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New Functional Device using Bio Nano Process

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[Abstract]

In order to fabricate new dye-sensitised solar cell, we proposed a new photoresponsive electrode structure using bio nano process. Carbon nanotube-TiO_2 hybrid materials having multitudious nano-sclae cavities were realized using a bifunctional cage-shaped protein possessing carbonaceous material-binding peptides and Ti-binding peptides. The obtained structure was confirmed to consist of a central nanotube, surrounding proteins, and a swathing titanium-layer. The process was carried out at room temperature, using an environmentally-friendly method.

[Summary]

In order to fabricate new dye-sensitised solar cell, we proposed a new photoresponsive electrode structure using bio nano process. Titanium dioxide (TiO_2) has been extensively applied to photovoltaic and photocatalytic materials due to its efficient photoactivity, high photostability, chemical inertness, biological inertness, and nontoxicity.[1] In order to realize an efficient photovoltaic reaction with TiO_2, the development of a TiO_2-based material having a large surface area and posessing a structure for preventing electron-hole recombination will be required. So far, various approaches have been attempted in order to develop such TiO_2-based materials. For example, carbon nanotubes (CNTs) are used to improve electric properties of TiO_2,[2] and various CNT/TiO_2 compounds using functionalized CNTs that were oxidatively treated by organic compounds have been produced.[3] However, it was anticipated that acid treatment of CNTs would reduce the electronic properties.[2] In contrast, Pender et al. created a bifunctional peptide aptamer that has titania-binding and CNT-binding motifs for construction of titania materials. The peptide aptamer can precipitate titania from water-soluble precursors at the surface of single-walled carbon nanotubes (SWNTs) without covalent functionalization of the nanotubes under mild conditions.[4] However, they reported that only a thin-layer with a small surface area was able to be constructed using the peptide aptamer, because aptamers were short polymers of amino acids. Here we report that a novel bifunctional cage-shaped protein able to fabricate a SWNT-titanium nanocompound containing nano-porous derived from protein complexs. The nanocompound has the potential to produce improved photoactive materials after sintering.

First, we designed variant proteins derived from Listeria innocua Dps.[5] Dps is composed of 12 identical subunits that assemble into robust cage-shaped structure, and is capable of holding metal oxide nano-particles (NPs) within its inner cavity. Cage-shaped proteins had been widely applied for fabricating nanostructures.[6-7] Two peptide aptamers, NHBP-1 (DYFSSPYYEQLF)[8] and minTBP-1 (RKLPDA)[9] were genetically fused at the

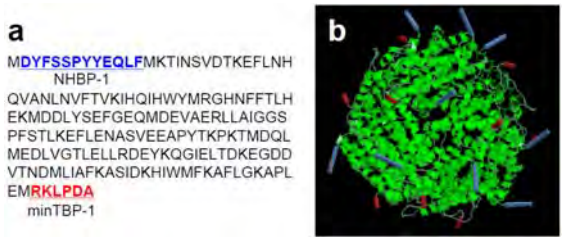

Fig. 1 (a) Amino acid sequence of CDT1. The under bars show carbonaceous material-binding peptide NHBP-1 and Ti-binding peptide minTBP-1, respectively. (b) Schematic drawing of CDT1. Blue rods and red rods indicate NHBP-1 peptide and minTBP-1, respectively.

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14. ABSTRACT
In order to fabricate new dye-sensitised solar cells, we studied a new photoresponsive electrode structure using bio-nano processes. Carbon nanotube-TiO2 hybrid materials having multitudious nano-sclae cavities were realized using a bifunctional cage-shaped protein possessing carbonaceous material-binding peptides and Ti-binding peptides. The obtained structure was confirmed to consist of a central nanotube, surrounding proteins, and a swathing titanium-layer. The process was carried out at room temperature, using an environmentally-friendly method

