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

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Abstract

We introduce a biological approach to prepare pure amylose microbeads using the amylosucrase from *Deinococcus geothermalis*. SWCNTs could be incorporated readily into the amylose structure during enzymatic synthesis to form well-defined amylose-SWCNT composite microbeads through a self-assembly process of the synthesized amylose molecules and SWCNTs.

Molecular self-assembly is a powerful approach for fabricating novel supramolecular structures with precise control of the size and shape.¹ This paper reports a facile bottom-up approach to produce well-defined amylose (AM) microbeads via an enzymatic reaction. As one of the most abundant polysaccharides in nature, amylose is a linear homopolymer of glucose linked with $\alpha(1,4)$ glycosidic bonds. Amylose is one of the major components in starch and serves as an energy reserve in nature along with another carbohydrate, amylopectin, which is a branched macromolecule composed of $\alpha(1,4)$ -D-glucan chains linked with 5-6% $\alpha(1,6)$ bonds. The self-association of amylose in aqueous solutions has been studied extensively to understand the formation, crystallization and structural change in starch granules and to produce amylose-base microstructures.^{2,3} Micro-sized amylose beads were prepared by heating and cooling high amylose starch solutions.⁴ The morphology and size of the amylose beads vary according to the speed of cooling the heated amylose solution. Cross-linking of an amylose emulsion and spray-drying of gelatinized high amylose starch can produce amylose micro beads.⁵ On the other hand, their amylose content is less than 70% because the complete separation of amylose from amylopectin in natural high amylose starch is difficult. In addition, the use of organic solvents and high energy processing steps, such as heating, pressing and stirring, make these approaches quite complicated. The only way to produce pure amylose with the desired molecular weight is through enzyme-mediated polymerization using phosphorylase and amylosucrase.⁶ Phosphorylase requires α -D-glucose 1-phosphate (G-1-P) and a glycosyl acceptor, maltooligosaccharide, with a degree of polymerization (DP) greater than 4. Phosphorylase transfers a glucose unit from G-1-P to the non-reducing end of the acceptor to form an $\alpha(1,4)$ -D-glucan chain.⁷ On the other hand, amylosucrase catalyzes amylose synthesis from sucrose without the need for an acceptor or G-1-P. Recently, the *in vitro* synthesis of amylose by these enzymes has attracted considerable attention because amylose can serve as a host molecule and forms amylose supramolecules by including a range of guest molecules that are otherwise insoluble or unstable in aqueous environments.^{8,9}

This paper presents a novel approach to prepare a pure amylose microbeads as well as AM-single walled carbon nanotube (SWCNT) composite microbeads by enzymatic synthesis using recombinant amylosucrase from *Deinococcus geothermalis* (DGAS). Two major advantages of this approach to use DGAS for the synthesis of AM microstructures are the low cost of the substrate, sucrose, and the stability of the enzyme at high temperatures,¹⁰ which is quite promising for practical applications in the industrial scale production of an amylose-

based functional microstructure. SWCNTs can be incorporated spontaneously into the amylose microstructure during enzymatic synthesis to form well-defined AM-SWCNT composite microbeads through a self-assembly process via a hydrophobic interaction between the amylose molecules and SWCNTs. To the best of the author's knowledge, this is the first report to present the preparation of well-defined amylose-nanomaterials 3D structure by a simple enzymatic reaction without chemical conjugation.

