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Research Article

Protein Adsorption on Hybrids of Thermoresponsive Polymers and Single-Walled Carbon Nanotubes

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Poly(*N*-isopropylacrylamide) (PNIPAAm) is one of the most popular thermoresponsive polymers. Adsorption of RecA proteins onto hybrids of PNIPAAm and single-walled carbon nanotubes (SWNTs) was observed in the presence and absence of DNA molecules. Although RecA molecules were adsorbed efficiently onto the hybrid surfaces at 37°C, even in the absence of DNA molecules, the adsorption of RecA was inhibited at 4°C. These results suggest that the thermoresponsive functions of PNIPAAm were effective, even on the SWNT surfaces, which supports the possibility of developing nanobiodevices using PNIPAAm-SWNT hybrids. However, although RecA is a DNA binding protein, there was no significant difference in the adsorption of RecA onto PNIPAAm-SWNT surfaces with and without DNA molecules. This study provides fundamental information for potential biological applications of PNIPAAm-SWNT hybrids.

1. Introduction

Poly(*N*-isopropylacrylamide) (PNIPAAm) is one of the most common thermoresponsive polymers owing to its lower critical solution temperature (LCST) of 32°C. It is well known that PNIPAAm molecules are hydrophobic at temperatures above 32°C and hydrophilic at temperatures below 32°C [1–4]. Moreover, the LCST does not depend on the polymer molecular weight, as long as M_w is greater than 50,000 Da [5]. Because of these unique properties, PNIPAAm and related polymers have been intensively studied [6, 7]. These polymers have been used for various biomedical applications, such as the preparation of cell sheets [3, 4]. In addition, the fabrication of PNIPAAm polymer brushes on reduced graphene oxide and hydrogel-forming polymer films have been reported, respectively [8, 9].

Carbon nanotubes (CNTs) are also promising nanomaterials for various industrial and biological applications [10–14]. For biological applications, CNT surfaces are typically wrapped with organic molecules or biomolecules such as DNA [15, 16]. This wrapping procedure has two purposes. First, although CNTs are insoluble in aqueous solutions, they can become soluble after being wrapped with hydrophilic organic molecules or biomolecules. Second, CNT surfaces can be functionalized by the aforementioned wrapping. For example, if CNT surfaces are wrapped with single-stranded DNA molecules, the resulting DNA-CNT hybrids can be used for hybridization with complementary DNA molecules.

The preparation of CNT surfaces with PNIPAAm or related molecules has been intensively studied by many authors. In most of the previously reported cases, PNIPAAm molecules were covalently attached to CNT surfaces [17–20]. However, in this procedure, the honeycomb carbon structures of the CNTs become disordered as a result of covalent bonding. Moreover, the unique optical and electronic properties of single-walled carbon nanotubes (SWNTs) are lost owing to this disorder. Wrapping molecules via physisorption is another approach for exploiting the

unique optical and electronic properties of SWNTs. However, to the best of our knowledge, there is only one report on the preparation of PNIPAAm-SWNT hybrids by physisorption [21].

In the present study, we demonstrate the reactions of RecA proteins and DNA with PNIPAAm-SWNT hybrids at two different temperatures (37°C and 4°C). RecA is one of the most important proteins associated with DNA recombination. RecA proteins bind to single-stranded DNA and form a helical filament structure [22]. Adsorption of RecA proteins onto single-stranded DNA molecules on SWNT surfaces was reported by Oura et al. [23]. PNIPAAm-SWNT hybrids have swelled rod-like structures; this suggests random physisorption of PNIPAAm onto the SWNT surfaces. Therefore, RecA proteins could randomly absorb onto PNIPAAm-SWNT hybrids. Accordingly, this study aims to provide fundamental information regarding the potential biological applications of PNIPAAm-SWNT hybrids.