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N-terminal and at the C-terminal ends of the Dps protein monomer respectively, to construct CDT1 (Carbonaceous material binding peptide-Dps-Titanium binding peptide, Fig. 1). To obtain the synthetic gene encoding CDT1, the DNA fragment was amplified by PCR using primers (5'-tttggatccg aattcgagct ccgtcg-3' and 5'-tttggatcct tacgcatccg gaagtttgcg catttctaat ggagcttttc-3') and pET20b-NHBP-LiDps plasmid [10] as a template. The pET20b-NHBP-LiDps plasmid contains the gene encoding Dps fused with only NHBP-1 at the N-terminal region (NHBP-LiDps). The PCR product was digested with BamHI and the resulting fragment was self-ligated. pET-CDT1 plasmid encoding CDT1 was obtained. In addition, pET-DT encoding Dps fused with minTBP-1 in a C-terminal region was also constructed following the procedures described above. The protein produced by pET-DT was designated DT (Dps-Titanium binding peptide). Three types of Dps variants were expressed in Escherichia coli BL21(DE3) which were cultivated in LB medium with ampicillin. CDT1 and DT were purified using a modified L. innocura Dps purification method which was reported previously (Fig. S1, ESI†).[11] NHBP-LiDps was purified by the modified protocol previously reported.[10]

TEM observation of overproduced CDT1 showed cage-shaped proteins, the size and shape of which are almost the same as native Dps (apo-CDT1, Fig. S2a, ESI†). Since native Dps is capable of forming various kinds of NPs within its cavity where source ions going through the channels in the protein shell,[7, 11] cores should be

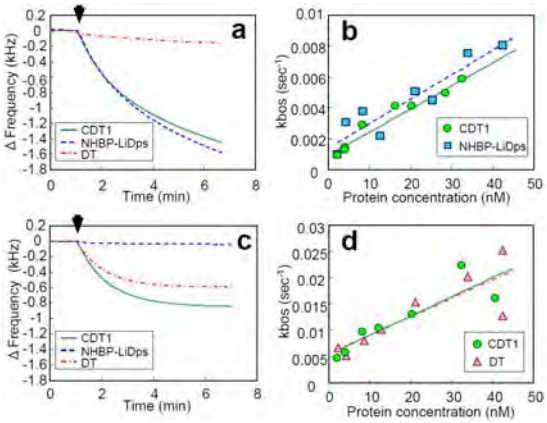

Fig. 2 Adsorption of CDT1 to the SWNT (a, b) or Ti (c, d) sensor of QCM. The changes in resonance frequency were measured as a function of time after the addition of 20 nM Dps-variant to (a) SWNT sensor, or (c) titania sensor. The proteins were injected to sensor chip at black arrow of graphs, respectively. The (b) and (d) show the correlations between the concentrations of Dps-variant and k_{obs}.

formed in the variant cavity, if the variant cage was complete. We tried iron oxide NP cores synthesis in the interior of CDT1 by using ammonium iron sulfate solution (Fe-cored CDT1, Fig. S2b, ESI†). It was clearly observed via TEM that iron oxide NPs with 4-5 nm in diameter were formed inside CDT1. It was shown that the mineralization activity of Dps is not hampered by addition of NHBP-1 and minTBP-1 at both terminal ends. These results indicated that CDT1-monomers spontaneously organized and were self-assembled into the same cage with the native one except having additional peptides.

Crystal structure analysis shows that both N-terminal and C-terminal regions of the Dps-monomer are located on the Dps protein shell. Therefore, two peptide aptamers of CDT1 are expected to protrude into solution and have a flexible conformation. We anticipated that CDT1 could interact with SWNTs and titania via the appended NHBP-1 and minTBP-1 which form the necessary structure for generation of an adhesive force. The interaction of CDT1 with SWNTs or titania was measured by using a quartz crystal microbalance (QCM) technique. Firstly, a sensor was prepared by deposition of SWNTs. SWNTs (Sigma Aldrich, MO, USA) were dispersed by sodium dodecyl sulfate and the obtained solution was applied to the gold electrode surface of quartz sensor chip (Initium Inc., Japan). After drying, the SWNT sensor chip was immersed into phosphate buffer, tween-20 (0.001 % (v/v) in 50 mM potassium phosphate, pH7.0 at 25°C. Upon injection of NHBP-LiDps having the NHBP-1 aptamer into a QCM-sensor cell, the resonance frequency of the SWNT sensor decreased. In a similar way, injection of CDT1 also decreased the resonance frequency (Fig. 2a). In contrast, the injection of DT which had no SWNT-binding peptide, showed little decrease of resonance frequency. The result indicated that the CDT1 was able to bind to the surface of SWNTs without acid treatment and that the NHBP-1 peptide of CDT1 retained the ability to bind SWNTs as expected.