Fig. 1 presents the overall scheme for the synthesis of pure AM microbeads and AM-SWCNT composite microbeads via a one-step enzymatic reaction using DGAS. The polymerization reaction begins by hydrolyzing sucrose to fructose and glucose. The glucose molecule is then used as the first acceptor for successive glucosyl moieties coming from the hydrolysis of sucrose. Through the cycle of reactions, malto-oligosaccharides or longer α -glucan polymers are produced and elongated further from their non-reducing end. The synthesized AM microbeads begin to precipitate from the reaction when a critical concentration and molecular weight are reached. The inset in Fig. 1 shows the precipitated AM microbeads and AM-SWCNT microbeads from 1 ml reactions containing 500 mM sucrose and 300 U DGAS at 30°C. A hydrogen bond and hydrophobic interaction between the synthesized amylose chains are believed to be responsible for the self-association of amylose in aqueous solution.^{2, 11} Purified SWCNTs dispersed in de-ionized (DI) water without a surfactant were aggregated and precipitated easily within a short period of time. On the other hand, the dispersibility of the SWCNTs in aqueous solutions was enhanced in the presence of a substrate, 500 mM sucrose, and the dispersed SWCNTs incorporated spontaneously into the amylose microstructure during synthesis. The enhanced solubility of SWCNTs in water by the vine-twining polymerization of amylose using potato phosphorylase was reported previously,¹² but the formation of a self-assembled AM-SWCNT composite microstructure has not been reported.

The morphology of the AM microbeads synthesized by an enzymatic reaction was examined by SEM (Fig. 1). For both reactions with or without SWCNTs, the AM microbeads were formed through self-association of the synthesized amylose molecules. As shown in the inset in Fig. 1, the synthesized AM microbeads were white, whereas the color was dark gray after the reaction with the SWCNTs, suggesting that all the SWCNTs were incorporated into the synthesized amylose molecule during the enzymatic reaction in solution. For SEM analysis, both samples were observed under the same operating conditions without a metal coating.

The charging effect was evident for pure AM microbeads due to the accumulation of electrons on the surface of non-conducting AM microbeads during imaging. On the other hand, the charging effect on the AM-SWCNT microbeads was reduced significantly during the SEM observations presumably because of the conducting nature of the SWCNTs, which were incorporated into the AM microbeads. The presence of SWCNTs in the amylose beads appear to enhance the stability and integrity of the microstructure forming a more discrete and spherical bead structure compared to the pure AM microbeads. The average degree of polymerization (\overline{DP}) of amylose self-assembled in the AM microbeads and AM-SWCNT microbeads are 47 and 43, respectively. The \overline{DP} of amylose was determined based on its relationship with the maximum absorption wavelength (λ_{\max}) of the amylose-iodine complexes (Fig. S2). The average size of AM microbeads was $2.78 \pm 0.46 \mu\text{m}$ while that of AM-SWCNT microbeads was $5.01 \pm 1.17 \mu\text{m}$. The size distributions of both microbeads were shown in Fig. S3. The enhanced aggregation of amylose chains in the presence of SWCNT might result in the increase in size of AM-SWCNT microbeads. SEM was also used to examine the formation of AM-SWCNT microbeads by sampling the reaction mixture at 1, 2, 3, and 6 h after the reaction began (Fig. 2). Amylose aggregates ($< 1 \mu\text{m}$ in diameter) entangled with CNTs were observed after 2 h of reaction. The amylose aggregates appeared to grow over the course of reaction because the CNTs were incorporated into the microstructure along with the synthesized amylose chains. The hydrophobic interaction between the amylose and CNT in the solution might induce the spontaneous entanglement of amylose chains and flexible CNTs. After 6 h of reaction, no observable CNTs remained in solution, suggesting that all the CNTs in the reaction mixture became embedded in the amylose microbeads during synthesis.

The characteristics of the AM-SWCNT and AM microbeads were examined by Raman spectroscopy (Fig. 3). As expected, the typical Raman bands of amylose appeared near 480, 860 and 941 cm^{-1} for both AM-SWCNT and AM microbeads. The Raman band at 480 cm^{-1} was attributed to the skeletal modes of the pyranose rings in amylose,¹³ whereas those at 860 and 941 cm^{-1} were assigned to six C-O-C bonds of glycosidic stretching modes.¹⁴ The SWCNTs used in this study showed strong asymmetric peaks at 1561 (G^-) and 1584 cm^{-1} (G^+), representing the semiconducting characteristics of the nanotubes.¹⁵ The same type of G band observed in the AM-SWCNT microbeads suggests that the semiconducting properties of SWCNTs were maintained even after complexation with the amylose chains (Fig. 3b). The

