Enhanced Production of Dopa-Incorporated Mussel Adhesive Protein Using Engineered Translational Machineries

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Abstract

Mussel adhesive proteins (MAPs) have great potential as bioglues, in particular in wet conditions. Although in vivo residue-specific incorporation of 3,4-dihydroxyphenylalanine (Dopa) in tyrosine-auxotrophic Escherichia coli cells allows production of bioengineered MAPs (bMAPs), the low production yield hinders the practical application of bMAPs. Such low production yield of Dopa-incorporated bMAPs (Dopa-bMAPs) was known to be caused by low translational activity of a noncanonical amino acid, Dopa, in E. coli cells. Herein, in order to enhance the production yield of Dopa-bMAPs, we investigated the coexpression of Dopa-recognizing tyrosyl-tRNA synthetases (TyrRSs). In order to use the Dopa-specific Methanococcus jannaschii TyrRS (MjTyrRS-Dopa), we altered the anti-codon of tyrosyl-tRNA amber suppressor into AUA (MjtRNATyrAUA) to recognize a tyrosine codon (MjtRNATyrAUA). Co-overexpression of MjTyrRS-Dopa and MjtRNATyrAUA increased the production yield of Dopa-MAP by 57%. Similarly, overexpression of E. coli TyrRS (EcTyrRS) led to a 72% higher production yield of Dopa-incorporated bMAP. Even with coexpression of Dopa-recognizing TyrRSs, Dopa-bMAPs have a high Dopa incorporation yield (over 90%) compared to Dopa-bMAPs prepared without any coexpression of TyrRS.

Introduction

Mussels can rapidly and strongly adhere to a variety of wet surfaces by secreting specialized adhesives, mussel adhesive proteins (MAPs), which are considered potential water-resistant bioglues (Cha et al., 2008; Lim et al., 2011; Silverman and Roberto, 2007; Waite et al., 2005). MAPs are characterized by the presence of specific post-translational modifications (Lim et al., 2011). In particular, hydroxylation of tyrosine residues results in a high content of 3,4-dihydroxy-L-phenylalanine (Dopa) with a reactive catechol group (Danner et al., 2012; Nicklisch et al., 2012). Dopa has been suggested as a key molecule for underwater mussel adhesion because Dopa mediates various interactions, including bidentate-hydrogen-bond formation, metal coordination, cation-π interaction, and oxidative cross-linking (Anderson et al., 2010; Harrington et al., 2010; Lee et al., 2006; Lu et al., 2012; Sever et al., 2004; Yu et al., 2013; Zeng et al., 2010). These interactions result in very strong underwater surface interaction and cohesion between proteins for integrity, water-resistance, self-healing properties, and so on (Harrington et al., 2010; Holten-Andersen et al., 2007; Holten-Andersen et al., 2011; Kim et al., 2014; Nicklisch and Waite, 2012).

To mimic mussel underwater adhesion in various fields, including medical, environmental, and industrial applications, biotechnology has been developed to produce bioengineered MAPs (bMAPs) via E. coli expression due to its superior productivity compared to direct extraction from mussels, and because it has less toxicity, less immunogenicity, and better biocompatibility than synthetic polymers (Cha et al., 2008; Choi et al., 2011; Cha et al., 2008; Yang et al., 2013). However, E. coli–derived bMAPs lack of Dopa due to its intrinsic inability for post-translational modification, and this critically limits underwater adhesion. Because of this,
there have been many trials to incorporate Dopa efficiently into bMAPs (Choi et al., 2012; Lim et al., 2011). To date, in vivo residue-specific Dopa incorporation is known as the most efficient method of incorporating Dopa into bMAPs via mis-aminoacylation of Dopa by endogenous tyrosyl-tRNA synthetase (TyrRS) in a tyrosine-auxotrophic strain under minimal conditions (Yang et al., 2014). While the conventional method, in vitro mushroom-tyrosinase modification, showed a low modification yield of less than 15% (Yang et al., 2013, 2014), and in vivo modification via tyrosinase coexpression showed high instability between reduced and oxidized forms of Dopa, as tyrosinase can also catalyze oxidation of Dopa to Dopa-quinone (Choi et al., 2012), in vivo residue-specific Dopa incorporation allowed a high incorporation yield, around 90%, close to the yields of natural MAPs, and allowed Dopa to stay in its reduced form (Yang et al., 2014). However, potential use of these high-quality bMAPs as practical bioglues was hindered by their low production yield, 3 ~ 5 mg/L (Yang et al., 2014). Therefore, there is a pressing need for increasing productivity toward commercialization. Such low production yield of protein obtained from residue-specific incorporation of a noncanonical amino acid is usually attributed to lower translational activity of a noncanonical amino acid compared to natural amino acids (Calendar and Berg, 1966; Hogenauer et al., 1978; Johnson, et al, 2010) (Fig. 1A). Therefore, in order to increase the productivity of Dopa-bMAPs, changes in translational machinery are required. Considering that the activation rates of noncanonical amino acids by aminocyl-tRNA synthetase (AARS) were usually lower than those of natural amino acids, overexpression of AARS recognizing noncanonical amino acids is one feasible option for overcoming low activation rates.

