Recognition

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Here we show the formation of the complex between DNA aptamer and single-walled carbon nanotube (SWNT) and its reaction with its target protein. The aptamer which is specifically bound with thrombin, target protein in this study, easily wraps and disperses the SWNT by noncovalent π-π stacking.

Aptamers are oligonucleotides which can recognize various targets such as small molecules, proteins, and cells as well as inhibit the function of the biomolecules.[1-5] The aptamers are regarded as an ideal alternative of the antibodies for biomolecular applications such as screening, diagnostics, and therapy due to their high affinity, selectivity, and stability.[6-9] They identify target molecules through conformational recognition which is formed by inter- or intra-hydrogen bondings in nucleic acids.[10] One of the unique properties of the DNA aptamers as compared with antibodies is degree of freedom of its conformations. The DNA aptamers can exist in the form of single-strand DNA (ssDNA) which can be freely suspended in the solution, double-strand DNA (dsDNA) which is produced by the reaction with its complementary DNA, complex with target molecule, and complex with other platforms to generate the signal or be delivered to a specific target.[11-13] To predict and understand the forms of the aptamer at a given condition and its transformation to others are important to use the aptamer suitably, especially when used as a complex form with other molecules.

The combination with nanomaterials has been studied for increasing the flexibility of the aptamers in recent years. Various nanomaterials such as gold nanoparticles, quantum dots, liposomes, and other nanoelements have been used as associating agents.[14-17] Single-walled carbon nanotubes (SWNTs) have also caught attention to combining with the aptamers due to their unique mechanical, electrical, and chemical properties.[18-20] Since DNA can disperse the SWNT through noncovalent π-π stacking, DNA aptamers can be linked to the SWNT without covalent bonding.[21-25] Our group previously showed the hybrid formation between ssDNA and the SWNT using the aforementioned noncovalent π-π stacking.[26] In addition, we verified the dissociation of the DNA from the SWNT because of the interaction with its complementary DNA.[27] Hydrogen bonding between the DNAs is stronger than the π-π stacking between the DNA and the SWNT. Thus DNA should be detached as it meet and react with its complementary DNA. Our interest has been moved to the interaction of the DNA aptamer in the aptamer–SWNT hybrid with its target protein.

Here we demonstrate the formation of aptamer–SWNT hybrids and the detachment of the aptamer from the SWNT due to the reaction with its target protein. Thrombin binding aptamer is selected as a wrapping agent on the SWNT, and thrombin is used as a target in this work. The changes in the electronic structure of the SWNT by wrapping and unwrapping processes were observed by Raman spectroscopy, and the conformational change of the aptamer as combining with the SWNT and reacting with its target protein was measured by circular dichroism spectroscopy. The experiments confirm that the aptamer helically wraps the SWNT as if it were dsDNA and this aptamer is released from the SWNT as reacting with target protein. Molecular dynamics (MD) simulations for binding free energy were performed to theoretically study the conformational change and its mechanism in detail.

The aptamer–SWNT hybrids have been produced through the sonication and centrifugation with the thrombin binding aptamer in D.I. water. The reaction of the aptamer–SWNT hybrid with the protein was carried out by adding thrombin as a target, and bovine serum albumin (BSA) as a negative control into aptamer–SWNT hybrid solution, respectively. Then the mixtures between the hybrids and the proteins were kept at

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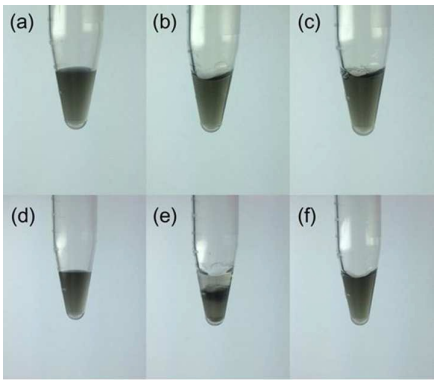

Figure 1 The photographs of the aptamer-SWNT hybrid before and after the reaction with protein, (a) and (d): the hybrid itself, (b) and (e): the mixture 1 between the hybrid and thrombin, (c) and (f): the mixture 2 between the hybrid and BSA. The uppers were taken right after the reaction and the lowers were done 1 hrs later. After 1 hrs, only the mixture 1 showed precipitation at the bottom.

