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Enzymatic synthesis of amylose nanocomposite microbeads using amylosucrase from *Deinococcus geothermalis*

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Materials and bacterial strains:

Sucrose, tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), sodium chloride (NaCl), ethylenediaminetetraacetic acid (EDTA), monosodium phosphate (NaH_2PO_4), sodium dodecyl sulfate (SDS), imidazole, ampicillin, and acetone were purchased from Sigma-Aldrich (St. Louis, MO, USA). Luria-Bertani (LB) broth was obtained from Becton, Dickinson and Company (Franklin Lakes, NJ, USA). The single-walled carbon nanotubes were acquired from ILJIN Nanotech (Seoul, Korea). The alumina membrane filter was supplied by Whatman (Anodisc, 0.1-μm pore size, Maidstone, UK). Distilled water was used in all experiments. *Escherichia coli* MC1061 was used as the host for the expression of recombinant DGAS.

Expression and purification of recombinant amylosucrase from *Deinococcus geothermalis*:

Escherichia coli MC1061 [F- araD139 Δ(*ara-leu*)7696 *gal*E15 *gal*K16 Δ(*lac*)X74 *rps*L(Str^r) *had*R2 ($r_k^-m_k^+$) *mrc*A *mrc*B1] harboring the cloned *dgas* in pHCE vector (pHCDGAS) was used for the production of DGAS.[1] The recombinant *E. coli* MC1061 harboring pHCDGAS cells were grown in 500 ml LB culture with 0.1 mg/ml ampicillin in 37°C for 24 h with agitation at 250 rpm. The cells were harvested by centrifugation (7,000 × g for 20 min at 4°C) and washed with a lysis buffer [50 mM NaH_2PO_4, 300 mM NaCl, and 10 mM imidazole (pH 8.0)]. The bacterial pellet was resuspended in a lysis buffer and disrupted by sonication (Sonifier 450, Branson, Danbury, CT, USA; output 4, 6×10s, constant duty) in an ice bath. The cellular debris was removed by centrifugation at 10,000 × g for 30 min at 4°C. The filtered supernatant was passed through a Ni-NTA affinity column (Qiagen Inc, Valencia, CA, USA). The Ni-NTA affinity column was washed with washing buffer [50 mM NaH_2PO_4, 300 mM NaCl, and 20 mM imidazole (pH 8.0)] and the recombinant DGAS was eluted with the elution buffer [50 mM NaH_2PO_4, 300 mM NaCl, and 250 mM imidazole (pH 8.0)]. The eluted fraction was dialyzed to remove the excess imidazole and NaCl.

Purification of single-walled carbon nanotube:

Single-walled carbon nanotubes (SWCNTs) were purified as described elsewhere.[2] Briefly, 4 mg of the SWCNT and 100 mg of SDS were mixed with 10 ml of DI water. The mixture was sonicated with a probe-ultrasonicator (VC 750, Sonics & Materials Inc., Newtown, CT, USA; output 40%, 15 sec disruption period with 25 sec interval, 30 min) in an ice bath. The sonicated mixture was centrifuged at 16,000 × g for 3 h at 4°C. The supernatant (~70%) was decanted and diluted with anhydrous acetone to remove the SDS from the SWCNT. The aggregated SWCNTs were collected by centrifugation and washed several times with acetone to completely remove the SDS.

Activity analysis of DGAS:

The activity of DGAS was measured using the dinitrosalicylic acid (DNS) method.[3] The reaction mixture was composed of 125 µl of 2M sucrose, 355 µl of distilled water, 0.2 mg of purified SWCNT and 500 µl of 100 mM Tris–HCl buffer (pH 8.0). The reaction was started by adding 300 U of the enzyme to the reaction mixture at 30°C. Fifty µl of the reaction was sampled every 2 h for 10 hr to determine the activity. The reducing sugars generated from the hydrolysis reaction were monitored by adding 150 µl of a DNS solution, followed by boiling for 5 min. The absorbance of the reaction was measured at 575 nm using microplate spectrophotometer (Infinite M200, Tecan, Durham, NC, USA). The reducing sugar concentration was calculated using fructose as a standard. The result showed that the activity of DGAS was higher for the reaction without the SWCNTs (Fig S1). The activity of DGAS was decreased slightly in the presence of SWCNTs. The reduced activity might have resulted from the physical adsorption of DGAS and substrate to the outer wall of the SWCNTs. Some of the sucrose adsorbed on the surface of SWCNT might not be available for polymerization by DGAS. The DGAS adsorbed on the SWCNTs may also have lower activity compared to the free enzyme.