Kinetic parameter k_{obs} was obtained from exponential curve fitting to the theoretical frequency variation.

There is a linear correlation between k_{obs} and protein concentration and the slope and intercept of the linear curve show the binding rate constant k_{on} and dissociate rate constant k_{off}, respectively (see ESI†).[12] The correlation between k_{obs} and CDT1 concentration shown in Fig. 2b indicated the k_{on} and the k_{off} of CDT1 for SWNTs were 1.5×10^{5} (M^{-1} s^{-1}) and 1.0×10^{-3} (s^{-1}), respectively. The k_{on} and the k_{off} of NHBP-LiDps for SWNTs were 1.6×10^{5} (M^{-1} s^{-1}) and 1.4×10^{-3} (s^{-1}), respectively. There is no significant difference in the affinities to SWNT between CDT1 and NHBP-LiDps. Similarly, the binding abilities of minTBP-1 appended to the C-terminal of CDT1 and DT were also measured by using thin-layer Ti deposited on a quartz sensor chip (Initium Inc., Japan) (Fig. 2c, d). There is no significantly difference in the kinetic parameters for titania between CDT1 [k_{on}; 3.6×10^{5} (M^{-1} s^{-1}) and k_{off}; 5.5×10^{-3} (s^{-1})] and DT [k_{on}; 3.5×10^{5} (M^{-1} s^{-1}) and k_{off}; 5.7×10^{-3} (s^{-1})].

Those results indicated that the CDT1 has the ability to bind both SWNTs and titanium. The result clearly showed the advantage of using Dps, the C-terminus and the N-terminus of the subunit peptide-chains are exposed at the surface and the those regions are not responsible for the cage assembly of tertiary structure.[5] We can replace the aptamers freely with apatmers specifically binding to other materials without hampering the Dps robustness.

The QCM analysis indicated that CDT1 could adsorb onto SWNTs without chemical treatment, but the best conditions for making bio-conjugate of CDT1 and SWNT where ideally, SWNT is covered fully by CDT1s was not clear. Especially it was anticipated that pH condition was critical for CDT1 full coverage. Solution pH greatly affect the electrostatic interaction among CDT1. Too strong electrostatic repulsive force will make SWNT covered sparsely by CDT1 and too weak leads to aggregation of CDT1s. Therefore, we tried to mix SWNT and CDT1 under various solution pHs and observe the interaction behaviors of CDT1s and SWNTs by TEM (JEM-2200FS, JEOL, Japan). First, SWNTs in 50 mM citrate buffer pH 4.0, 50 mM potassium phosphate buffer pH 6.0, 50 mM HEPES buffer pH 7.5, 50 mM Tris-HCl buffer pH 9.0 and 50 mM glycine buffer pH 10.5 were prepared. The SWNT solutions were mixed with CDT1 and made to a final concentration of 0.5 mg/ml CDT1 and 0.3 mg/ml SWNT. The mixtures were treated by ultrasonic sonication (Digital Sonifier 450, Branson, USA) in an ice bath for 5 min. After ultrasonic treatment, the unreacted SWNTs were removed by centrifugation. The supernatant was retrieved and aliquots were observed by TEM (Fig. 3). The adsorption of CDT1 with SWNTs was confirmed in buffers of pH 6.0, pH 7.5, and pH 9.0. As pH value increases from 6 to 9, CDT1s showed a tendency to bind with not only SWNT, but also each other. Therefore, CDT1s could bind around SWNT efficiently at pH 6.0 buffer. In contrast, the CDT1 was not able to bind SWNTs in buffers at pH 4.0 and pH 10.5. It was concluded that the best condition for making conjugates of CDT1 and SWNT was 50 mM potassium phosphate buffer at pH 6.0. In contrast, complexes of SWNT and DT protein lacking the NHBP-1 aptamer could not be obtained.

