Supplementary Material For:

Auto-induction medium containing glyphosate for high-level incorporation unusual aromatic amino acids into proteins

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Stock solutions

The recipes and rationale for preparation of the auto-induction medium derive from the work of Studier (1), with a modification of the media for incorporation of unusual aromatic amino acids. The preparation of vitamin and metal mixtures were as described by Sreenath et al. (2), and the preparation of the glyphosate solution (1 g/L) was as described by Kim et al. (3).

All media components were prepared using distilled and deionized water and were either filter-sterilized (0.2, 0.45, and 0.75 µm filters; Nalgene, Rochester, New York, USA) or heat-sterilized. Solutions of glyphosate (1 g/L), *p*-aminobenzoic acid (10 µM), *p*-hydroxybenzoic acid (10 µM), phenylalanine (1 g/100 mL), tryptophan (1 g/100 mL), tyrosine (50 mg/L), tyrosine (1 g/100 mL), and 6-fluoro-D,L-tryptophan (1 g/120 mL; Sigma-Aldrich, St. Louis, MO, USA) were filter-sterilized. The following stock solutions were used in constructing the media (see Supplementary Table S1).

- NPS (to achieve final concentrations): 25 mM $(NH_4)_2SO_4$, 50 mM KH_2PO_4, 50 mM Na_2HPO_4.
- 80155 mixture (to achieve final concentrations): 0.8% (v/v) glycerol, 0.015% (w/v) glucose, 0.5% (w/v) α-lactose.
- Aspartic acid, 15% (w/v): add 25 mL/L [final concentration 0.25% (w/v)].
- $MgSO_4$, 1 M: add 2 mL/L (final concentration 2 mM).
- Amino acid mixture I: 0.36% (w/v) of all 20 standard amino acids with the exception of cysteine and tryptophan; add 20 mL/L.
- Amino acid mixture II: 0.36% (w/v) of each of the 20 standard amino acids with the exception of cysteine, tyrosine, and tryptophan; add 20 mL/L.
- Metal mixture used with the auto-induction medium (to achieve final concentrations): 20 µM $CaCl_2$, 10 µM $MnCl_2 \cdot 4H_2O$, 10 µM $ZnSO_4 \cdot 7H_2O$, 2 µM $CoCl_2 \cdot 6H_2O$, 2 µM $CuCl_2 \cdot 2H_2O$, 2 µM $NiCl_2 \cdot 6H_2O$, 2 µM $Na_2MoO_4 \cdot 5H_2O$, 2 µM $Na_2SeO_3 \cdot 5H_2O$, and 2 µM H_3BO_3.
- Vitamin mixture used with the auto-induction media (to achieve final concentrations): 0.2 µM nicotinic acid, 0.2 µM pryridoxine, 0.2 µM thiamine, 0.2 µM *p*-aminobenzoic acid, 0.005 µM folic acid, 0.005 µM riboflavin, 0.2 µM vitamin B12.
- Kim medium: M9 medium containing 1 mL concentrated metal mixture, 1 mL 1 M $MgSO_4$, 30 mM glucose, 0.2 mM thiamine hydrochloride, 0.8 mM arginine hydrochloride, 50 mg/L tyrosine, 50 mg/L phenylalanine, 6 mg/L tryptophan, and 0.2 mM uracil.
- Concentrated metal mixture used with the Kim medium (see above) to achieve final concentrations: 4 mM $ZnSO_4$, 1 mM $MnSO_4$, 4.7 mM H_3BO_3, 0.7 mM $CuSO_4$, 2.5 mM $CaCl_2$, and 1.8 mM $FeCl_3$.

Cell growth

Auto-induction medium

BL21-Codon Plus(DE3)-RIL (Stratagene, La Jolla, CA, USA) *Escherichia coli* strain carrying pMAL-p2E (New England BioLabs, Ipswich, MA, USA) was used for expressing maltose binding protein (MBP) as a fusion with α fragment. A single colony of the transformant was inoculated into 10 mL MDAG (a noninducing medium containing 100 µg/mL ampicillin) medium (1) and grown overnight at 37°C with shaking at 225 rpm. The cell culture (2.5 mL) was centrifuged, washed twice with auto-induction medium, and inoculated into a 50-mL working volume.