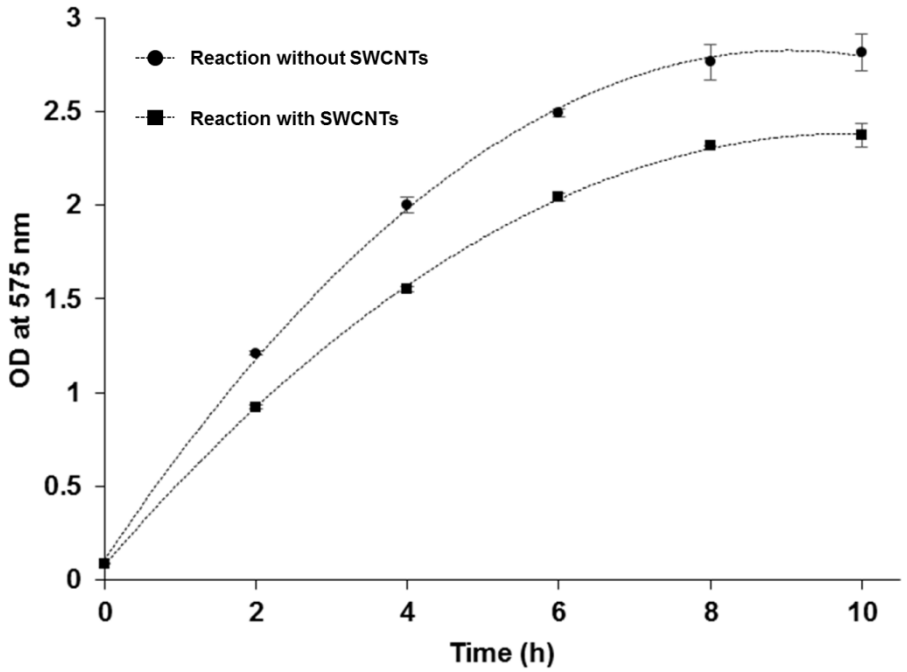

Fig. S1 Activities of DGAS over the course of a 10 h reaction at 30°C with and without the SWCNTs. The reactions with (square marker) and without (circle marker) the SWCNTs were analyzed using the DNS method under the same conditions.

Preparation of amylose microbeads and AM-SWCNT microbead:

Amylose microbeads were synthesized in 1 mL of 50 mM Tris-HCl buffer (pH 7.0) containing 500 mM sucrose and 300 U amylosucrase (DGAS) at 30˚C for 24 hr. To synthesize the AM-SWCNT microbeads, 0.2 mg of the purified SWCNTs was added to the reaction mixture. The reaction mixture was composed of 125 µl of 2M sucrose, 355 µl of distilled water, 0.2 mg of purified SWCNT and 500 µl of 100 mM Tris–HCl buffer (pH 8.0). The reaction was started by adding 300 U of the enzyme to the reaction mixture at 30°C. The synthesized amylose microbeads and AM-SWCNNT microbeads were harvested by centrifugation, washed three times with DI water and dried in a vacuum dryer for further analysis.

Characterizations of amylose microbeads and AM-SWCNT microbeads:

The reaction mixture (100 µl) was filtered through an Anodisc® membrane with a 0.1-µm

pore size, followed by washing with 1 ml of DI water. The morphology of the amylose-based microbeads was investigated by scanning electron microscopy (SEM, Stereoscan 440, Leica Cambridge, Cambridge, UK; accelerating voltage of 20 kV) without a metal coating. Raman scattering was performed using a high resolution Raman spectrometer (InVia Raman microscope, Renishaw InC., Gloucestershire, UK) with a laser excitation wavelength at 514 nm with 50 mW Argon Ion laser grating from 100 to 3200 cm^{-1}. The three samples, SWCNTs, amylose microbeads and AM-SWCNT microbeads, were measured on a clean glass microscope slide.[4] Power X-ray diffraction (XRD, D8 Advance, Bruker, Karlsruhe, Germany) of the three samples were performed using Cu Kα radiation (0.154 nm) at 40 kV and 40 mA. Scanning was performed from 2° to 30° 2θ with a step size of 0.020° with an exposure time of 12.4 s.[5]

Average degree of polymerization ($\bar{DP}$) of amylose:

The AM microbeads and AM-SWCNT microbeads were washed with DI water for tree times and dried in vacuum dryer. The dried samples were solubilized in 1 M KOH to the final concentration of 10 mg/ml. The solution was mixed with 10.5 volume of 0.1 M HCl, 111.5 volume of DI water, and 2 volume of iodine solution (2% KI and 0.2% I2). The maximum absorption wavelength (λmax) of the amylose-iodine complexes was determined from the absorption spectra from 450 to 750 nm with 2 nm step using microplate spectrophotometer (Infinite M200, Tecan, Durham, NC, USA). The corresponding $\bar{DP}$ of amylose from AM microbeads and AM-SWCNT microbeads was calculated based on the following equation.[6]

$$103/\lambda_{max} = 11.6/\bar{DP} + 1.537$$

Figure S2 shows the absorption spectra of amylose-iodine complexes from two samples. From the spectra, the maximum absorption wavelength of AM microbeads was 560 nm while that of AM-SWCNT microbeads was 554 nm. Based on the equation, the $\bar{DP}$ of amylose self-assembled in the AM microbeads and AM-SWCNT microbeads are 47 and 43, respectively.