inset in Fig. 3b showed the unique radical breathing modes (RBMs) at 148, 152, 163, and 172 cm^{-1} . Because the RBMs band is quite sensitive to the diameter and local conformation of the SWCNTs, the up-shift of the RBMs for the AM-SWCNTs is probably caused by the amylose-assisted dissociation of the non-covalently bundled SWCNTs.¹¹ Raman spectroscopy showed that the amylose molecules and SWCNTs were complexed by non-covalent interactions and both amylose and SWCNT maintained their characteristics after complexation.

The microstructures were also examined by X-ray diffraction (XRD) to determine the crystallinity of amylose and the intertube packing of the SWCNTs. The peaks corresponding to typical B-type crystal structure of amylose near 5, 15, 17, 22, and 24° 2 θ were evident for AM and AM-SWCNT microbeads.⁸ On the other hand, the amplitude of those peaks in the AM-SWCNT microbeads was slightly lower, suggesting that the crystallinity of the self-assembled amylose structure was disrupted slightly by the embedded SWCNTs. Considering that the peak at lower angles (<5°) is related to the intertube packing of SWCNTs,¹⁶ the significantly decreased peak below 5° in AM-SWCNT suggests that the SWCNTs were dissociated by amylose molecules and were well dispersed within the microbeads. From these results, it is clear that DGAS can create pure B-type amylose microbeads through a bottom-up self-assembly process and guest molecules, such as CNT, can be introduced into the microbeads during the synthesis and self-assembly of amylose chains.

In conclusion, this paper reported a novel approach to produce pure amylose microbeads via an amylosucrase-catalyzed synthesis reaction followed by spontaneous self-association of the synthesized amylose molecules. The SWCNTs could be incorporated into the amylose microbeads during synthesis through a self-assembly process via hydrophobic interactions between the amylose molecules and SWCNTs, forming well-defined amylose-SWCNT composite microbeads. The functionality of the amylose microbeads can be expanded using a variety of guest materials. A biological system was used to synthesize the amylose based microstructure in a simple and cost-effective manner. This method is expected to have many applications ranging from chromatography to amylose-based encapsulation systems in the near future.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Detailed experimental procedures including cloning, expression, activity analysis of DGAS; enzymatic synthesis of AM microbeads and AM-SWCNT microbead; characterization of AM-SWCNT microbeads by SEM, Raman spectroscopy and XRD; and determination of average degree of polymerization and size distribution of both microbeads. See DOI: 10.1039/c000000x/

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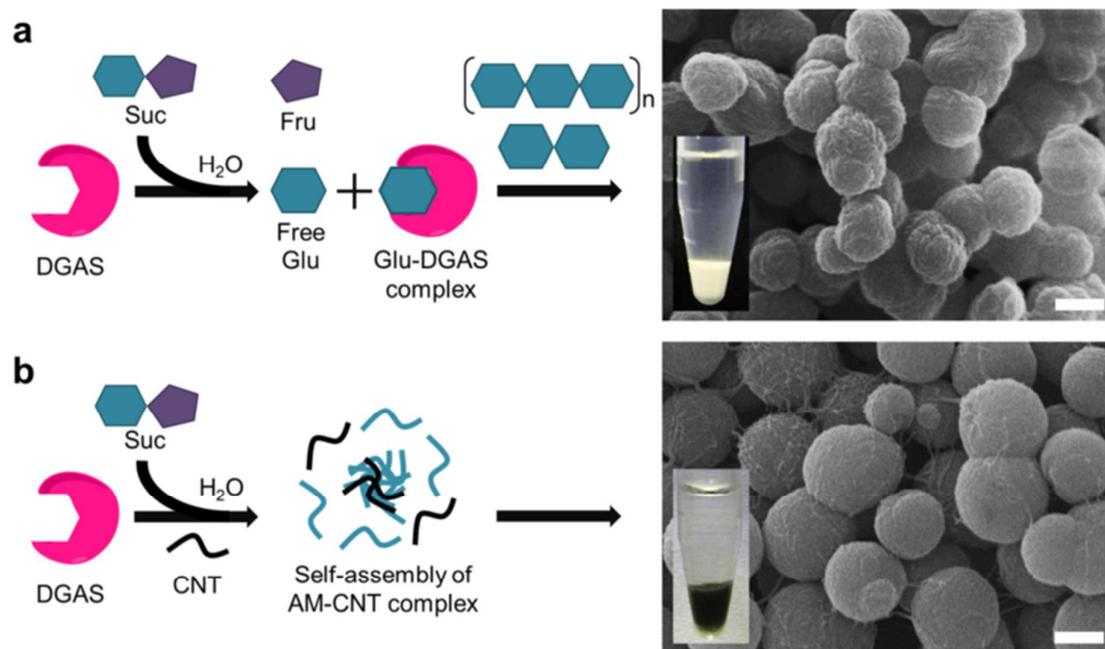


