

Supporting Information

Ribosomal Synthesis of Dehydroalanine Containing Peptides

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Materials. Chemicals were purchased from Sigma, Aldrich, Fluka or New England Biolabs unless noted otherwise and used without further purification.

mRNA Templates. DNA templates for in the in vitro transcription of mRNAs coding for peptides 1-3 (Figures 1 & 2) were constructed by ligating complementary primers (Table S1) to Nde1/Xho1 digested pMG209,¹ which is derived from the pET22b vector. The resulting plasmids were transformed into the *E. coli* strain TOP10. Monoclonal plasmids were isolated, sequenced and used as PCR templates. Taq-amplified PCR products were washed with phenol:chloroform:isoamyl alcohol (50:49:1), ethanol precipitated, and then used as template for standard T7 RNA polymerase in vitro transcription reactions.² The full length transcripts were isolated by denaturing a preparative 5 % polyacrylamide gel electrophoresis.

Table S1

peptide1s	5' -TATGAGTAAGC
peptide1a	5' -TCGAGCTTACTCA
peptide2s	5' -TATGAGTAAGAAGAAAC
peptide2a	5' -TCGAGTTTCTTCTTACTCA
peptide3s	5' -TATGTGTAGTAAACGTGGTTGTC
peptide3a	5' -TCGAGACAACCACGTTTACTACACA

Selenalysine. L-selenocystine (65 mg, 0.2 mmol) was suspended under argon in degassed 50 mM KOH (2.5 ml) and ethanol (0.75 ml) and cooled on ice. Sodium borohydride (25 mg, 0.65 mmol) was added and the reaction was allowed to warm up to room temperature. After the reaction became colorless, the mixture was placed on ice and its pH was adjusted to 6 with concentrated HCl. Then 2-bromo-ethylamine (48 mg, 0.4 mmol) was added and the mixture was stirred for 12 h under argon at 4°C. The resulting material was dried under vacuum, desolved in H₂O and centrifuged to remove insoluble material. The supernatant was desalted on an ion retardation resin using AG[®] 11 A8 resin from BIORAD. MS (ESI) +m/z 213.0 (80Se).

Peptide translation. The composition of the reconstituted *Escherichia coli* has been described previously.^{3,4} Usually these systems employ a polymix buffer containing a complex mixture of cations.⁵ For the sake of simplicity we used a simplified buffer with the following composition: 10 mM Tris-HCl, 10 mM Mg(OAc)₂, 100 mM NH₄Cl pH 7.5 (at 37°C). Translation reactions (50 µl) were typically incubated for 1 h at 37°C. The reactions were diluted with 100 µl wash buffer (50 mM Tris-HCl, 300 mM NaCl, pH 8) and supplemented with 50 µl suspended NTA-agarose beads (Quiagen, D). After incubation (30 min) agarose beads were washed (wash buffer + 0.2 mM tris(carboxyethyl)-phosphine) and then eluted with 0.2 % TFA. The yield of translated peptides was quantified by liquid scintillation counting of the specific activity of ³⁵S-methionine. For MALDI-TOF analysis, peptides were desalted and concentrated by reversed phase micro-chromatography (C18 Zip Tips, Millipore, MA) and eluted with a 70 % acetonitrile, 0.1 % TFA solution saturated with *o*-cyano-4-hydroxycinnamic acid. Mass measurements were performed using an Applied Biosystems Voyager MALDI-TOF.

References

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