Non-covalent Attachment of Proteins to Single-Walled Carbon Nanotubes

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Abstract

A method for the non-covalent attachment of proteins to single-walled carbon nanotubes (SWNTs) is described. In this method, the protein is adsorbed to SWNTs that are suspended using sodium cholate, a surfactant and bile salt. The sodium cholate is then removed by dialysis with retention of the protein on the SWNTs. This method has resulted in good protein loadings and good retention of protein activity.

Key words: Non-covalent attachment, Adsorption, Dialysis, Proteins, Single-walled carbon nanotubes, Sodium cholate, Surfactant

1. Introduction

Single-walled carbon nanotubes (SWNTs) have important optical, thermal, mechanical, and electronic properties (1), and are being developed for applications in various biological systems (2). For some of these biological applications, it is necessary to attach proteins to the SWNTs, for example, to target the SWNTs to specific cells or to construct a biosensor. In this chapter, we provide detailed information on a method that we have used successfully to attach proteins to SWNTs by adsorption, a non-covalent form of attachment (3). This method is advantageous in that important features in the UV-vis-NIR adsorption spectra of the SWNTs are preserved, which is critical in applications where it is desired, for example, that the SWNTs strongly absorb energy when NIR radiation is applied. One problem with direct covalent
attachment of molecules to the SWNTs is that this method results in the complete elimination of the UV-vis-NIR adsorption bands, which is thought to be due to the SWNT’s π system being disrupted (4, 5).

The adsorption method we will describe was first used for proteins by Graff et al. (6). The first step in this method is to completely suspend the SWNTs in an aqueous solution of sodium cholate, which is a surfactant and a bile salt. After centrifugation, the protein is added, and the suspension is dialyzed using a dialysis membrane that will retain the protein but allow the sodium cholate to pass through. The suspension is centrifuged again, and the supernatant containing the suspended SWNTs with protein adsorbed is retained. This method for us has given good protein loading and good retention of biological activity for horseradish peroxidase (HRP, MW = 40 kDa) (3) and glucose oxidase (MW = 160 kDa) (unpublished data): HRP loading of 2 mg protein/mg SWNTs and 98% retention of native enzyme activity; glucose oxidase loading of 22 mg protein/mg SWNTs and 87% retention of native enzyme activity. Atomic force microscopy (AFM) analysis was performed on SWNTs with HRP adsorbed, and it is possible to visualize the HRP adsorbed on the nanotubes (Fig. 1) (3). In addition, for SWNTs with HRP adsorbed, there was retention of a substantial fraction of the NIR absorption at 980 nm (Fig. 2) (3).

Other methods that have been used to adsorb proteins on SWNTs are the organic solvent displacement method (7) and the

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Fig. 1. AFM images showing SWNT with sodium cholate and HRP protein. (a) SWNT in sodium cholate after sonication and centrifugation. Arrows indicate solid sodium cholate with a height of 1.0–1.5 nm (does not include 0.8 nm SWNT height). (b) SWNT/protein after dialysis and centrifugation. Arrows indicate protein associated with SWNT. The height is 3.8–6.0 nm (does not include 0.8 nm SWNT height). Reproduced from ref. (3) with permission from IOP Publishing Ltd.
aqueous sonication method (8); however, these methods gave problems with complete dispersion of SWNTs and with the maintenance of protein activity or structure.

2. Materials

2.1. SWNT Preparation and Suspension

1. Purified and freeze dried CoMoCAT SG65 SWNTs, rich in (6,5) type with an average diameter of 0.75 nm, were provided by Southwest Nanotechnologies, Inc. (Norman, OK).
2. Sodium cholate (Sigma-Aldrich) was used as a dispersant.
3. A horn sonicator equipped with a microtip of 3.2 mm in diameter was used (CPX750, Cole Parmer).
4. An ultracentrifuge was used to centrifuge the SWNT suspension (Optima XL series preparative ultracentrifuge, Beckman Coulter).

