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Supporting Information

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Supporting Information

for

Ribosomal Synthesis of Peptides with C-Terminal Lactams, Thiolactones, and Alkylamides

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1. Experimental

1.1 Synthesis of DBE and CME substrates: Synthesis of azidohomoalanine[1], azidonorvaline[1], 2-mercaptoethanol-homocysteine[2] and 2-mercaptoethanol-mercapto-norvaline[2] were reported elsewhere. Phenyllactic acid was purchased from Tokyo Chemical Industry. 3,5-Dinitrobenzyl ester (DBE) derivatives of the above nonproteinogenic amino acids and cyanomethyl ester (CME) of phenyllactic acid were synthesized using the same procedure as previously reported[3].

1.2 Preparation of flexizyme, microhelix RNA, and suppressor tRNA$^{Asn\text{-}E1}$: Flexizyme, microhelix RNA (for the aminoacylation test), and suppressor tRNA$^{Asn\text{-}E1}$ with GGU and GAG anticodon were prepared as previously described[3-5].

1.3 Preparation of DNA templates coding peptides: All oligonucleotides, listed in Table S2, were purchased from OPERON, Japan. For the synthesis of DNA template D1 (Table S1) encoding P1, O5-1 and O3-1 were annealed and extended by Taq DNA polymerase. The resulting product was diluted 20 times with PCR reaction buffer and amplified by using O5-2 and O3-2 as the 5'- and 3'-primers, respectively. For the synthesis of DNA template D2 encoding P2, O5-1 and O3-3 were annealed and extended by Taq DNA polymerase. The resulting product was diluted 20 times with PCR reaction buffer and amplified by using O5-2 and O3-4 as the 5'- and 3'-primers, respectively. For the synthesis of DNA template D3 encoding P3, O5-1 and O3-5 were annealed and extended by Taq DNA polymerase. The resulting product was diluted 20 times with PCR reaction buffer and amplified by using O5-2 and O3-6 as the 5'- and 3'-primers, respectively. Prepared DNA templates were purified by the extraction with phenol/chloroform and ethanol precipitation.

1.4 Aminoacylation of microhelix RNA by flexizyme: Aminoacylation assay was performed according to the following procedure: 2.5 µL of 20 µM microhelix RNA in 0.2 M tris-HCl (pH 8.0) was heated at 95 °C for 1 min an d cooled to room temperature over 5 min. 1 µL of 0.1 M $MgCl_2$ and 0.5 µL of 0.1 mM dFx or eFx were added to the solution. 1 µL of 25 mM substrate (DBE or CME) in DMSO was then added to the mixture and incubated on ice for 2 h. The acylation reaction was quenched by addition of 15 µL of the loading buffer [150 mM sodium acetate (pH 5.0), 10 mM EDTA and 83% formamide]. This sample was analyzed by 20% acid denaturing PAGE, stained with ethidium bromide and quantified by using FLA-5100 (Fuji, Japan).

2. Supporting figures

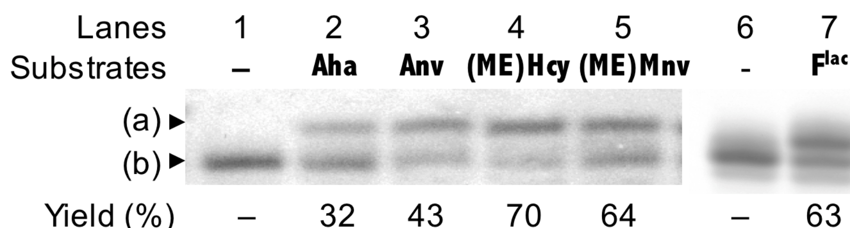

Figure S1. Flexizyme catalyzing acylation of microhelix RNA analyzed by denaturing acid PAGE. Lanes 1 and 6, negative controls in the absence of substrates by using dinitroflexizyme (dFx) and enhanced-flexizyme (eFx), respectively; Lanes 2–5, dFx-catalyzing aminoacylation with designated Xaa 3,5-dinitrobenzyl esters; lanes 6, eFx-catalyzing aminoacylation with designated F^{lac} cyanomethyl esters; The band (a) indicates acylated microhelix RNA and the band (b) indicates microhelix RNA. The efficiency of acylated microhelix RNA was calculated based on the fluorescence intensity of bands (a) and (b), fitted to (a)/[(a)+(b)]. Each yield was determined by a mean score of duplicates.