Fig. 1 Schematic diagram and SEM images representing the enzymatic synthesis of AM microbeads (a) and AM-SWCNT microbeads (b). Inset shows synthesized AM microbeads and AM-SWCNT microbeads from a 1 ml enzymatic reaction. The scale bar is 2 μm .

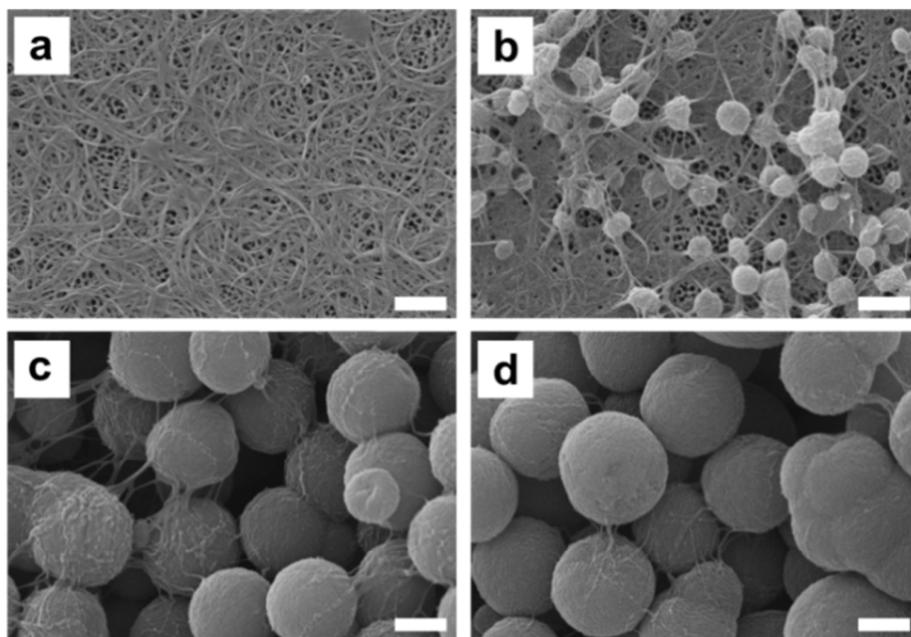


Fig. 2 Sequential SEM images showing the formation of AM-SWCNT microbeads over the reaction for 1 h (a), 2 h (b), 3 h (c), and 6 h (d). The scale bar is 2 μm .