2. Materials and Methods

SWNT powder (Super Purified HiPco SWNTs) was purchased from Unidym, Inc. (Menlo Park, CA, USA). PNIPAAm was obtained by telomerization using *N*-isopropylacrylamide, 2-mercaptoethanol (MeEtOH), and 2,2′-azobis(isobutyronitrile) (AIBN) [24]. The molecular weight of PNIPAAm-OH was characterized using 1H NMR (INOVA 400; Varian, Palo Alto, CA, USA) and gel permeation chromatography (GPC) (HLC-8320GPC; Tosoh, Tokyo, Japan). The GPC system was equipped with three sequentially connected columns (TSKgel Super Aw2500, TSKgel Super AW3000, and TSKgel Super AW4000; Tosoh) and calibrated using polystyrenes with various defined molecular weights (Sigma-Aldrich, St. Louis, MO, USA). The GPC experiments were carried out at 40°C using DMF containing 50 mM LiCl as the eluent (flow rate, 0.6 mL/min). Thus, the molecular weight of synthesized PNIPAAm-OH was confirmed to be 6,000 Da. DNA (thymine 30 mers, T30) and RecA proteins (1.0 mg/mL) were purchased from Life Technologies Inc. (Tokyo, Japan) and Bio Academia (Osaka, Japan), respectively.

Wang and Chen reported that PNIPAAm molecules can adsorb onto SWNT surfaces [21]. To prepare hybrids of PNIPAAm and SWNT, we used the sonication method. SWNT powder (1 mg) was added to 4 mL of PNIPAAm solution (1 mg/mL). The mixture was sonicated using an ultrasonic homogenizer (VCX 130, Sonics & Materials Inc., Newtown, CT, USA) at 5 W for 90 min in ice water. To remove excess PNIPAAm molecules, the samples were centrifuged (20130 ×g, 10 min) after adding 20 μL of 1 M tris(hydroxymethyl)aminomethane-HCl (Tris-HCl) buffer solution to 180 μL of the mixture. Then, the supernatant was replaced with pure water. After repeating this treatment 10 times, the precipitates with pure water were sonicated in an ultrasonic bath (LEO-80, Steady Ultrasonic Sdn. Bhd., Selangor, Malaysia) for 10 min in ice water to resuspend the mixture. These resuspended mixtures were used as the PNIPAAm-SWNT suspension (stock suspension A). For DNA adsorption, 500 μL of DNA solution (1 mg/mL) and 500 μL of the stock suspension A were mixed and then stored at 4°C (stock suspension B).

For characterization, eight kinds of mixtures were prepared as follows: 8 μL of stock suspension A and 13 μL of water were mixed. The mixture was incubated for 60 min at 4°C (sample #1) or 37°C (sample #5). For DNA samples, 16 μL of stock suspension B and 5 μL of water were mixed. The mixture was incubated for 60 min at 4°C (sample #2) or 37°C (sample #6). For experiments measuring RecA absorption onto PNIPAAm-SWNT, 8 μL of stock suspension A, 5 μL of RecA with adenosine 5′-[γ-thio]triphosphate tetralithium salt (ATPγS), and 8 μL of water were mixed. The mixture was incubated for 60 min at 4°C (sample #3) or 37°C (sample #7). Finally, for experiments measuring RecA adsorption onto PNIPAAm-SWNT with DNA, 16 μL of stock suspension B and 5 μL of RecA solution with ATPγS were mixed. The mixture was then incubated for 60 min at 4°C (sample #4) or 37°C (sample #8). In samples #3, #4, #7, and #8, the final concentrations of RecA and ATPγS were 160 μg/mL and 2 mM, respectively. The contents of each sample are summarized in Table 1.

For atomic force microscopy (AFM) observations, 20 μL of each mixture was dropped on a mica surface pretreated with 3-aminopropyltriethoxysilane (AP-mica), incubated at room temperature for 10 min, and rinsed with pure water. The samples were dried overnight before AFM observation. The AFM observations were performed using AC-AFM mode (MFP-3D microscope; Asylum Research, Santa Barbara, CA, USA) with a silicon cantilever (NANOSENSORS PPP-NCSTR-W; NanoWorld AG, Neuchâtel, Switzerland) in air. Cross-sectional analysis was carried out (100 cross-sections, five positions for 20 hybrids) to estimate the heights of the hybrids.