Kim medium

For this experiment, a single colony of the transformant was inoculated and grown in 10 mL LB overnight at 37°C, shaking at 225 rpm, and inoculated (7.5%) into a 50-mL (containing 100 µg/mL ampicillin) working volume of Kim et al. (3) medium. Because the 6F-Trp is a D,L mixture, a higher concentration (74 mg/L) of 6F-Trp was used. We confirmed that no significant growth difference was observed between the use of 37 and 74 mg/L 6F-Trp.

Protein product

The *mal*E gene on vector pMAL-p2E codes for an amino-terminal signal peptide that directs the MBP protein to the periplasm and a sequence at the C terminus of the MBP gene that codes for a lacZ-α fusion along with an enterokinase cleavage site (New England Biolabs). The primary product, as a fusion, should be approximately 53 kDa. However, the α fragment is not particularly stable (Paul Riggs, New England BioLabs; personal communication); thus, PAGE exhibits multiple bands including a stable breakdown product about the size of MBP (~43 kDa; see main text, Figure 2, A and B). Extraction of the periplasmic fluid from cells and amylose resin affinity purification of MBP were as described by the manufacturer's protocol (New England BioLabs). The eluted fractions were stored at 4°C overnight to yield MBP from the primary fusion by autocleavage. The periplasmic fluids were concentrated (8×) using a 5-kDa cutoff Centricon filter (Millipore, Billerica, MA, USA) prior to applying to the amylose resin column. Preparation of soluble and insoluble fractions was obtained by centrifuging the osmotic fluids at 16,500 rpm for 15 min; the pellet of centrifuged osmotic fluids was resuspended in the same amount of buffer. The molecular mass was determined for the purified unlabeled and labeled MBP by electrospray ionization mass spectrometry (ESI-MS; using Sciex API 365 triple quadrupole mass spectrometer (Perkin-Elmer, Boston, MA, USA), and liquid chromatography ESI-MS (LC-ESI-MS) maintained at the University of Wisconsin Biotechnology Center (Madison, WI, USA). The labeling efficiency (%) was calculated from the experimentally determined and calculated molecular weights of the labeled and unlabeled protein as described by Tyler et al. (4).

The matured product of MBP after self-cleavage of the α fragment from the fusion had the following amino acid sequence:

Table 1. Composition of the media investigated (see main-text Figure 1]).

Media and other components	Auto-induction medium without glyphosate (●)	Auto-induction medium with glyphosate, amino acid mixture I, and Trp(◊)	Auto-induction medium with glyphosate, amino acid mixture I, and 6F-Trp + Trp (■)	Auto-induction medium with glyphosate, amino acid mixture I, and 6F-Trp (▲)	Auto-induction medium with glyphosate, amino acid mixture I, and Tyr (×)	Auto-induction medium with glyphosate, amino acid mixture II, and Trp(✳)	Kim et al. medium containing glyphosate and 6F-Trp (+)
Kim medium plus metal mixture							+
NPS		+	+	+	+	+	-
80155 mixture	+	+	+	+	+	+	
Amino acid mixture I		+	+	+	+		
Amino acid mixture II						+	
Metal mixture for auto-induction		+	+	+	+	+	
Glyphosate (1 g/L)		+	+	+	+	+	+
p-Aminobenzoic acid (10 µM)							+
p-Hydroxybenzoic acid (10 µM)							+
Tyrosine (200 µg/IL)					+		
Tryptophan (200 µg/L)		+				+	
6-Fluoro-D,L-tryptophan (316 mg/L)			+	+			
6-Fluoro-D,L-tryptophan (74 mg/L)							+

KIEEGKLVIWINGDKGYNG-
LAEVGKKFEKDTGIKVTVEH-
PDKLEEKFPQVAATGDGPDIIF-
WAHDRFGGYAQSGLLAEITP-
DKAFQDKLYPFTWDAVRYNG-
KLIAYPIAVEALSLIYNKDLLP-
NPPKTWEEIPALDKELKAKGK-
SALMFNLQEPYFTWPLIAADGG-
YAFKYENGKYDIKDVGVDNA-
GAKAGLTFLVDLIKNKHM-
NADTDYSIAEAAFNKGETAM-
TINGPWAWSNIDTSKVNYGVT-
VLPTFKGQPSKPFVGVLSAGI-
NAASPNKELAKEFLENYLLT-
DEGLEAVNKDKPLGAVALKSY-
EEELAKDPRIAATMENAQK-
GEIMPNIPQMSAFWYAVRTAVI-
NAASGRQTVDEALKDAQTNS-
SSNNNNNNNNNN LGDDDDKVPE
FGS.

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