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Effects of Microwave Radiation on Proteins Coexisting with Carbon Nanotubes

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Microwave (MW) radiation is known to heat carbon nanotubes (CNTs) very efficiently. The maximum allowable MW irradiance to avoid proteins from denaturing was determined. The MW irradiance of 100–300 J was sufficient to denature a recognizable amount of hemoglobin with CNTs, but the same irradiance had no effect without CNTs. Additionally, CNT under MW radiation was found to accelerate reduction of ferric cytochrome *c*.

Over the past decade, increasingly large quantities of carbon nanotubes (CNTs) have been used in both academic research and industrial applications. Frequent contact increases the possibility of inhaling airborne CNTs or taking them through wounds. Additionally, there is a growing interest in applying CNTs to medical and pharmaceutical fields, for instance, in biomolecular sensing,[1] tissue engineering,[2] and delivery systems.[3] Whether taken accidentally or administered intentionally, CNTs can be brought inside living bodies. Inevitably, hazardous effects of CNTs have become a focus point of the present time. All previous studies, however, have been restricted to toxicology.[4]

It is well known that carbon compounds are heated easily by microwave (MW) radiation. Temperatures can reach over glass-melting temperatures in short exposures. In fact, MW heating of CNTs has been shown to be a versatile technique for CNT chemistry. A reaction efficiency of CNTs increased an order of magnitude by MW heating in comparison with oil-bath heating.[5] Local heating properties were applied to afford Pt nanoparticles only on the CNT surfaces[6] and to improve elastic response of the CNT/polymer composite.[7] These studies demonstrate that a very small MW irradiance suffices for chemical changes, typically a few tens of watts (W) irradiated for less than a minute.

In the present, we are constantly irradiated with MW radiation. For example, mobile phones use nearly 1 W radiation to communicate with base stations, which radiate as much as 30 W MW. Most digital devices in computers and appliances operate at MW frequencies. Thus, it is of great importance to determine the safe level of MW dose on biosystems that are coexisting with CNTs. In this study, we report the maximum MW irradiance allowable to avoid denaturing proteins, namely, hemoglobin (Hb) and cytochrome-*c* (Cyt-*c*), coexisting with single-walled carbon nanotubes (SWCNTs) in solution. Such a simple system under controlled MW radiation gives fundamental data that can be used as a guideline for future clinical studies.

It is reported that Hb is adsorbed onto SWCNTs without denaturation[8,9] and responds specifically to surface functionalities on SWCNTs.[10] Hb can be denatured by heat in complicated ways, involving disintegration into dimers, detachment of the heme group, and destruction of secondary and primary structures. Most of these changes are monitored by diminished Q-band (500–600 nm) intensity in absorption spectra. In the case of extensive denaturation, Hb coagulates heavily which results in scattering of light and lifting a baseline. A degree of denaturation is expressed as a fractional change of spectral area under the Q-bands above the baseline.

Purified SWCNTs (HiPco) were sonicated in a mixture of H_2SO_4/HNO_3 for several hours, further treated in H_2SO_4/H_2O_2, and then thoroughly washed with water. The SWCNT dispersion was sorted with centrifugation, by retaining the supernatant part under 3500 *g* and the sediment part under 45000 *g*. All solvents were deoxygenated prior to use. A set of data presented in each figure, was performed by dividing a single batch of the resulting SWCNTs to equal portions. Typically, 18 μM of deoxy-state bovine Hb was mixed with 0.01 mg mL^{-1} of the above SWCNTs in Tris buffer (pH 7.4) in a quartz cell equipped with a Thunberg tube. The cell was vacuum-pumped and refilled with N_2 gas three times to exclude gaseous oxygen. The tube was completely sealed and was kept at 4 °C. After assuring the interaction between Hb and SWCNTs reached equilibrium,[10] the mixture was irradiated with MW radiation at 2455 MHz while monitoring the average solution temperature. All spectroscopic measurements were taken at room temperature. The identical procedure was followed with Cyt-*c*.

Because water is heated at 2455 MHz, Hb without SWCNTs is also denatured upon irradiation. First, MW power was fixed at 20W, a typical level of base stations for mobile phones. A 30-s irradiation caused total denaturation of Hb without SWCNTs (Figure 1). With the presence of SWCNTs, Hb was denatured faster at all irradiation times. It can be inferred from the graph that approximately 15 s is the maximum irradiation that Hb with SWCNTs is denatured but one without SWCNTs is intact.

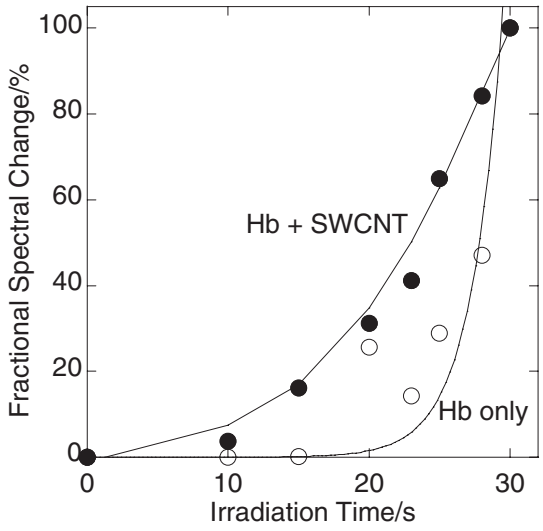

Figure 1. Denaturation of Hb at various MW irradiation time. The closed circles denote Hb with SWCNTs and the open circles correspond to Hb only.