In the present work, we engineered the translational machinery in E. coli to improve production yield of Dopa-bMAP with a high DOPA incorporation yield using two strategies. First, overexpression of Dopa-specific AARS and its cognate tRNA was investigated. Mutations at the binding pocket of AARS might be required for recognition of a specific noncanonical amino acid (Alfonta et al., 2003) (Fig. 1B). Previously, AARS with high selectivity for Dopa has been developed by screening the mutant libraries of M. jannaschii tyrosyl tRNA-synthetase (MjTyrRS) for site-specific Dopa incorporation into myoglobin with its cognate mutant tyrosine amber suppressor tRNA (MjtRNA_TyrCUA) in response to an amber codon (Alfonta et al., 2003). After mutation of the anticodon of MjtRNA_TyrCUA into AUA to recognize a Tyr codon (UAU), we designed a coexpression vector for Dopa-specific aminocyl-tRNA synthetase (MjTyrRS-Dopa) and its cognate tRNA (MjtRNA_TyrAUA) and examined the improvement of production yield. Second, overexpression of E. coli tyrosyl-tRNA synthetase (EcTyrRS) was investigated. Overexpression of endogenous AARS in E. coli cells was shown to increase the production yield of recombinant proteins containing phenylalanine or methionine analogs (Kiick et al., 2000) (Fig. 1C). Although Tyr is a better substrate than Dopa for EcTyrRS, in minimal media deficient of tyrosine, EcTyrRS still activates Dopa, resulting in Dopa incorporation. Therefore, overexpression of EcTyrRS was expected to compensate low affinity of EcTyrRS for Dopa, which finally results in improved production yield, with plenty Dopa-charged E. coli tyrosyl tRNAs.

Results and Discussion

Expression and purification of Dopa-free bMAP and Dopa-bMAP without overexpression of any AARS. Dopa incorporation into bMAP was performed using pQE80-bMAP containing the gene of dMAP (dfp-3) according to a residue-specific incorporation protocol (Johnson et al., 2010; Link and Tirrell, 2005). Briefly, the pQE80-bMAP plasmid was transformed into AY12 Tyr auxotrophic E. coli strain generating AY12[pQE80-bMAP]. An overnight culture of the AY12[pQE80-bMAP] E. coli cells in LB media was inoculated into M9 minimal media containing 20 natural amino acids. When the cell density reached a set point, a media shift into Tyr-deficient M9 minimal media supplemented with Dopa was performed. Expression of bMAP was induced by the addition of isopropyl-D-thiogalactopyranoside (IPTG). The cell lysates before induction (BI) and after induction (AI) were subjected to SDS-PAGE analysis. The visible molecular weight of Dopa-incorporated bMAP on an SDS-PAGE gel appeared to be about 10 kDa (Fig. 2A, B), which is greater than the calculated molecular weight from amino acid sequence (about 7 kDa). Such a band shift was made likely by the high pl value of bMAP (10.24) and was consistent with band shifts reported previously (Yang et al., 2014). The purification of Dopa-bMAP was performed as described previously (Yang
et al., 2014). The nitroblue tetrazolium (NBT) staining was reported to be a specific detection method of proteins containing quinone by redox-cycling (Paz et al., 1991). With NBT staining, bMAP bands in the after induction sample and purified sample were clearly observed in a blue-purple color, whereas no bMAP band in the before induction sample was detected (Fig. 2B). These results demonstrated that the Dopa were successfully incorporated into bMAP (Yang et al., 2014). In order to obtain Dopa-free bMAP samples, AY12[pQE80-bMAP] cells were cultured in LB media and bMAP was expressed using 1 mM IPTG. Dopa incorporation into bMAP was performed at 30 °C. The purification of Dopa-free bMAP was performed via metal affinity chromatography using a hexahistidine tag according to the manufacturer’s protocol (Qiagen).