2.2. Adsorption of Proteins on SWNTs

1. Sodium phosphate (20 mM, pH 7.4) for buffering.
2. Dialysis membranes (10 kDa and 100 kDa, Spectrum Laboratories).
3. Methods

3.1. SWNT Preparation and Suspension

1. Add 3.0 mg of SWNTs to 7 mL of a 2 wt% sodium cholate solution and sonicate at a power of 7 W for 30 min.
2. Centrifuge the resulting suspension at 29,600×g for 30 min (see Note 1).

3.2. Adsorption of Proteins on SWNTs

1. Mix sodium phosphate buffer with the SWNT suspension.
2. Add 20 mg of protein to the suspension at 4°C.
3. Dialyze the solution at 4°C for 12 h against 2 L of sodium phosphate buffer using a 10 kDa dialysis membrane to remove unadsorbed sodium cholate (see Note 2).
4. Transfer the solution to a 100 kDa dialysis membrane and then dialyze at 4°C against 2 L of sodium phosphate buffer, with a change of the 2 L of buffer at 2, 4, 16, and 24 h from the start of dialysis (see Note 3). Carry out the final dialysis for 4 h.
5. Centrifuge the solution at 29,600×g for 1 h at 4°C.
6. Save the supernatant (see Note 4).
7. Perform the Bradford protein assay in order to quantify the protein concentration in the solution (see Note 5). Also, measure the protein concentration in the dialysate (the solution on the outside of the dialysis membrane).
8. Measure the absorbance at 800 nm of the initial suspension (SWNTs dispersed using sodium cholate) and the final suspension (SWNT/protein complex) for determination of the SWNT concentration (see Note 6).

4. Notes

1. The centrifugation step that is performed during the production of the suspension is important, since it eliminates nanotubes aggregates. The presence of aggregates in suspension is undesirable; the existence of aggregates can contribute to significant changes in the properties of the suspension. During the centrifugation step, it is important to use at least 29,600×g centrifugal force when using single-walled carbon nanotubes, in order to assure a good nanotube dispersion of single-walled nanotubes, and to remove any aggregated nanotubes.

2. The reason for the use of a 10 kDa dialysis membrane is because the sodium cholate (MW = 431 Da) will be able to
pass through the membrane pores freely and therefore will permit the removal of unadsorbed sodium cholate. Proteins with molecular weights higher than 10 kDa will be retained inside the membrane.

3. A membrane with a larger pore size (100 kDa molecular weight cutoff) is used in the second dialysis, in order to remove unadsorbed protein through the membrane. Extensive dialysis is used to ensure complete removal of unadsorbed protein; less dialysis can possibly be used for some proteins, especially low molecular weight proteins. The removal of unadsorbed protein can be evaluated by measuring the protein concentration in the dialysate. For low protein concentrations, it may be necessary to use a micro protein assay (for example, the micro BCA protein assay from Pierce). It is important to note that the membrane pore size should always be larger than the protein molecular size. Different membrane pore sizes are commercially available for the dialysis of proteins with a range between 10 and 300 kDa. It is also important to note that higher than a 100 kDa molecular weight cutoff of the membrane can lead to some loss of nanotubes through the membrane.

4. The initial suspension (SWNT dispersed using sodium cholate) and the final suspension (SWNT/protein complex) can be analyzed by atomic force microscopy (see the AFM images in Fig. 1 for the adsorption of HRP using this procedure).

5. The amount of protein used gives a relatively low coverage of the SWNT surface area. For example, using the weights of SWNTs and protein in Subheading 3 and assuming the protein is HRP, the ratio of the number of protein molecules to the number of six-carbon groups in the SWNTs is calculated to be 0.012. Assuming the protein is globular, the diameter of the protein is approximately 7.5 times the diameter of a SWNT. These calculations are verified by the AFM image of HRP adsorbed on SWNTs shown in Fig. 1.1, where the coverage by the protein on the SWNTs is relatively sparse and the protein is much larger than a SWNT. Seven and a half times the SWNT average height of 0.75 nm is 5.6 nm, which falls within the 3.8–6.0 nm height of the HRP protein measured.

6. A wavelength of 800 nm is used to measure the concentration of the SWNTs, since bands are not present on the absorption spectra at this wavelength (see Fig. 2 for SWNTs with HRP adsorbed). A calibration curve can be made from a plot of absorbance at 800 nm versus SWNT concentration.
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