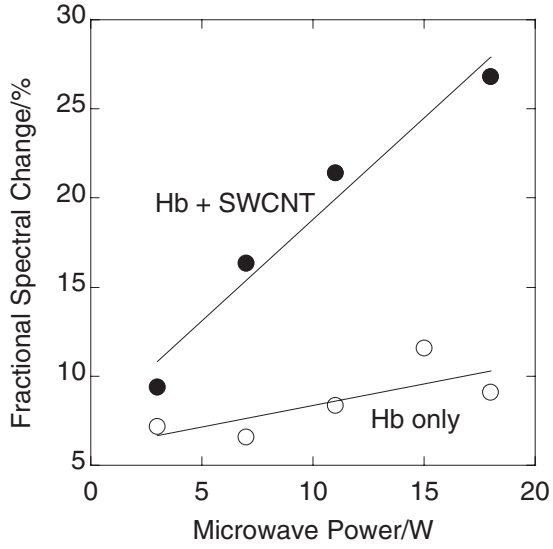

Figure 2. Denaturation of Hb at various MW power.

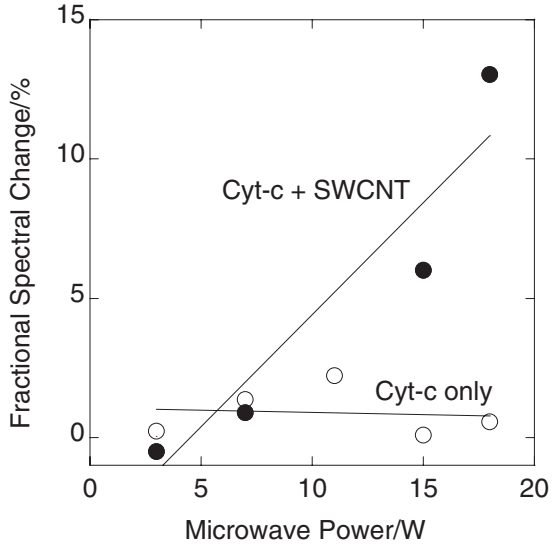

Figure 3. Denaturation of Cyt-*c* at various MW power.

Figure 2 shows a similar comparison with various MW powers at a constant irradiation time of 25 s, enough time to have a short conversation or to send an e-mail. The presence of SWCNTs enhances denaturation at all powers. We estimate that about 4 W is the maximum power to recognize the effect of SWCNTs. Both results indicate that the MW irradiance of 100–300 W s (J: joule) causes recognizable differences by the presence of SWCNTs.

Hb, however, is very vulnerable to heat. The average solution temperature at the maximum irradiance in the above experiments was measured to be 40 °C, yet we expected that the temperature of SWCNTs must be much higher.[5] There is a possibility that some Hb are denatured not by heated SWCNTs but by heated water around hot SWCNTs. To clarify this point, the same experiment was repeated with another heme protein, Cyt-*c*, found in mitochondria. It is not destroyed even near the boiling temperature and has a reversible folding/unfolding transition at 67 °C.[11] It is known to adsorb on SWCNTs without denaturation.[12–14] All spectral changes were monitored after cooling to room temperature. Cyt-*c* alone is hardly denatured, but the presence of SWCNTs clearly promotes denaturation (Figure 3). The power at which SWCNTs have a clear influence is about 7 W. Cyt-*c* requires slightly higher MW power than Hb. Because the data presented in Figures 2 and 3 have been obtained using the identical SWCNT dispersion, their magnitudes may be compared. These plots indicate that the denatured fraction of Cyt-*c* is smaller than Hb under the same MW irradiation. Either more Hb than Cyt-*c* has been adsorbed on SWCNTs or unadsorbed Hb has been denatured by heated water.

Upon irradiation, denatured Hb coagulated heavily and formed large aggregates with SWCNTs, making further analyses difficult. On the other hand, Cyt-*c* mixtures stayed reasonably clear to allow further examinations, probably due to the smaller quantity of denatured proteins. UV–vis absorption spectroscopy and circular dichroism spectroscopy reveal that some ferric Cyt-*c* is transformed into ferrous form while denaturation is in progress.[15] On the contrary, we have not observed an enhanced reduction of Hb. This fact can be explained by the protein structure. Because heme is exposed in Cyt-*c*, electron transfer takes place by simple collision. For Hb, heme is hidden inside globins and it requires adsorption and restructuring for transfer.[10]

The present study demonstrates that SWCNTs under MW radiation, with a magnitude level of base stations irradiated for a few tens of seconds, have considerable effects on the denaturation and redox equilibrium of heme proteins.

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