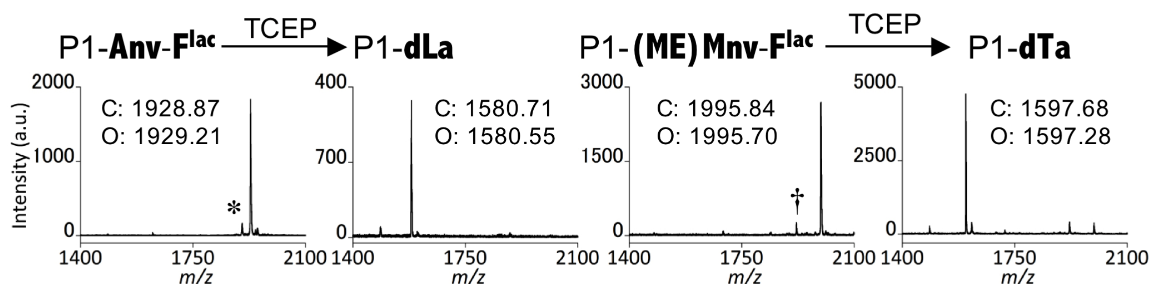

Figure S2. Ribosomal synthesis of P1-dLa and P1-dTa. MALDI-TOF MS spectra of P1-Anv-F^{lac}, P1-dLa, P1-(ME)Mnv-F^{lac} and P1-dTa are shown. Asterisk (*) denotes a peak of P1-Anv-F^{lac} in which Anv was presumably reduced by DTT present in the translation mixture; the calculated molecular mass (C) is 1904.00, while the observed molecular mass (O) is 1904.76. Dagger (†) denotes a peak of P1-(ME)Mnv-F^{lac} in which Mnv was presumably reduced by DTT present in the translation mixture; C is 1920.97, while O is 1920.70.

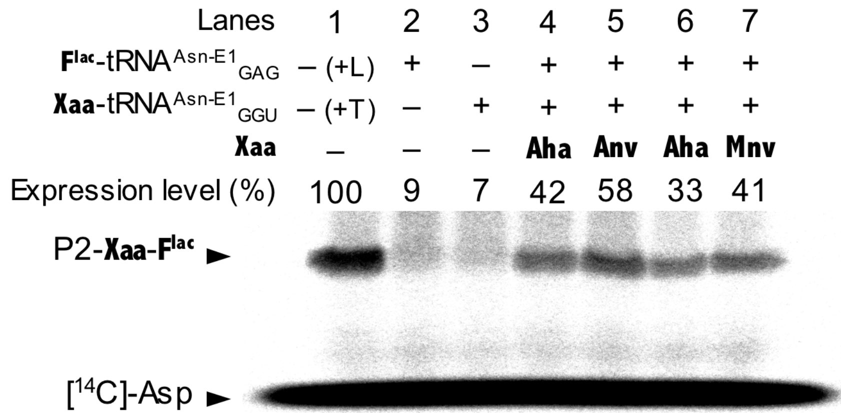

Figure S3. Tolerance of the successive incorporations of Xaa followed by F^{lac} in ribosomal peptide synthesis. (A) Sequences of mR2 and P2-Xaa-F^{lac}. D was replaced by [^{14}C]-D for tricine-SDS-PAGE analysis. (B) Tricine-SDS-PAGE analysis of the expressed peptides labeled with [^{14}C]-D detected by autoradiography. Lane 1, the wildtype peptide expressed in the Thr, Leu-containing ordinary PURE system where ACC and CUC assign Thr and Leu, respectively; lane 2, a negative control using Thr, Lue-withdrawn PURE (*w*PURE) system in the absence of Xaa-$tRNA^{Asn\text{-}E1}{}_{GGU}$; lanes 3, a negative control *w*PURE system in the absence of Xaa-$tRNA^{Asn\text{-}E1}{}_{GAG}$; lane 4-7, expression of the peptide containing the respective tRNAs charged with Xaa and F^{lac}. The expression efficiency relative to wildtype was determined by a mean score of duplicates.