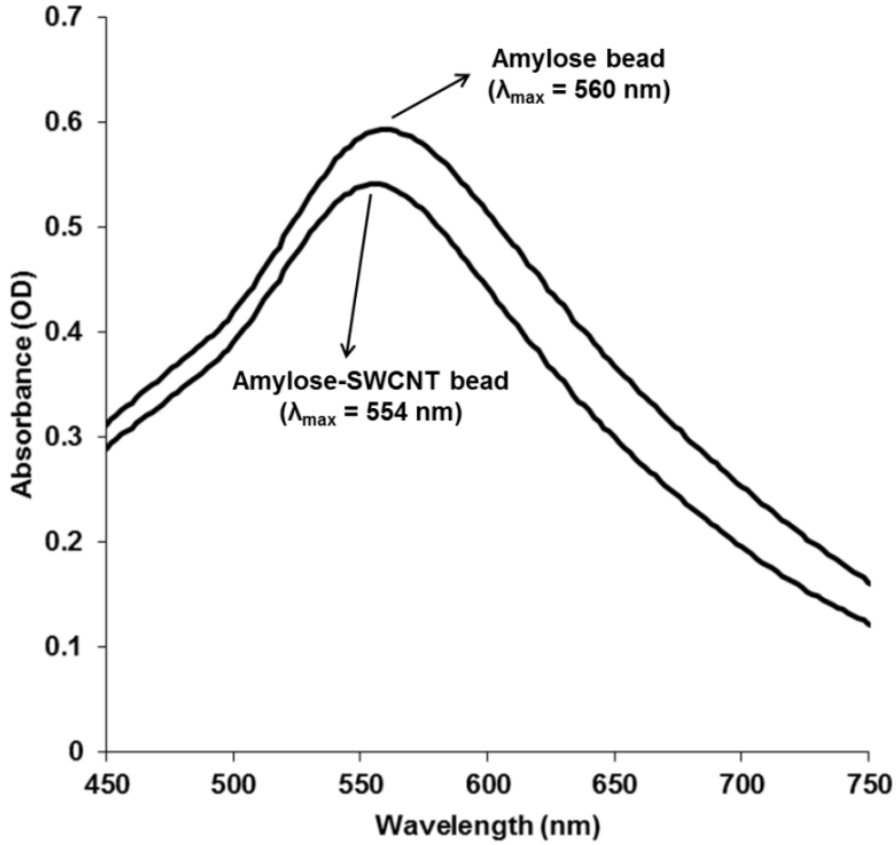

Figure S2. Absorption spectra of amylose-iodine complexes from AM microbeads and AM-SWCNT microbeads.

Size distribution of AM microbeads and AM-SWCNT microbeads:

The average sizes of AM microbeads and AM-SWCNT microbeads were determined by measuring the diameter of 100 microbeads from SEM images.

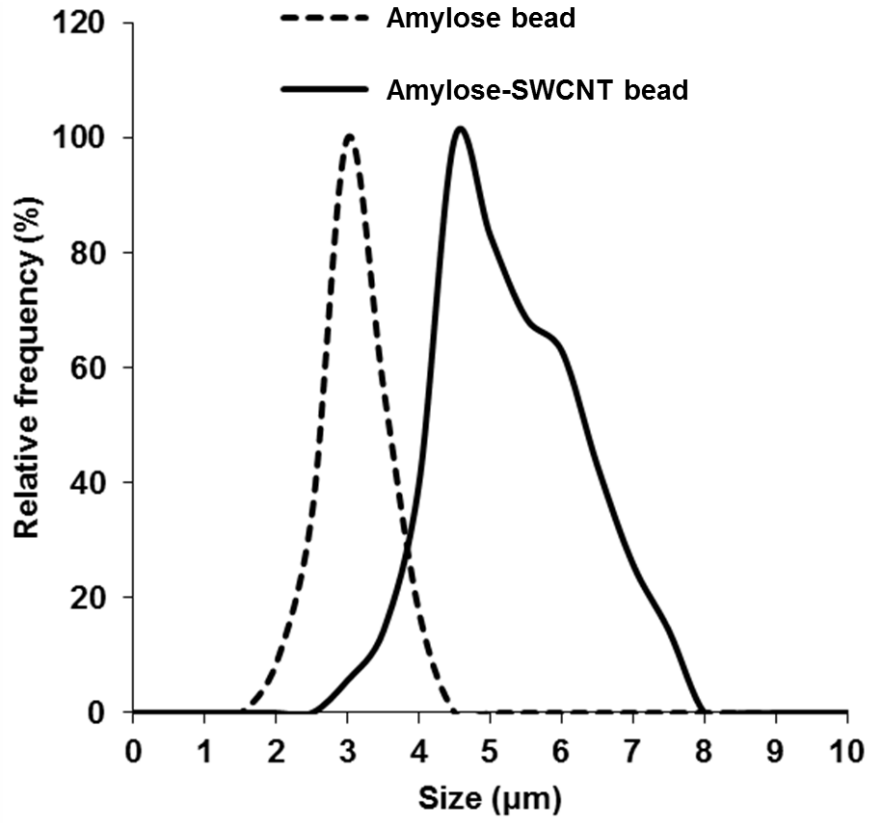

Figure S3. The size distribution of AM microbeads and AM-SWCNT microbeads.

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