For agarose gel electrophoresis, 2 μL of glycerol was added to 10 μL of the sample mixture. The electrophoresis was carried out at 50 V for 60 min in Tris-Acetate buffer solution with ethylenediaminetetraacetic acid (TAE) using a 0.5% agarose gel. All characterizations were carried out at room temperature.

3. Results and Discussion

Figure 1 shows a schematic representation of the reactions examined in our experiments. DNA molecules and/or RecA proteins reacted with the PNIPAAm-SWNT hybrids at 4°C and 37°C. For experiments carried out on the reactions of both DNA and RecA, the mixture of DNA and PNIPAAm-SWNT was preincubated before adding the RecA proteins. AFM images of the PNIPAAm-SWNT hybrids prepared at 4°C and 37°C in the presence of DNA molecules (samples #2 and #6, resp.) are shown in Figure SP1 (see Supplementary Material available online at http://dx.doi.org/10.1155/2016/3539609); AFM images of the hybrids prepared in the absence of DNA (samples #1 and #5, resp.) are shown in Figure SP2. No apparent differences in the morphologies were observed among these four samples. In comparison with hybrids of DNA and SWNTs [23, 25, 26], the variance of height values that were obtained from the cross-sectional analysis of the AFM images was quite large. The average height values were 4.5±3.7, 6.8±14.0, 5.9±4.4, and 5.0±4.6 nm

Table 1: Contents of each sample.

Sample number	PNIPAAm-SWNT suspension (μL)	DNA solution (μL)	RecA solution with ATPγS (μL)	Water (μL)	Incubation temperature (°C)	Average height values (nm)
#1	8	0	0	13	4	4.5 ± 3.7
#2	8	8	0	5	4	6.8 ± 14.0
#3	8	0	5	8	4	7.4 ± 6.0
#4	8	8	5	0	4	7.0 ± 5.4
#5	8	0	0	13	37	5.9 ± 4.4
#6	8	8	0	5	37	5.0 ± 4.6
#7	8	0	5	8	37	N.A.
#8	8	8	5	0	37	7.8 ± 5.7

In each sample, the final concentration of PNIPAAm-SWNT was 380 μg/mL. In samples #2, #4, #6, and #8, the final concentration of DNA was 380 μg/mL. In samples #3, #4, #7, and #8, the final concentrations of RecA and ATPγS were 160 μg/mL and 2 mM, respectively.

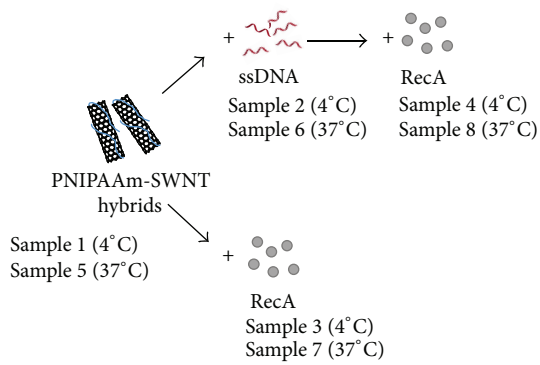

Figure 1: Schematic representation of the reactions studied.

for PNIPAAm-SWNT without DNA at 4°C (sample #1), PNIPAAm-SWNT with DNA at 4°C (sample #2), PNIPAAm-SWNT without DNA at 37°C (sample #5), and PNIPAAm-SWNT with DNA at 37°C (sample #6), respectively. Although the average height of each sample was slightly different, it is not obvious whether DNA was attached to PNIPAAm-SWNT because the standard deviation of each sample, especially that of sample #2, was large. This irregularity of the standard deviations may be caused by non-Gaussian distributions.