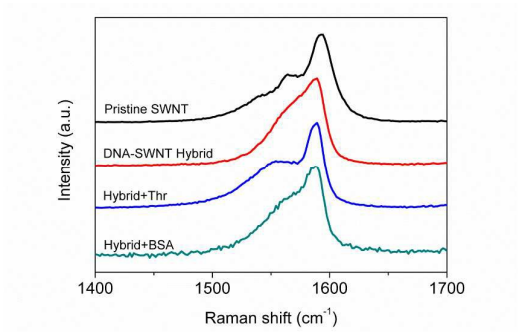

Figure 2 G-modes of the four samples; pristine SWNT, hybrid, the mixture 1 between the hybrid and thrombin, and mixture 2 between the hybrid and BSA. The Breit-Wigner-Fano lineshape which was shown in pristine SWNTs disappeared after hybrid formation with the aptamer, and then it returned back to initial lineshape due to the reaction with thrombin.

room temperature without any further treatment. The SWNTs in the sample which thrombin was introduced began to be aggregated and precipitated at the bottom of the solution and they were fully deposited at the bottom after 1 hr (in Figure 1), which is caused by the detachment of the aptamers from the SWNTs due to affinity difference. While the aptamer–SWNT hybrid is formed only by π-π stacking between the bases of the DNA and the hexagonal ring structure in the SWNT, the aptamer–target protein binding structure is formed by stacking interactions, shape complementary, electrostatic interactions, and hydrogen bonding.[28] Therefore, the binding of the aptamer with the target protein instead of the SWNT is more favourable. On the contrary to the reacted sample with thrombin, the aptamer-SWNT hybrids with BSA or nothing were not aggregated or precipitated. This means that there were no reactions in these solutions because the hybrids were stable in DI water and the affinity with non-target protein was not high enough to detach the wrapping aptamer from the SWNT. UV/Vis/NiR spectra of the samples clearly showed relatively quantitative results regarding the amount of remained hybrids after the reaction. (Supporting information 3) After BSA injection, the intensity of the spectrum showed no difference according to the concentration of the BSA. However, the intensity after thrombin injection became lower as increasing the concentration of the thrombin. Based on these observations, we concluded that unwrapping of the aptamer by recognition of protein was target-specific event.

To investigate the detachment of the aptamer from the SWNT, Raman spectra of the samples were observed, which is useful to see unwrapping of the DNA from the SWNT by measuring the change in Breit-Wigner-Fano (BWF) lineshape.[26] The majority of the diameter of the SWNTs was estimated to about 1.1 nm for every sample through analyzing the peak at radial breathing modes (RBMs, 270 cm-1).[29] (Supporting information 5) To see the change in BWF lineshape according to the reaction with protein, every measurement was carried out in pH 3. This acidic condition was a key factor in this study because basic environment of the solution induced by the protein distorted the BWF lineshape of the SWNT. (Supporting information 4) After wrapping of the aptamer around the SWNT, the BWF lineshape was shifted to higher frequency and its magnitude was diminished, which indicates that the electronic structure of the SWNT was changed by wrapping of the aptamer. This transition of the BWF lineshape by wrapped DNA around the SWNT was previously explored.[26]

This transition has returned back by the injection of the thrombin into the hybrid solution. The SWNT after the reaction with thrombin showed strong BWF lineshape again unlike the hybrid before the reaction. It means that the reaction with target protein leads to the release of the aptamer from the SWNT and then the electronic property of the SWNT returned back to its original state representing the strong BWF lineshape. This phenomenon looks very similar to previous result done with its complementary DNA.[27] On the contrary, the hybrid with BSA showed no significant difference in BWF lineshape from hybrid. This is clear evidence for specific reaction of the hybrid.

