Conditions on Single-walled Carbon Nanotubes for "Specific" Interactions with "Nonspecifically" Adsorbed Hemoglobin

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"Nonspecificity" is commonly observed in adsorption of proteins onto single-walled carbon nanotubes (SWCNTs). We show that, although any forms of SWCNT adsorb hemoglobin, only individually isolated, small diameter SWCNTs can induce specific responses of hemoglobin against a functional group attached to the SWCNTs.

Single-walled carbon nanotubes (SWCNTs) are known to adsorb various proteins nonspecifically.[1] In fact, implementing specificity to protein adsorption is one of the most important tasks in SWCNT-based biotechnology.[2] Recent studies indicate that the observed adsorption cannot be reasoned on a unique interaction. In addition to intuitively favorable hydrophobic or electrostatic interactions,[3,4] other contributions, like a charge transfer from amines,[5] may be involved. One of the problems in identifying the relevant interaction is the fact that all available nanotube samples are mixtures of SWCNTs with various structures; i.e., lengths, diameters, bundle sizes, defects, etc. are not controlled. There is a possibility that, although a protein–SWCNT interaction is actually specific and poses conditions to be fulfilled, the presence of other nonspecifically adsorbing SWCNTs that do not satisfy these conditions hinders the specificity. In this study, we examine if SWCNTs are capable of specific interactions even though they adsorb proteins non-specifically. SWCNTs were chemically modified with different functionalities. After recognizing specific responses of a protein against each functional group, only those SWCNTs that were interacting with the protein were collected and analyzed. We show that, although any forms of SWCNT adsorb the protein, only individually isolated, small diameter SWCNTs are able to induce specific responses.

Hemogloin (Hb) was chosen as the protein, because it disperses SWCNTs well in a buffer solution by nonspecific adsorption[6] (SWCNTs coagulate without Hb in the buffer) and the state of Hb is easily followed by UV–vis adsorption spectroscopy. Two types of functional groups were implemented on defect sites of purified SWCNTs (HiPco). The commonly employed acid-treatment[7] is known to introduce various oxygen-containing groups and the resulting tubes are designated as SWCNT-O. Another sample containing nitroso groups was also prepared[8] and denoted as SWCNT-N. Unless otherwise stated, the SWCNT dispersion was "sorted" with centrifugation, by retaining the supernatant part under a centrifugal force of 3500 g and the sediment part under 45,000 g. The resulting SWCNT samples consisted of thick bundles with an average length of ca. 1 μm. These SWCNTs in tris-buffer (pH = 7.0) were mixed with deoxyhemoglobin (deoxy-Hb) in a quartz cell equipped with a Thunberg tube. The concentrations were adjusted so that the absorption peaks due to SWCNTs remain small compared with those of Hb. The cell was vacuum-pumped and refilled with N_2 gas 3 times to exclude gaseous oxygen. The tube was completely sealed and was kept at 4 °C. All samples used in a single sequence of the experiment were made from a single batch of the SWCNT dispersion to retain the same reference state.

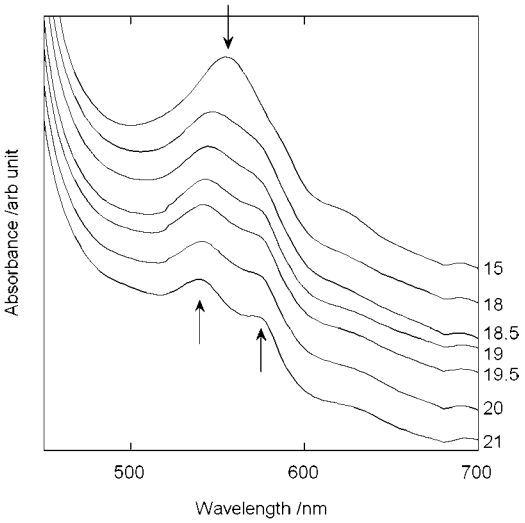

Figure 1. UV–vis absorption spectra of Hb and SWCNT-O. The numbers on the right axis indicate the time after mixing in hour. The top (15 h) was identical to the spectrum just after mixing.

Figure 1 shows a temporal development of absorption spectra of a deoxy-Hb and SWCNT-O mixture. Initially the spectrum shows only deoxy-state ($\lambda_{max} = 555$ nm). It remains the same for 15 h. After this long induction period, the maxima at 540 and 570 nm start to grow gradually and reach a steady state. Contrarily, when SWCNT-N was mixed with deoxy-Hb, the maximum at 555 nm changed to 545 and 575 nm after waiting for a long time (Figure 2). This change was followed by a slow increase at 500 and 630 nm. Thus, Hb responds specifically to each functional group. Although these spectral changes are similar to Hb interacting with gaseous CO or NO (then to a methoxy state), there are no gases involved in the present case. If any gases are introduced into a mixture by accident, a spectrum changes instantly. The observed long time scale is a characteristic of the SWCNT mixing.

The spectral changes are not due to denaturation of Hb possibly caused by adsorption. A pH dependence study[8] shows that the spectral changes at pH 7.2 or 7.8 proceed much faster than one at pH 6.8. This pH dependence is consistent with the allosteric effect of Hb commonly observed in oxygen binding. Also, after the reaction with SWCNTs, bubbling gaseous O_2 recovered the ordinary spectrum of oxy-Hb.

