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Electronic Supplementary Information (ESI)

Amylosucrase-mediated synthesis and self-assembly of amylose magnetic microparticles

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Synthesis of the amylose magnetic beads and purification of MBP-tagged proteins

Chemicals and bacterial strains:

Sucrose, maltose, tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), sodium chloride (NaCl), monosodium phosphate (NaH_2PO_4), sodium dodecyl sulfate (SDS), imidazole, ampicillin, isopropyl-β-D-thiogalactopyranoside (IPTG), and magnetic iron oxide nanoparticle were purchased from Sigma-Aldrich (St. Louis, MO, USA). Luria-Bertani (LB) broth was obtained from Becton, Dickinson and Company (Franklin Lakes, NJ, USA). Agar powder was provided by Daejung Chemicals & Metals Co., Ltd. (Gyeonggi, Korea). Electro-cuvette 0.2 cm is a product of the Bio-Rad Company (Hercules, CA, USA). Ampicillin was acquired from Biosesang Company (Gyeonggi, Korea). The other chemicals, such as T4 DNA ligase, *EcoR*I, *Xba*I, and amylose resin, were supplied by New England Biolabs (Ipswich, MA, USA). Ni-NTA resin was purchased from Qiagen (Valencia, CA, USA). The PCR SV kit for PCR product purification was obtained from GeneAll (Seoul, Korea). Distilled water was used in all experiments. *Escherichia coli* MC1061 was used as the host pHCE vector (pHCDGAS) encoding the recombinant DGAS. *E. coli* DH5α containing pMal-c2x::*histag/gfp* was used for overexpression of the MBP-tagged proteins.

Synthesis of amylose magnetic beads by amylosucrase:

DGAS were prepared as described earlier.[1] Briefly, recombinant *E. coli* MC1061 harboring the pHCDGAS cells were grown in 500 mL LB culture (0.1 mg/mL ampicillin) in 37°C for 24 hr 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 in an ice bath and the cellular debris was spun down by centrifugation at 10,000 × g for 30 min at 4°C. The supernatant was passed through a Ni-NTA affinity column (Qiagen Inc., Valencia, CA, USA) and the column was washed with a washing buffer (50 mM NaH_2PO_4, 300 mM NaCl, and 20 mM imidazole (pH 8.0)). Finally, the recombinant DGAS was eluted with an elution buffer (50 mM NaH_2PO_4, 300 mM NaCl, and 250 mM imidazole (pH 8.0)). The enzyme activity was measured by determining the hydrolysis activity using the dinitrosalicylic acid (DNS) method with fructose as the standard.[2] To produce the amylose bead, an aqueous solution containing 500 mM sucrose, 300 U DGAS in 1 mL of 50 mM Tris-HCl buffer (pH 7.0) was prepared and the enzyme reaction was typically performed at 30°C for 24 hr. The amylose magnetic beads were then formed by the addition of 200 μl of heptane containing iron oxide nanoparticles (<14 mg/mL) to the 1 mL of aqueous reaction solution. Before adding the DGAS, the reaction solution was mixed to disperse the organic phase in an aqueous solution by bath sonication for 5 min. Subsequently, 300 U of DGAS was added to the mixed reaction solution and the reaction was performed under the same conditions described above. After the enzymatic amylose synthesis reaction was complete, the formed amylose beads and amylose magnetic beads were harvested by centrifugation and a magnet, respectively. The collected beads were washed several times with pure water and stored in 20% ethanol at 4°C until further use.

Characterization of amylose magnetic beads:

The produced amylose magnetic beads solution was dropped on the plasma-treated silicon substrate and dried at ambient temperature. The morphology of the amylose magnetic beads was examined by field emission-scanning electron microscopy (FE-SEM, Leo Supra 55, Genesis 2000, Carl Zeiss, Oberkochen, Germany) at an accelerating voltage of 5 kV. The mean size of the produced amylose magnetic beads was determined by measuring the diameter of 100 beads from the SEM images. The shape of the amylose magnetic bead was observed by transmission electron microscopy (TEM, JEM-2010F, JEOL, Tokyo, Japan). Elemental mapping of the samples was performed by energy dispersive X-ray spectrometry (EDS, Oxford INCA, Oxford Instruments, Oxfordshire, UK). The power X-ray diffraction (XRD) patterns of the three kinds of samples, iron oxide nanoparticles, amylose beads and amylose magnetic beads, were collected from 10 to 80°(2θ) using Cu Kα radiation on a Bruker D8 Advance diffractometer (Bruker, Karlsruhe, Germany). To prepare the XRD samples, several drops of an iron oxide nanoparticle solution were spread over glass covers and dried. Amylose beads and amylose magnetic beads were dehydrated in a vacuum desiccator and analyzed by XRD. Vibrating sample magnetometry (VSM, LakeShore 7404, Lake Shore Cryotronics, Inc., Westerville, OH, USA) was performed to characterize the magnetic properties of the amylose beads and amylose magnetic beads at room temperature. Solution samples of the amylose beads and amylose magnetic beads were prepared at the same weight per volume concentration.