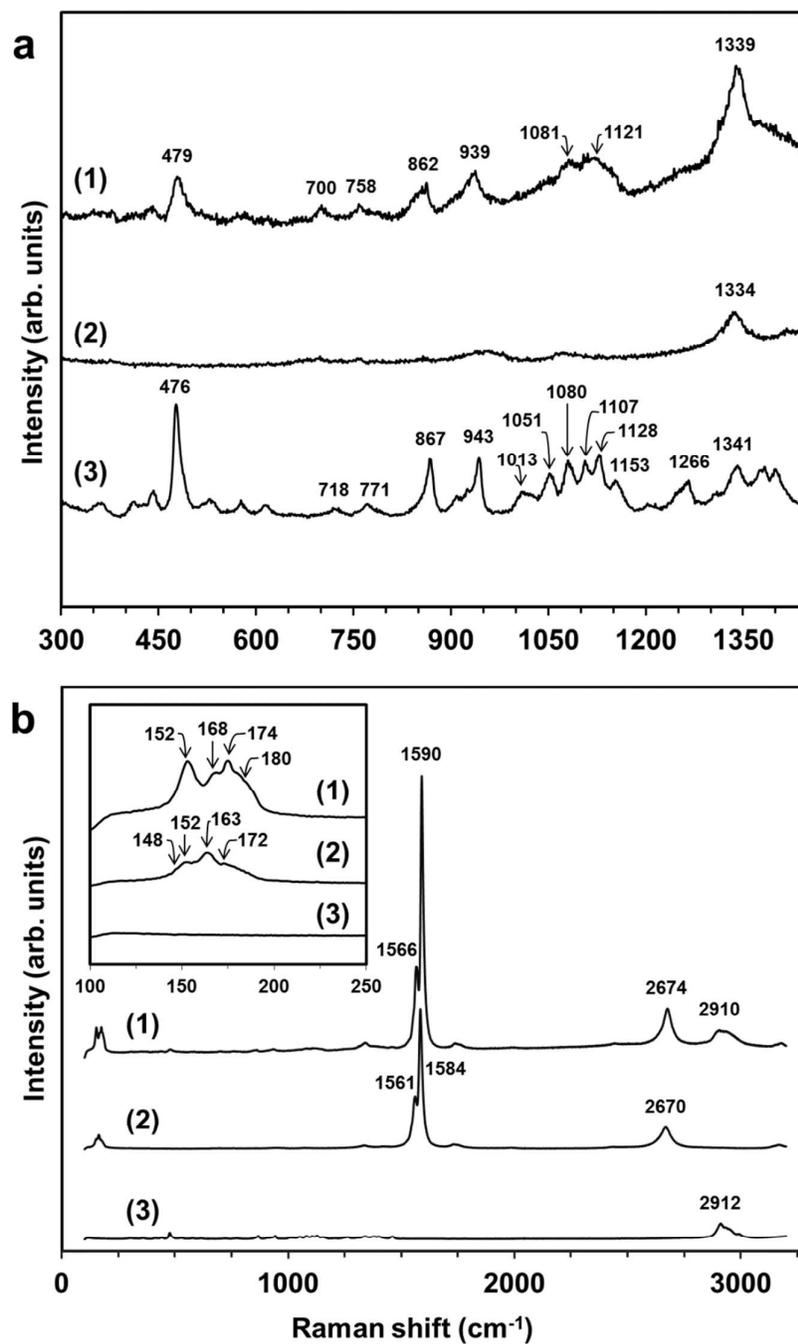


Fig. 3 Raman spectra of AM-SWCNT microbeads (1), SWCNT (2), and AM microbeads (3). The Raman spectra are shown at different scales to resolve the peaks in a lower shift (a and b). Inset shows the enlarged Raman spectra of the RBMs region of SWCNTs (b).