An explanation for the long induction periods is suggested

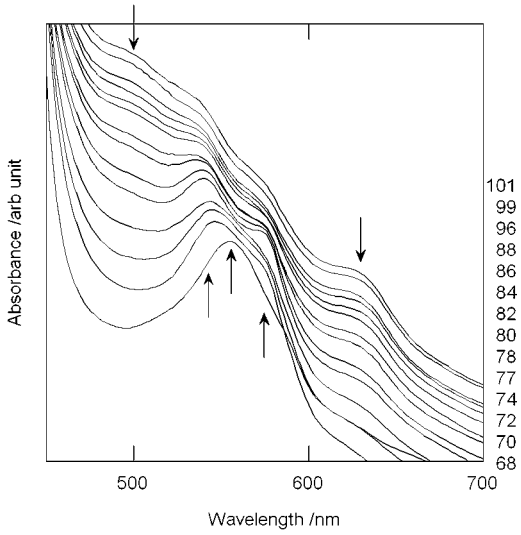

Figure 2. UV–vis absorption spectra of Hb and SWCNT-N. The numbers on the right axis indicate the time after mixing in hour. The bottom (68 h) was identical to the spectrum just after mixing.

by an observation that when a SWCNT solution containing individually isolated tubes and small bundles was mixed with deoxy-Hb, the spectral change took place faster than the minimum time required to measure a spectrum. This indicates that size is relevant for Hb. To see the size selectivity, the thick bundled SWCNTs were mixed with deoxy-Hb and the supernatant under 45,000 g was collected after the spectral change had been observed. Under this centrifugal force, the original thick bundled SWCNTs alone sediment but Hb alone stay in the supernatant. Atomic force microscopy of a cast film of the supernatant reveals that the supernatant contains individually isolated SWCNTs (Figure 3). Additionally, the resonance Raman spectrum of the same cast film shows a single pronounced peak at 273 cm^{-1} in the radial breathing mode region (Figure 4). In this region, the Raman shift is inversely proportional to the tube diameter (the above peak corresponds to a diameter of 0.86 nm).[9] The Raman spectrum of the original SWCNTs exhibits many peaks, implying that it is a mixture of different diameter tubes. These results indicate that, although any SWCNTs adsorb Hb, only individually isolated, small diameter SWCNTs are able to induce spectral changes. Thus, the long induction period is explained by

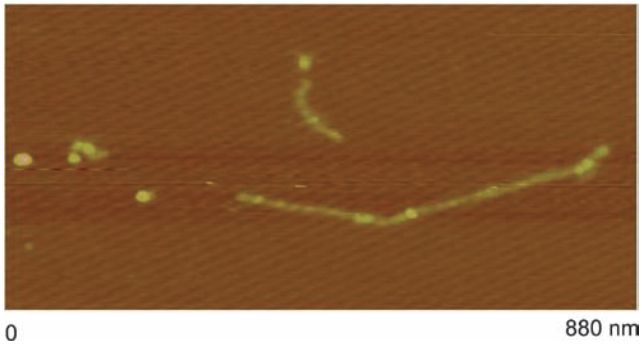

Figure 3. AFM image of a SWCNT with adsorbed Hb. Usually, SWCNTs are covered by thick layers of Hb. Here, the concentration of Hb is lowered to access the SWCNT height, which is ca. 1 nm.

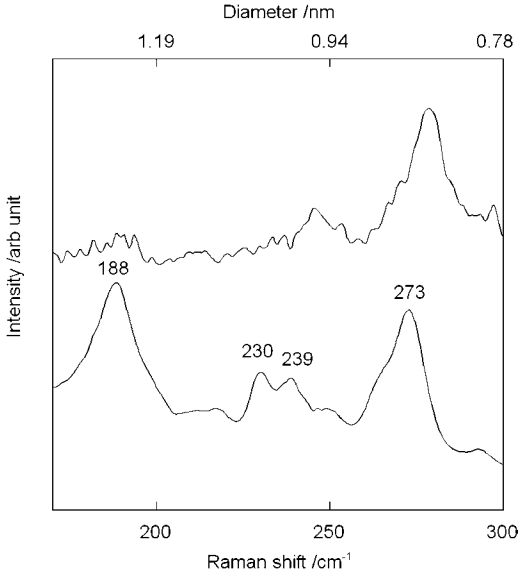

Figure 4. Raman spectra of solid films made from the supernatant of Hb-SWCNT mixture (top) and the original SWCNT-O (bottom), excited by 532 nm laser.

the time required for the small diameter SWCNTs to be freed from the thick bundles.

The present study raises many questions. Whether debundling took place as a result of Hb adsorption or adsorption occurred after SWCNTs were debundled by thermal fluctuation is not known. Also, a mechanism of SWCNTs contacting the heme group is not clear. Although 0.86-nm SWCNT may be able to penetrate 5-nm wide Hb, it cannot make a direct contact with the heme groups without inducing protein deformation. We speculate that certain defects, such as the dangling fragments[10] from the SWCNT wall, may be able to reach the heme groups. Lastly, chemical groups that are responsible for the spectral changes are not yet identified. These points are the subjects of further studies.

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Published on the web (Advance View) August 23, 2006; doi:10.1246/cl.2006.1062