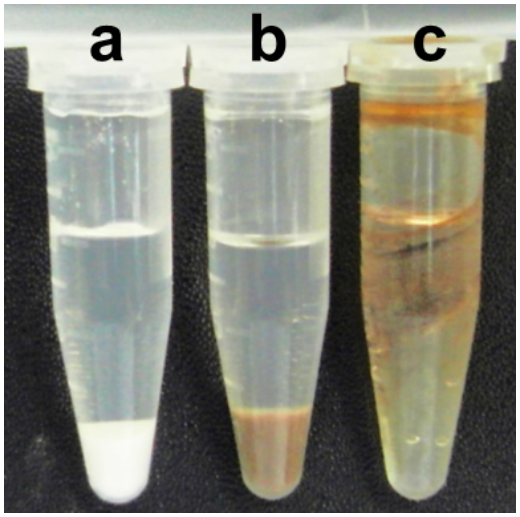

Fig. S1. Reaction tubes after the enzymatic reaction was complete. (a) Reaction without iron oxide nanoparticles, (b) reaction with iron oxide nanoparticles, and (c) reaction without DGAS.

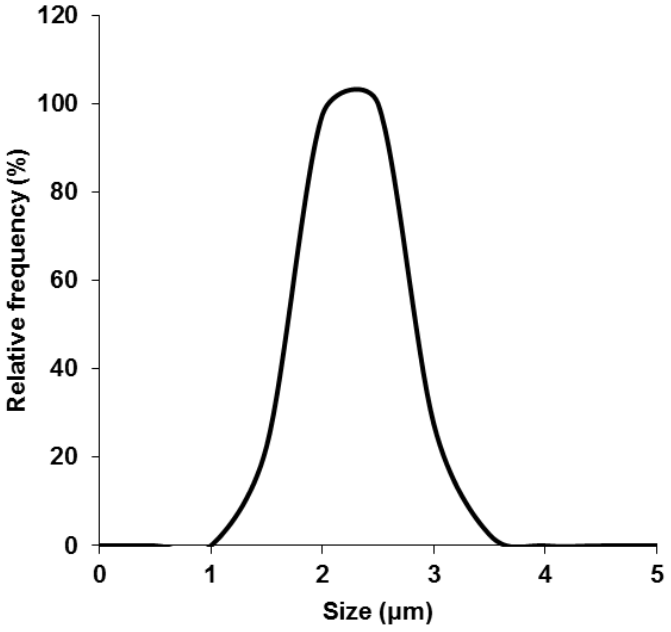

Fig. S2. Size distribution of the amylose magnetic beads. The mean diameter of the amylose magnetic beads was 2.09 ± 0.42 μm.

Expression and purification of MBP-tagged proteins from cell lysate:

E. coli DH5α containing pMal-c2x::*histag/gfp* was cultured for overexpression of the MBP-tagged green fluorescent protein (GFP). 100 mL of the cells was grown at 37°C with constant shaking in LB medium containing ampicillin (100 μg/mL). After reaching an OD_{600} of 0.8, the culture was induced with 0.1 mM IPTG at 18°C overnight. After induction, the cells were pelleted by centrifugation at 3,000 × g for 20 min at 4°C and redispersed in 5 mL of a column buffer (20 mM Tris-HCl, 200 mM NaCl, and 1 mM ETDA (pH 8.0)). Disruption of the cells was performed by sonication (VC 750, Sonics & Materials Inc., Newtown, CT, USA) with a 15 sec disruption period at 25 sec intervals in an ice bath for 10 min with a power of 225 W (duty cycle 50%). The soluble fractions from the cell disruption treatment were obtained by centrifugation at 3,000 × g for 20 min at 4°C. A 1 mL sample of the soluble fractions was incubated with 5 mg of amylose magnetic beads at 4°C for 30 min using a tube rotator (AG, FinePCR, Seoul, Korea). The amylose magnetic beads were separated by a magnet and washed 3 times with 1 mL of column buffer to remove the unbound lysates. Subsequently, 200 μl of an elution buffer (column buffer + 10 mM maltose) was added and incubated at 4°C for 10 min to elute the MBP-GFP. All fractions from each step were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and the amount of purified MBP-GFP was analyzed using the Bradford assay. The recyclability of the amylose magnetic beads for the purification of the MBP-tagged protein was investigated using the same procedures described above with the regeneration step. After the final elution, the amylose magnetic beads were washed in the following sequence: 300 μl of water, 300 μl of 0.1% SDS, 100 μl of water, and 500 μl of column buffer. The recyclability was tested 3 times and the elution fractions were analyzed by SDS-PAGE and the Bradford assay.

