Supporting Information

Structures of two bacterial resistance factors mediating tRNA-dependent aminoacylation of phosphatidylglycerol with lysine or alanine

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Figure Legends

Fig. S1: Structure based sequence alignment of the catalytic domain of A-PGS from *Pseudomonas aeruginosa*, L-PGS from *Bacillus licheniformis,* and FemX from *Weissela viridescens* (1, 2). Alignments were calculated using UCSF Chimera (38, 39). Clustal Omega (45) was used for primary sequence analysis. Conserved and partly conserved residues are indicated by an asterisk (*) and colon (:) or period (.), respectively, and named Cons1 for conservation between A-PGS sequences, Cons2 for L-PGS sequences and Cons3 for the overall alignment of A-PGS, L-PGS, and FemX. Helices (boxes) and β-strands (arrows) are marked. GNAT domain 1 is colored in light blue, GNAT domain 2 in blue, secondary structure elements of A-PGS and L-PGS, which are part of both GNAT domains are highlighted in purple, inserted elements are colored in gray. Underlined fonts indicate L-PGS residues which are involved in L-lysine amide interaction; green fonts label conserved residues of the lipid tunnel. Red fonts mark residues of FemX (and A-PGS and L-PGS), which are involved (might be involved) in the interaction with the CCA end of tRNA, orange fonts highlight residues proposed for tRNA acceptor stem interaction and blue fonts indicate FemX residues which are involved in pentapeptide interaction (20).

Fig. S2: Incorporation of radioactively labeled ^{14}C-lysine and ^{14}C-alanine into the organic lipid phase by the enzymatic activities of the catalytic domain of A-PGS and L-PGS.
The crude cellular extract of *E. coli* cells overproducing A-PGS$_{543\text{-}881}$ and L-PGS$_{519\text{-}850}$, respectively, were supplemented with radioactively labeled ^{14}C-lysine **(A)** or ^{14}C-alanine **(B)** and an ATP regenerating system as described (5). Samples were subjected to lipid extraction and synthesis of radioactively labeled lipids was analyzed by liquid scintillation counting.

Figure S3. Electron density map of L-lysine amide in the L-PGS structure. A feature enhanced electron density map (46) at 1.5 σ is shown as black mesh, an Fo-Fc omit density map at 2.1 σ is shown in magenta.

Fig. S4: Comparison of the tRNA acceptor stem of Ala-tRNA^{Ala}, Lys-tRNA^{Lys} and of misacylated Ala-tRNA^{Lys}C70U, identity elements of aminoacyl-tRNA synthetases and substrate recognition of A-PGS and L-PGS. The acceptor stem of Ala-tRNA^{Ala} from *B. licheniformis*, Lys-tRNA^{Lys} from *P. aeruginosa* and from misacylated Ala-tRNA^{Lys}C70U (point mutation highlighted by asterisk) is shown. Enzymatic misacylation of tRNA^{Ala} or tRNA^{Lys} requires the respective tRNA identity elements (highlighted gray) of alanyl-tRNA synthetase (AlaRS; A73 discriminator base, G3-U70 (3, 4)) or lysyl-tRNA synthetase (LysRS; A73 discriminator base, G3-C70, UUU34-36 anticodon (5, 6)), respectively. Sequence differences for tRNA^{Ala} and tRNA^{Lys} are highlighted by blue boxes. Substrate recognition of aaPGS was analyzed using the artificial substrate Ala-tRNA^{Lys}C70U. Synthesis of this misacylated tRNA by AlaRS solely requires a single base mutation (5). Ala-tRNA^{Lys}C70U was not accepted by L-PGS but was efficiently converted by A-PGS. Notice that tRNA^{Lys}C70U is not accepted as a substrate of LysRS due to the C70U mutation (36).

Fig. S5: L-PG synthesis of L-PGS and A-PGS using ^{14}C-Lys-tRNA^{Lys} as substrate. Isolated ^{14}C-Lys-tRNA^{Lys} was used as substrate in the presence of purified A-PGS (1 µM) or L-PGS (1 µM) in the presence of PG. L-PG synthesis was analyzed by lipid extraction and liquid scintillation counting.

Fig. S6: Model for L-PGS and A-PGS tRNA interaction. A tRNA molecule (pdb ID 1TN1) was manually positioned onto the structure of L-PGS **(A)** and A-PGS **(B)** using the aminoacyl binding pocket and the CCA binding pocket as point of reference. Substitution of amino acid residues Lys676, Arg684, Arg687 located on helix 5 results in impaired A-PGS activity. The terminal base pairings G2-C71, G3-U70, G4-C69 have been previously identified as tRNA recognition elements (compare Table S2 and (15)). The theoretical position of the C70 or U70 base in the model of L-PGS or A-PGS is indicated by asterisk, respectively.