To confirm incorporation of Dopa into Tyr residues of bMAP, Dopa-bMAP samples and Dopa-free bMAP were analyzed by matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) mass spectrometry (Bruker Daltonics). bMAP has 10 Tyr residues. The mass of the major peak of Dopa-free bMAP was 6,652 Da (Fig. 3A), which is almost identical to the theoretical mass of Dopa-free bMAP (6,654 Da). The mass difference between Dopa and Tyr is 16 Da. The masses of the major peaks of Dopa-bMAP_Control were 6,811; 6,796; 6,780; and 6,765 Da, which matched well with the theoretical masses of bMAP samples containing 10, 9, 8, and 7 Dopa residues (6,814; 6,798; 6,782; and 6,766 Da) with less than 1% error, respectively. Since the average number of Dopa in the Dopa-bMAP sample was 9.1, the Dopa incorporation yield was 91%, consistent with the yields in the literature (Yang et al., 2014).

Expression and purification of Dopa-bMAP coexpressed with Dopa-specific AARS. In order to enhance the production yield of Dopa-bMAP, we explored the coexpression of Dopa-specific AARS. We chose MjTyrRS-Dopa as a Dopa-specific AARS. The original plasmid prepared for site-specific incorporation of Dopa into an amber codon has the gene for M. jannascii tyrosyl-tRNA amber suppressor (MjtRNA\text{\textsubscript{TyrCUA}}). In this study, since Dopa should be incorporated into Tyr residues (UAU codon), the anticodon of amber suppressor was mutated into AUA-generating MjtRNA\text{\textsubscript{TyrAUA}} using site-directed mutagenesis. In the final construction of pEVOL-Dopa plasmid, the gene of MjTyrRS-Dopa is under control of an inducible ara promoter and the gene of MjtRNA\text{\textsubscript{TyrAUA}} is under control of a constitutive proK promoter.

Deficiency of Tyr in minimal culture media would not allow translation of functional MjTyrRS-Dopa. All 20 natural amino acids in minimal culture media are required to produce functional MjTyrRS-Dopa in E. coli cells. Therefore, MjTyrRS-Dopa should be expressed in the presence of all 20 natural amino acids in minimal media before media shift to Tyr-deficient minimal media. In this study, when the optical density at 600 nm (OD\textsubscript{600}) of cultured cells reached 0.7, arabinose was added to culture media to induce MjTyrRS-Dopa. After 30-min expression of MjTyrRS-Dopa, media shift was performed. Then bMAP expression was induced by the addition of IPTG and Dopa. Dopa-incorporated bMAP coexpressed with MjTyrRS-Dopa (Dopa-bMAP_MjTyrRS) and purification of Dopa-bMAP with coexpression were performed similar to preparation of Dopa-bMAP expressed without coexpression of any AARS, except the arabinose addition and 30-min incubation prior to media shift. The protein band of Dopa-bMAP_MjTyrRS was observed to be similar to that of Dopa-bMAP_Control (Fig. 2A, B). The spectrum of MALDI-TOF MS analysis of Dopa-bMAP_MjTyrRS exhibited a pattern similar to that of Dopa-bMAP_Control (Fig. 3C). The masses of the major peaks of Dopa-bMAP_MjTyrRS were 6,812; 6,796; 6,781; and 6,765 Da, which correspond with Dopa-bMAP samples containing 10, 9, 8, and 7 Dopa residues, respectively. The Dopa incorporation yield of Dopa-bMAP_MjTyrRS was 93%, which is comparable to that of Dopa-bMAP_Control.

Expression and purification of Dopa-bMAP coexpressed with E. coli TyrRS. As an alternative strategy to enhance production yield of Dopa-bMAP, we explored coexpression of E. coli TyrRS. The gene of E. coli TyrRS (EcTyrRS) was amplified from E. coli genomic DNA and was subcloned into pEVOL-Dopa to replace the MjTyrRS-Dopa gene, generating pEVOL-EcTyrRS. Therefore, the gene of EcTyrRS was also under control of the ara promoter; the amino acid sequence of EcTyrRS is shown in Supporting Information Table 1. Both pEVOL-EcTyrRS and pQE80-bMAP plasmids were transformed into AY12 cells to generate AY12[pEVOL-EcTyrRS][pQE80-bMAP] cells. Expression and purification of Dopa-bMAP in the presence of EcTyrRS (Dopa-bMAP_EcTyrRS) was performed similarly to Dopa-bMAP_MjTyrRS.