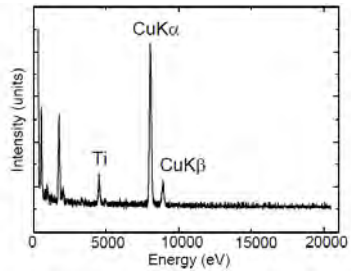

Fig. 4 The EDS spectrum of the SWNT/CDT1/Ti complex. The Cu peaks are due to the TEM grid.

We use the obtained CDT1 and SWNT bio-conjugate for fabrication of SWNT-titanium nanocompound. It has been shown that the aptamers that bind specific inorganic materials are able to mediate mineralization of

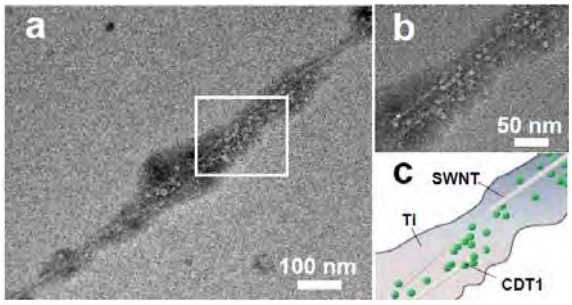

Fig. 5 (a) TEM image of SWNT/CDT1s coated with titanium matrix. (b) The enlarged microphotograph of white box in (a). (c) Schematic drawing of SWNT/CDT1/Ti complex shown in (b). The samples were stained with 3 % PTA.

their target materials. For instance, the Ti-recognizing peptide aptamers, minTBP-1 and R5, are able to mineralize titania using soluble precursor of titanium, titanium (IV) bis-(ammonium lactato)-dihydroxide (Ti[BALDH], Sigma Aldrich, MO, USA).[4, 13] If the minTBP-1 is exposed freely in the solution, CDT1 also should have the ability to accelerate the formation of titanium precipitate and CDT1 on the SWNT could selectively coat SWNT/CDT1 conjugates with titanium. We added a 50 %wt solution of Ti[BALDH] to 50 mM potassium phosphate buffer (pH6.0) containing SWNT/CDT1 nanocomplexs and left at 24°C for 16 hour. After incubation, the solution produced some precipitate. The precipitates were recovered by centrifugation and analyzed by TEM. The TEM image showed the precipitates contained a matrix of black fibrous structures. The matrix enclosed the individual and small boundless of SWNTs that coated several CDT1 proteins (Fig. 5). The matrix was analyzed using energy X-ray dispersive spectroscopy (EDS). As a result, the presence of titanium in the black fibers was confirmed (Fig. 4). The length and diameter of the SWNT/CDT1/Ti nanocomplexes were 1 μm and 50 - 100 nm, respectively. The titanium coated SWNTs were not able to be obtained without bifunctional cage-shaped protein (Fig. S3, ESI†). The results suggested that CDT1 selectively modified the SWNTs without covalent functionalization of the nanotube, under mild conditions and deposited a Ti-layer at the surface of the SWNTs. Moreover, the surface area of Ti-layer deposited on SWNTs could be increased by the nano-sized cavity of CDT1. Therefore, a SWNT/TiO_2 hybrid material that has a large surface area and a structure for preventing electron-hole recombination would be fabricated using the SWNT/CDT1/Ti nanocomplexes.

In summary, we have succeeded in construction of a novel bifunctional cage-shaped protein that is able to selectively adsorb SWNTs without special treatments using oxidizing conditions. The titanium-coated SWNTs were obtained using CDT1 at room temperature and will be used as a precursor material of photocatalysis compounds. This will be promising for the fabrication of photoresponsive electrode structure in dye-sensitised solar cell.

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