The RecA proteins reacted with PNIPAAm-SWNT with and without DNA molecules at 4°C and 37°C. Because RecA is one of the major DNA binding proteins, we were interested in the binding of RecA proteins to PNIPAAm-SWNT hybrids. At 4°C, no significant changes were observed, even after adding RecA proteins. Figure 2(a) shows an AFM image of the PNIPAAm-SWNT hybrids with RecA (sample #3). In this case, RecA proteins directly reacted with PNIPAAm-SWNT hybrids without DNA. On the other hand, as shown in Figure 2(b), when DNA was added to PNIPAAm-SWNT hybrids first, the RecA proteins reacted with the mixture of DNA and PNIPAAm-SWNT hybrids (sample #4). There were no significant changes in the morphologies of these samples when compared with the results shown in Figures SP1 and SP2. The average heights were 7.4 ± 6.0 and 7.0 ± 5.4 nm for PNIPAAm-SWNT with RecA (sample #3) and PNIPAAm-SWNT with DNA and RecA (sample #4), respectively. These values are obviously larger than that for PNIPAAm-SWNT without RecA and DNA (sample #1), which suggests that RecA proteins are bound to PNIPAAm-SWNT surfaces both with and without DNA molecules.

Similar experiments were carried out at 37°C. In this case, the morphologies of the PNIPAAm-SWNT hybrids were drastically changed compared with those at 4°C. Many large hybrids were observed in the images of both sample #7 (Figure 2(c)) and sample #8 (Figure 2(d)). In particular, many aggregated hybrids were found in PNIPAAm-SWNT with RecA (Figure 2(c); sample #7), and a cross-sectional analysis was therefore not possible in the case. An additional AFM image is shown in Figure 2(c) instead of a histogram. The average height of PNIPAAm-SWNT with DNA and RecA (sample #8) was 7.8 ± 5.7 nm, which is a higher value than those for PNIPAAm-SWNT hybrids with and without DNA (samples #5 and #6, resp.).

From the above results, we can conclude that RecA proteins tend to bind with the PNIPAAm-SWNT hybrids at 37°C, even in the absence of DNA molecules. For PNIPAAm-SWNT with RecA in the absence of DNA, more RecA proteins were attached to PNIPAAm-SWNT at 37°C (sample #7) than at 4°C (sample #3). However, in the presence of DNA molecules, the change of the average height between 4°C (sample #4) and 37°C (sample #8) was not significant. Thus, the thermoresponsive properties of PNIPAAm were useful for regulating RecA protein attachment to the SWNT surfaces in the absence of DNA molecules. On the other hand, the aggregation of the hybrids in the absence of DNA molecules is not well understood. Further experiments are necessary to clarify the mechanisms underlying the adhesion of RecA proteins onto the PNIPAAm-SWNT surface without DNA and the aggregation of the hybrids.

Finally, the samples were analyzed using agarose gel electrophoresis (Figure 3). The location of each band of SWNTs can be observed without staining. Lanes 1 and 5 show