SDS-PAGE analysis demonstrated that the molecular weight of Dopa-bMAP_EcTyrRS was very similar to
the molecular weight of Dopa-bMAP\textsubscript{Control} and Dopa-bMAP\textsubscript{MjTyrRS} (Fig. 2A, B). The spectrum of MALDI-TOF MS analysis of Dopa-bMAP\textsubscript{EcTyrRS} exhibited a pattern similar to that of Dopa-bMAP\textsubscript{Control} (Fig. 3D). The masses of the major peaks of Dopa-bMAP\textsubscript{EcTyrRS} were 6,814; 6,799; and 6,783 Da, which correspond to Dopa-bMAP\textsubscript{Control} samples containing 10, 9, and 8 Dopa residues, respectively. The Dopa incorporation yield of Dopa-bMAP\textsubscript{EcTyrRS} was about 90%, which is comparable to that of Dopa-bMAP\textsubscript{Control}. The characterization results of Dopa-bMAP\textsubscript{MjTyrRS} and Dopa-bMAP\textsubscript{EcTyrRS} led to a conclusion that they have properties comparable to those of Dopa-bMAP\textsubscript{Control}.

**Improvement of production yield of Dopa-bMAP with coexpression of MjTyrRS or EcTyrRS.**

To compare the production yield of purified Dopa-bMAP samples, the concentrations of purified Dopa-bMAP samples were calculated by measuring the absorbance at 280 nm and applying the Beer-Lambert law. The production yield of Dopa-bMAP without coexpression of any AARS (Dopa-bMAP\textsubscript{Control}) was 4.18 ± 0.02 mg/L. With coexpression of MjTyrRS and EcTyrRS, the production yield of Dopa-bMAP was 6.56 ± 0.04 and 7.11 ± 0.05 mg/L, which is greater than that of Dopa-bMAP\textsubscript{Control} by 57% and 70%, respectively (Fig 4). Considering that the Dopa incorporation yields of three Dopa-bMAP samples (Dopa-bMAP\textsubscript{Control}, Dopa-bMAP\textsubscript{MjTyrRS}, and Dopa-bMAP\textsubscript{EcTyrRS}) were comparable, these results successfully demonstrated that coexpression of Dopa-recognizing AARS (MjTyrRS and EcTyrRS) is an effective strategy to enhance the production yield of bMAP with a high Dopa incorporation yield (greater than 90%).

**Conclusions**

In vivo residue-specific incorporation of Dopa in \textit{A. \textit{Y.}} tyrosine-auxotrophic \textit{E. coli} cells resulted in 4.19 mg/L of purified Dopa-MAP with over 90% Dopa incorporation yield. In order to enhance the production yield of Dopa-MAP, coexpression of two Dopa-recognizing TyrRSs (MjTyrRS-Dopa and EcTyrRS) was performed. Coexpression of MjTyrRS as well as MjtRNA\textsubscript{TyrAUA} increased the production yield of Dopa-MAP by 57%. Coexpression of EcTyrRS resulted in a 72% enhanced production yield of Dopa-MAP. Reactivities of Dopa in the Dopa-bMAP samples were confirmed by NBT staining of protein gels. MALDI-TOF MS analysis showed that all Dopa-bMAP samples have over 90% Dopa incorporation yield. Although the increase in the production yield of Dopa-MAP was moderate in this study, application of these strategies to other MAP proteins with different Dopa contents, especially those with lower Dopa contents, may result in a greater increase in production yield.

**Materials and Methods**

**Materials.** Ni-nitrilotriacetic acid (NTA) agarose was purchased from Qiagen (Valencia, CA, USA). 0.20 μm syringe filters were purchased from Advantec (Dublin, CA, USA). Zeba spin desalting columns were purchased from Thermo Fisher Scientific (Waltham, MA, USA). PD-10 desalting columns were obtained from GE Healthcare (Piscataway, NJ, USA). All other chemicals and reagents were purchased from Sigma-Aldrich (Saint Louis, MO, USA) and used without further purification unless otherwise indicated.