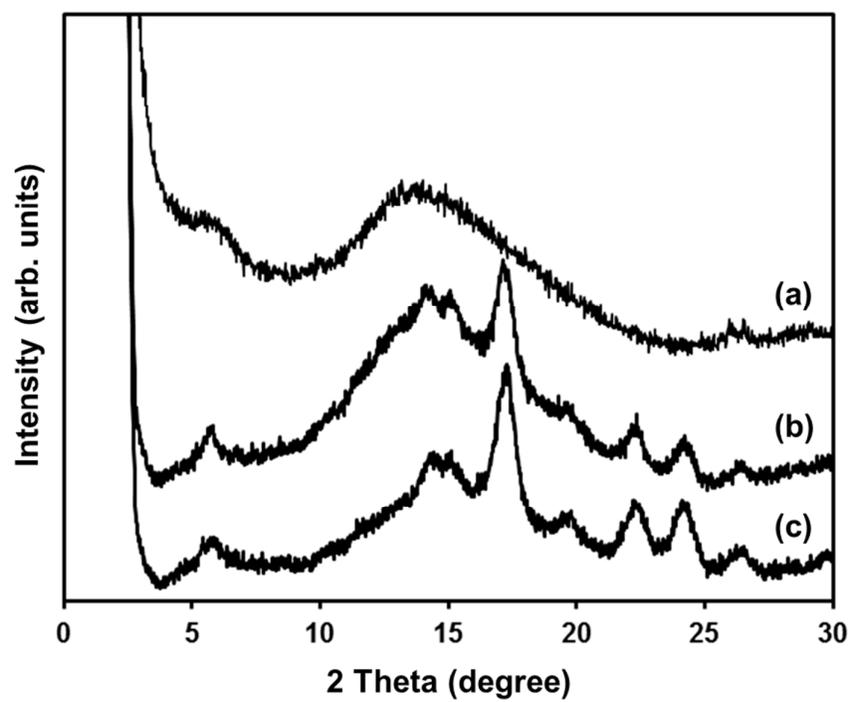
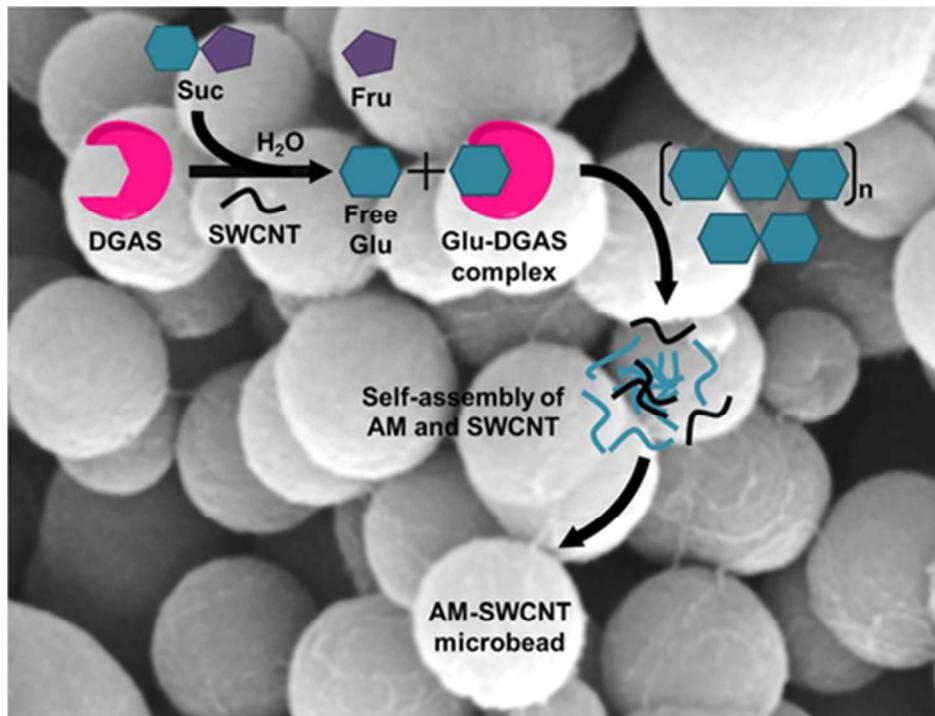


Fig. 4 X-ray diffraction patterns of SWCNT (a), AM-SWCNT microbeads (b), and AM microbeads (c).

This communication reports a biological approach to synthesize pure amylose microbeads and amylose-SWCNT composite microbeads using the amylosucrase from *Deinococcus geothermalis*



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