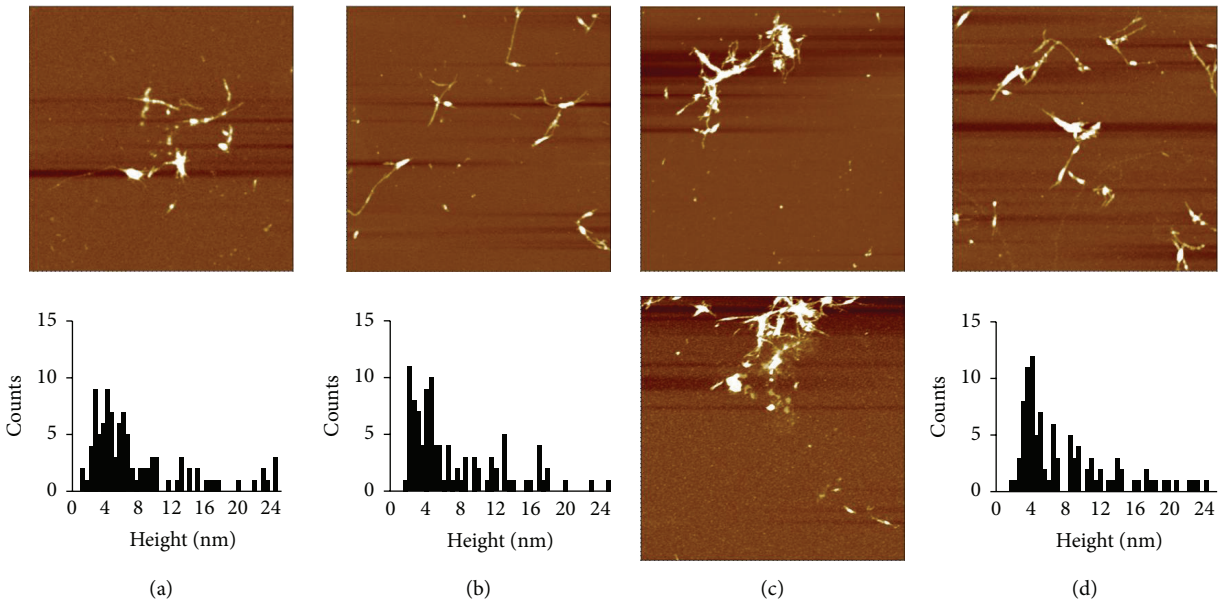

Figure 2: AFM images and height distributions of PNIPAAm-SWNT hybrids with DNA and/or RecA. (a) PNIPAAm-SWNT with RecA incubated at 4°C (sample #3). (b) PNIPAAm-SWNT with DNA and RecA incubated at 4°C (sample #4). (c) PNIPAAm-SWNT with RecA incubated at 37°C (sample #7). (d) PNIPAAm-SWNT with DNA and RecA incubated at 37°C (sample #8). Scan size: 3.0 μm × 3.0 μm.

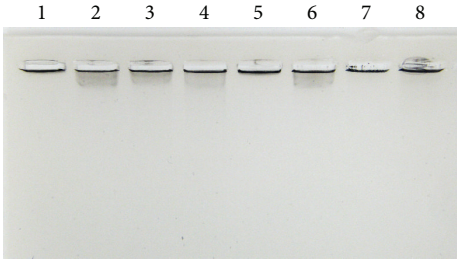

Figure 3: Agarose gel electrophoresis of PNIPAAm-SWNT hybrids. Lanes 1–4: samples incubated at 4°C. Lanes 5–8: samples incubated at 37°C. Lanes 1 and 5: PNIPAAm-SWNT. Lanes 2 and 6: PNIPAAm-SWNT with DNA. Lanes 3 and 7: PNIPAAm-SWNT with RecA. Lanes 4 and 8: PNIPAAm-SWNT with DNA and RecA.

the PNIPAAm-SWNT hybrids prepared at 4°C (sample #1) and 37°C (sample #5), respectively. In these cases, the SWNTs remained in the loading well, even after 1 h electrophoresis. Lanes 2 and 6 show the mixtures of DNA and PNIPAAm-SWNT at 4°C (sample #2) and 37°C (sample #6), respectively. In these cases, some of the SWNTs migrated; thus, the results suggest that DNA adsorbed onto the PNIPAAm-SWNT surfaces, although this was not obvious in the cross-sectional analysis of the AFM images. When RecA proteins were added at 4°C (Lanes 3 and 4; samples #3 and #4, resp.), the SWNT bands were similar to that in Lane 2 (sample #2). In our experiments, RecA proteins are negatively charged [27, 28]. Therefore, this result suggests that a small amount of RecA proteins adsorbed onto the PNIPAAm-SWNT surfaces at 4°C. On the other hand, with the addition of RecA proteins at 37°C, the SWNTs remained in the loading wells in Lanes 7 (sample #7) and 8 (sample #8) and in Lanes 1 (sample #1) and 5 (sample #5). This result might suggest an increase in the molecular weight of the hybrids due to the binding of large amounts of RecA proteins.

4. Conclusion

In this work, we demonstrated the attachment of RecA proteins onto PNIPAAm-SWNT hybrids. The amount of adsorbed RecA proteins was found to be regulated by temperature in the absence of DNA molecules. To regulate the reactions of RecA and DNA molecules on PNIPAAm-SWNT surfaces, further improvement of sample conditions is necessary.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

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