**Plasmid construction for overexpression of AARS and bMAP.** The engineered pair of \textit{M. \textit{jannaschii}} tyrosyl tRNA synthetase specific for Dopa (MjTyrRS-Dopa) and tyrosyl-tRNA amber suppressor (MjtRNA\textsubscript{TyrCUA}) were originally developed by Dr. Schultz and the colleagues (Alfonta et al., 2003). The plasmid-encoding MjTyrRS-Dopa and MjtRNA\textsubscript{TyrCUA} (pSPEL 143 plasmid) were obtained from Dr. Tae Hyeon Yoo at Ajou University in South Korea. Based on the previous work enhancing the enzymatic activity of MjTyrRS (Amiram et al., 2015), site-directed mutagenesis was performed at the tRNA anticodon binding interface of MjTyrRS-Dopa, using pSPEL 143 as a template and primers (CCG769GCC(t1)–F: 5’-AAATTTCTCCGGGACCCTTAAATGCAGTCGGGATTTT-3’; CCG769GCC(t1)–R: 5’-GGATACCCACTGACGATTAAGGGCCCGGAGAAATTT-3’). Then the anticodon of MjtRNA\textsubscript{TyrCUA} was mutated into AUA by site-directed PCR mutagenesis using primers (tRNAc35a-F: 5’-TGCCATGCGGATTTATAGTCCGCCGTTCTGC-3’; tRNAc35a-R: 5’-GCAGAACGGCGGACTATAAATCCGCATGGCA-3’), generating pEvol-Dopa plasmid. The \textit{E. coli} TyrRS (EcTyrRS) gene was amplified by PCR using genomic DNA of Top10 \textit{E. coli} cells as a template and primers (F: 5’-ATGGCAAGCAGTAACTTG-3’; R: 5’-TTATTTCCAGGAATTCAGCAG-3’). The EcTyrRS gene
fragment digested by Bgl II and Sal I was cloned between Bgl II and Sal I sites of pEvol-Dopa, generating pEvol-EcTyrRS. The fp-3 gene encoding MAP with an N-term histidine tag (Yang et al., 2014) was cloned into pQE80 plasmid (Qiagen) to generate pQE80-bMAP plasmid.

Preparation of Dopa-incorporated bioengineered MAP samples. For expression of bMAP without incorporation of Dopa (Dopa-free bMAP), pQE80-bMAP plasmid was transformed into TOP10 cells. The transformants were cultured in LB media. When OD$_{600}$ of the cell culture reached 1.0, 1 mM IPTG was added to induce expression of Dopa-free bMAP. For expression of Dopa-bMAP using overexpressed MjTyrRS-Dopa/MjtRNA$^{TyrAUA}$ or EcTyrRS, pQE80-bMAP and either pEVOL-Dopa or pEVOL-EcTyrRS plasmid were cotransformed into JW2581 E. coli cells (a tyrosine-auxotrophic E. coli strain obtained from Yale Genetic Stock Center) to generate JW[pQE80-bMAP/pEVOL-Dopa] or JW[pQE80-bMAP/pEVOL-EcTyrRS] expression cells, respectively. For expression of Dopa-incorporated bMAP (Dopa-bMAP), cotransformed cells were inoculated into M9 minimal medium (100 mL of M9 salt [67.8 g of Na$_2$HPO$_4$, 30.0 g of KH$_2$PO$_4$, 5.0 g of NaCl, and 10.0 g of NH$_4$Cl in 1 L distilled water (DW)], 20 mL of 20% glucose, 1 mL of 2 M MgSO$_4$, 1 mL of 0.1 M CaCl$_2$, and 1 mg of thiamine-hydrochloric acid in 1 L DW) with 20 natural amino acids (40 mg/L), ampicillin (100 μg/mL), and chloramphenicol (35 μg/mL), and were incubated at 37 °C with 210 rpm shaking overnight. The overnight culture was transferred to fresh M9 minimal medium with 20 natural amino acids and was cultured at 37 °C with 210 rpm shaking. When the optical density at 600 nm (OD$_{600}$) reached 0.7, L-(+)-arabinose was added to the culture to the final concentration 0.2%. When OD$_{600}$ reached 1.0, a medium shift was performed. To remove residual tyrosine in the medium, cell pellets were collected by centrifugation at 6,000 rpm for 10 min and the supernatant was decanted. The cell pellets were resuspended in ice-cold 0.9% NaCl solution. This procedure was performed three times. Then the cell pellets were resuspended in M9 minimal medium with 19 natural amino acids deficient of tyrosine. After the culture was incubated at 30 °C with 210 rpm shaking for 10 min, 1 mM IPTG and 1 mM Dopa were added to the culture in order to induce bMAP-Dopa. The cells were cultured at 30 °C with 210 rpm shaking for 15 hr and then harvested by centrifugation at 6,000 rpm for 10 min at 4 °C. The cell pellets were stored at -80 °C until required. For expression of bMAP without overexpression of any AARS (Dopa-bMAP Control), pQE80-bMAP plasmid was transformed into JW2581 E. coli cells to generate JW[pQE80-bMAP] expression cells. Then the expression of Dopa-bMAP Control was achieved by the same procedure except that neither chloramphenicol nor L-(+)-arabinose was added to the culture.