The structures of DNA aptamer when wrapping the SWNT or detached from the SWNT are important for the estimation of the aptamer/SWNT hybrid structure. In case of thrombin binding aptamer we used in this study, two main peaks are shown in CD spectrum when it constructs G-quartet structure; one is negative around 270 nm and another is positive around 295 nm.[30] The intensity of spectrum represents the amount of G-quartet structures in the analyte. Figure S1 shows the CD spectra of aptamer, thrombin, aptamer-SWNT hybrids, SWNT after reaction with thrombin, and the hybrids reacted with thrombin. (Supporting information 2) When the aptamer was dissolved in D.I. water, its spectrum clearly represented the formation of G-quartet structure which is secondary structure of the thrombin binding aptamer. On the contrary, when the aptamer wrapped the SWNT, the resulting hybrid showed different shape from initial CD spectrum of only aptamer,

Table 1 Relative binding free energy of aptamer DNA-thrombin hybrid and aptamer DNA-SWNT hybrid. The energy unit is kcal·mol^{-1}.

Configura-tions	Aptamer-Thrombin complexand separated SWNT [Figure3(a)]	Aptamer-Thrombinhybrid and separated thrombin [Figure3(b)]	SWNT, aptamer and thrombin. All molecules are separated. (reference)
Interaction energy	-221.5	-125	0
TS	-51.9	-41	0
Free energy	-169.6	-84	0

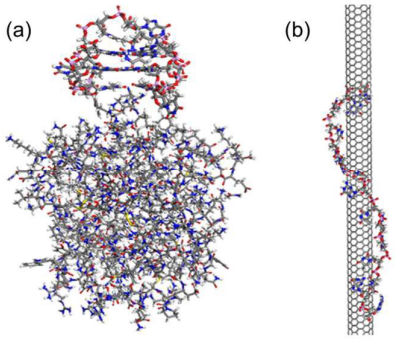

Figure 3 (a) Simulation snapshot of aptamer-thrombin complex. (b) Simulation snapshot of aptamer-SWNT hybrid. Water molecules are omitted for visual clarity.

which has reverse peaks; positive around 270 nm and negative around 295 nm. This spectrum is similar to the general spectrum of b-type double strand DNA, which indicates helical wrapping of the aptamer around the SWNT as if double strand DNA.[31] CD spectra showed the structural change of the aptamer by the reaction with thrombin. To see pure spectrum of the solution that the strong thrombin peak was excluded, the spectrum of the solution after the reaction with thrombin was obtained by the subtraction of the thrombin spectrum from that after the reaction between the hybrid and thrombin. It showed lower peak around 260 nm than before the reaction and the spectrum of the solution after the reaction except for the hybrid showed the peaks for G-quartet structures. It means that DNA aptamer was released from SWNT by the reaction with thrombin and formed the G-quartet structure as reacting with thrombin and some aptamer still remained on SWNT even after the reaction. The unwrapping efficiency seemed to be half but we believe that it can be improved by external energy to raise the possibility for the hybrid and thrombin to meet together in the solution. From CD spectroscopic analyses, it was confirmed that the structure of the aptamer with the SWNT is "helically wrapping" and this has been changed by the detachment of the aptamer from the SWNT as reacting with thrombin.

Next, we performed molecular dynamics (MD) simulations using the Large-scale Atomic/Molecular Massively Parallel Simulator (LAMMPS) program to understand the atomistic mechanism of the formation of the DNA aptamer–SWNT hybrid and the reaction with its target protein observed in our experiment. The computational cost prohibited us from simulating the full time atomic dynamics in which the DNA aptamer is detached from the SWNT due to the binding with the target protein. Therefore, we just calculated the total free energies of various equilibrium atomic configurations and compared them. In each simulation, the binding free energies of biomolecule complexes were obtained by calculating the time-averaged free energy at equilibrium. Binding free energies consisted of the molecular energies of biomolecule complexes and water-biomolecule interaction energy and the entropic contribution. For every 1ps, the total energy was calculated and 20-ns time average was used for analysis. The equilibrated atomic configuration was used to compute the entropic contribution to the binding free energy. The vibrational, translational, and rotational entropies were given by the following formulas:

$$-TS_{vibration} = -Tk_B \sum_{\omega_i} [\frac{\beta h \omega_i}{e^{\beta h \omega_i} - 1} - \log(1 - e^{-\beta h \omega_i})]$$

$$-TS_{translation} = -Tk_B [\frac{5}{2} + \frac{3}{2} \log(\frac{2\pi m}{\beta h^2}) - \log \rho]$$