Construction of MBP-His-GFP:

To construct MBP-His-GFP expression vector, the *histag-gfp* gene was amplified from the pET28a::GFP vector by PCR using a primer set (5'*GCA TCA GAA TTC CAT CAT CAT CAT CAT CAC3'* and 5'*TAC AGT TCT AGA TTA TTT GTA TAG TTC ATC CAT3')*.* The amplified *histag-gfp* gene was purified and digested with *EcoR*I and *Xba*I. The pMal-c2x vector was also treated with those two restriction enzymes. The digested PCR product was ligated into the pMal-c2x vector according to the manufacturer's instructions using T4 ligase (Fig. S3) and the resulting construct, pMal-c2x::*histag/gfp* vector, was transferred to *E. coli* DH5α by electroporation. *E. coli* DH5α harboring pMal-c2x::*histag/gfp* was screened by growing in selective media containing ampicillin and colony PCR using the same primer set described above.

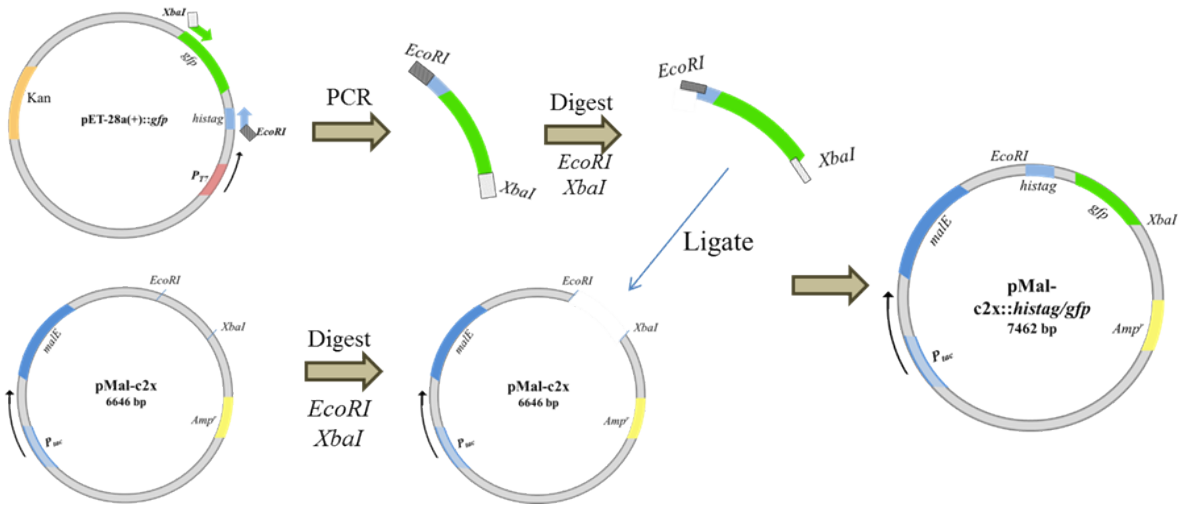

Fig. S3. Construction of the pMal-c2x::*histag/gfp* vector. *Histag-gfp* fragment with *EcoR*I and *Xba*I sites was ligated into pMal-c2x using T4 DNA ligase.

References

1. M.-C. Lim, D.-H. Seo, J.-H. Jung, C.-S. Park and Y.-R. Kim, *RSC Advances*, 2014, 4, 26421-26424.
2. J. B. Sumner and S. F. Howell, *Journal of Biological Chemistry*, 1935, 108, 51-54.