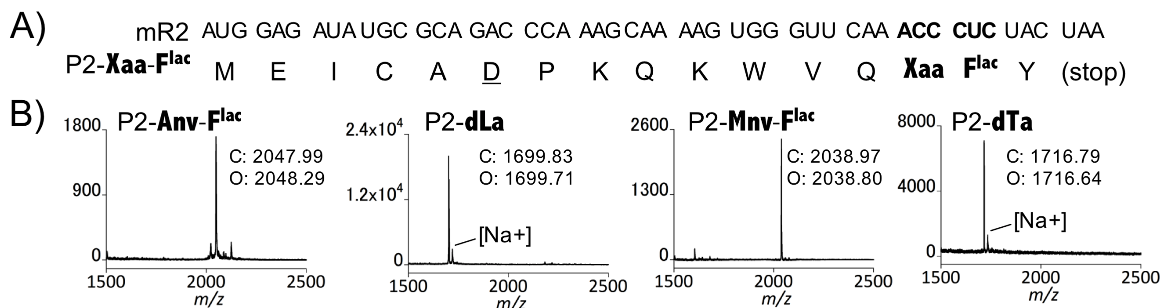

Figure S4. Ribosomal synthesis of P2-dLa and P2-dTa. A) Sequences of mR2 and P2-Xaa-F^{lac}. Xaa and F^{lac} are assigned to codons, ACC and CUC on the mRNA, respectively. B) MALDI-TOF analysis of the peptides. C and O for singly charged species, $[M\text{+H}]^{+1}$, of the peptide are shown in each spectrum. TCEP reduction of P2-Anv-F^{lac} and P2-(ME)Mnv-F^{lac} resulted in the formation of ™-lactam and δ-thiolactone at the peptide C terminus. [Na^{+}] denotes a peak of sodium adducts of the peptide in which molecular mass increases by approximately 22. Asterisk (*) denotes a peak of P2-Anv-F^{lac} in which Ava was presumably reduced by DTT present in the translation mixture; C is 2022.00, while O is 2022.48.

Supporting Tables

Table 1. DNA templates for the preparation of peptides. Forward sequences of dsDNA are listed.

Template	Sequence
D1	5'-TAATACGACTCACTATAGGGTTAACTTTAACAAGGAGAAAAAC ATG CGT ACC CTC CGT GAC TAC AAG GAC GAC GAC GAC AAG TAA GCTTCG-3'
D2	5'- TAATACGACTCACTATAGGGTTAACTTTAACAAGGAGAAAAAC ATG GAG ATA TGC GCA GAC CCA AAG CAA AAG TGG GTT CAA ACC CTC CGT TAA GCTTCG-3'
D3	5'-TAATACGACTCACTATAGGGTTAACTTTAACAAGGAGAAAAAC ATG GAC AAG CCA GAC ATC TTC GGT GAA TGG ACC CTC GAC TAC AAG GAC GAC GAC GAC AAG TAA GCTTCG-3'

Table 2. Primers used for the preparation of DNA templates encoding peptides.

Primer	Sequence
O5-1	5'-TAATA CGACT CACTA TAGGG TTAAC TTTAA CAAGG AGAAA AACAT G-3'
O5-2	5'-GGCGT AATAC GACTC ACTAT AG-3'
O3-1	5'-GTCGT CCTTG TAGTC ACGGA GGGTA CGCAT GTTTT TCTCC TTGTT-3'
O3-2	5'- TTATT AACGG AGTGG ACGCT TGTCG TCGTC GTCCT T-3'
O3-3	5'-CTTTT GCTTT GGGTC TGCGC ATATC TCCAT GTTTT TCTCC TTGTT-3'
O3-4	5'-TTATT AACGC AGGGT TTGAA CCCAC TTTTG CTTTG GGTC-3'
O3-5	5'-GAGGG TCCAT TCACC GAAGA TGTCT GGCTT GTCCA TGTTT TTCTC CTTGT T-3'
O3-6	5'-TTATT ACTTG TCGTC GTCGT CCTTG TAGTC GAGGG TCCAT TCACC GAA-3'

References

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