$$-TS_{rotation} = -Tk_B [\frac{3}{2} + \frac{1}{2} \log(I_a I_b I_c) + \frac{3}{2} \log(\frac{8\pi^2}{\beta h^2}) - \log \sigma]$$

where, m is the mass of the molecule, $\beta = 1/k_B T$ is inverse temperature, h is the Planck constant, ρ is the number density, $I_{a/b/c}$ is moment of inertia, σ is the symmetry factor of the molecule, and ω_i is i-th vibrational eigenfrequency of the molecule.[27] In this calculation, we set the number density and symmetry factor as 1M/L and unity, respectively. These values are different from those of real materials, but its correction is very small because entropy depends logarithmically on them. Since the molecule has a large size (~5,000 atoms in thrombin), Since it is very difficult to calculate exact vibrational eigenfrequencies, we roughly estimated the vibrational entropy as described in the previous work.[27]

Table 1 summarizes the total binding free energies of the aptamer–SWNT hybrid (aptamer DNA wrapped around SWNT) and aptamer–thrombin complex. In this MD simulation, atomic coordinates of the aptamer–SWNT hybrid and the aptamer-thrombin complex were taken from previous MD simulations by Johnson et al. and by Jayapal et al. respectively.[32, 33] The total binding free energy of the aptamer-thrombin complex is larger by 85.6 kcal·mol^{-1} than that of the aptamer–SWNT hybrid. This affinity difference induces aptamer to become detached from the SWNT and to bind with the thrombin. To verify that the SWNT is completely detached from the aptamer-thrombin complex, we compared the binding free energies of possible SWNT-aptamer-thrombin three molecular complexes shown in Figure 4. The SWNT was completely detached and separated from the aptamer-thrombin complex. This computational result is in good agreement with our

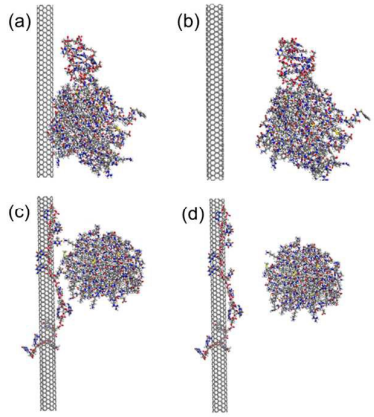

Figure 4 Simulation snapshots of DNA aptamer, thrombin, and SWNT complexes. (a) Aptamer-thrombin complex near the SWNT. (b) Aptamer-thrombin complex separated from SWNT. (c) Thrombin near the aptamer-SWNT hybrid. (d) Thrombin separated from the aptamer-SWNT hybrid. Water molecules are omitted for visual clarity.

Table 2 Relative free energy of aptamer-thrombin-SWNT (complex type 1). The energy is in unit of kcal·mol^{-1}.

	Aptamer-thrombin complex and SWNT	Aptamer-thrombin-SWNT complex
Free energy	Figure 5(a) -1527	Figure 5(b) 0
	Figure 5(c) -1739	Figure 5(d) 0

experimental findings. The detailed values are summarized in Tables 2.

In summary, we demonstrated the structure of the aptamer when interacting with the SWNT and the detachment of the aptamer from the SWNT by the reaction with target protein by the optical measurement such as Raman and CD spectroscopy. In Raman spectra, while the pristine SWNT showed strong BWF lineshape, the SWNT after the aptamer wrapping showed the attenuated BWF lineshape due to the interaction among the SWNT and the aptamer. The reaction with thrombin made its optical property returned to the initial state through unwrapping process of the aptamer from the SWNT. The CD spectrum of the aptamer after wrapping the SWNT showed no G-quartet structure but the peaks similar to double strand DNA due to helical wrapping around the SWNT. On the other hand, it showed the peaks representing G-quartet structure after the specific reaction with thrombin. The MD simulations and binding energy calculations provided atomistic description for the pathway to this phenomenon. Based on the calculations, we verified that the reaction of the aptamer with thrombin is more favourable than that with the SWNT and aptamer-thrombin complex is completely detached from the SWNT. We believe that this observation will improve the understanding of the structure of nanomaterial-biomolecule conjugates and open up a new approach for biological applications such as apta-sensor for various molecules.

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