To purify bMAP samples, cell pellets were resuspended in lysis buffer (100 mM NaH$_2$PO$_4$, 10 mM Tris-Cl, 10 mM ascorbic acid, and 8 M urea, pH 6.9) and sonicated for 20 min (cycled 3 sec on and 7 sec off). The cell lysate was centrifuged at 10,000 rpm for 30 min. The supernatant of cell lysate was mixed with Ni-NTA agarose beads (Qiagen) for 30 min at 18 °C and then loaded onto a column. The column was washed with wash buffer (100 mM NaH$_2$PO$_4$, 10 mM Tris-Cl, 10 mM ascorbic acid, and 8 M urea, pH 6.9). The bMAP samples were eluted with 0.5 M HCl. To exchange the buffer condition, a PD-10 desalting column (GE Healthcare) was used. SDS-PAGE analysis was performed to confirm the expression of Dopa-bMAP samples. For NBT staining of Dopa-bMAP, the resolved protein samples on SDS-PAGE gel were transferred to nitrocellulose blotting membranes (BioTrace NT, Pall Life Science, Pensacola, FL) at 100 V for 2 h using a pH 8.3 transfer buffer containing 25 mM Tris, 190 mM glycine, and 20% methanol. Then the NBT staining of Dopa-bMAP was achieved by the similar procedure as previously reported (Paz et al., 1991).

The concentration of purified bMAP samples was calculated using molar absorbance at 280 nm and the Beer-Lambert law. The molar extinction coefficient of bMAP at 280 nm was calculated using the following equation (Pace et al., 1995):

$$\varepsilon_{280} = (5500 \times n_{Trp}) + (1490 \times n_{Tyr}) + (125 \times n_{Cys}) + (2630 \times n_{DOPA})$$

where 5,500; 1,490; 125; and 2,630 are the molar extinction coefficients of tryptophan, tyrosine, cysteine, and Dopa, respectively. The $\varepsilon_{280}$ of Dopa-bMAP was calculated to be 42,800 M$^{-1}$cm$^{-1}$. The absorbance of purified proteins was measured using a Synergy microplate reader (BioTek, Winooski, VT, USA).
Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) analysis of purified bMAP samples. Purified bMAP samples were desalted with ZipTip pipette tips (Merck Millipore, Burlington, MA, USA) and diluted in α-cyano-4-hydroxycinnamic acid matrix solution in a buffer (30:70 [v/v] of acetonitrile: 0.1% TFA aqueous solution). A small quantity of sample matrix mixture was spotted on the polished steel target plate (Bruker Daltonics, Billerica, MA, USA). The sample was analyzed with an autoflex MALDI-TOF/TOF mass spectrometer (Bruker Daltonics).

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References


List of Figures

Figure 1. Scheme of preparation of Dopa-incorporated bioengineered MAP (bMAP) proteins. In vivo residue-specific incorporation of Dopa (A) without coexpression of any AARS, or with coexpression of (B) Dopa-specific Methanococcus jannascii TyrRS (MjTyrRS) or (C) E. coli TyrRS (EcTyrRS)

Figure 2. Protein gels of expressed and purified bMAP samples stained with Coomassie blue (A) or NBT (B). Lanes are denoted as molecular weight markers (Mw), cell lysate before induction (BI), cell lysate after induction (AI), purified bMAP (E), Dopa-bMAP without over-expression of any AARS (Control), or Dopa-bMAP with co-expression of either MjTyrRS-Dopa (+MjTyrRS) or EcTyrRS (+EcTyrRS). The protein bands of bMAP samples are marked with an arrow.

Figure 3. MALDI-TOF MS spectra of bMAP samples. (A) Dopa-free bMAP, (B) Dopa-bMAP_Control, (C) Dopa-bMAP_MjTyrRS, and (D) Dopa-bMAP_EcTyrRS

Figure 4. Production yields of purified Dopa-bMAP samples without any AARS (Control) with either MjTyrRS-Dopa (+MjTyrRS) or EcTyrRS (+EcTyrRS). Experiments were performed in triplicates (error bars: standard deviations, *: P < 0.05 (two-tailed student’s t test)).

Figure 1
Figure 2

Figure 3
Figure 4