Isolated Peptidoglycan Glycosyltransferases from Different Organisms Produce Different Glycan Chain Lengths

Tsung-Shing Andrew Wang[†], Sara Aviva Manning[†], Suzanne Walker[‡*], and Daniel Kahne[†*]

Department of Chemistry and Chemical Biology, Harvard University, Cambridge, Massachusetts 02138

and

Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115

kahne@chemistry.harvard.edu; suzanne_walker@hms.harvard.edu

Supporting information

Materials:

Heptaprenyl-[^{14}C]-Lipid II **1** was synthesized as described previously.[1] Full-length *E. coli* PBP1A, *E. coli* PBP1B, *E. faecalis* PBP2A, and *S. aureus* PBP2 were purified as described previously.[2-5] *E. faecalis* ΔTMPBP2A was cloned and purified as described below. Moenomycin A was isolated from flavomycin feedstock as described.[4] All other chemicals were purchased from Sigma-Aldrich, unless otherwise indicated.

Cloning and Expression of *E. faecalis* ΔTMPBP2A:

The *pbp2a (A68-N728)* gene encoding truncated *E. faecalis* ΔTMPBP2A was PCR amplified from pET42b(+)::*pbp2a* (lab stock) by the forward primer (5'-gag cat gtg cat atg gca aac gtt gag acc cta aaa-3') and the backward primer (5'-att aat gtt gcg gcc gcc taa ttt cct aat aag cct cc-3'). After digestion with *NdeI* and *NotI*, the PCR fragment was ligated into pET28b(+) (Novagen) vector to produce pWTS645. The inserted *pbp2a (A68-N728)* gene was confirmed by sequencing.

The pWTS645 plasmid encoding truncated *E. faecalis* ΔTMPBP2A with an N-terminal His_6 tag was transformed into *E. coli* Rosetta 2 (DE3) cells (Novagen) for overexpression. Cells were grown in 1.5 L LB medium supplemented with 50 μg/mL carbenicillin and 30 μg/mL chloramphenical at 37 °C to OD600 = 1.0. Cells were cooled to 24 °C before induction with 0.01 mM IPTG for 20 h and were harvested by centrifugation (5255 × g, 20 min, 4 °C). The pellet was resuspended in 25 mL of 20 mM Tris-HCl buffer, pH 8.0 with 50 μg/mL DNase I, 10 mM $MgCl_2$, 50 μg/mL RNase A, 100 μg/mL lysozyme and 1 mM PMSF and lysed by two passages through a French pressure cell (16000 psi, 4 °C). The cell lysate was then pelleted by centrifugation (70000 × g, 45 min, 4 °C) and the pellet was extracted with 25 mL of extraction buffer (10 mM Tris-HCl, 150 mM NaCl, 2% Triton X-100, 1 mM PMSF, pH 6.8) at 4 °C for 20 h. The extract was centrifuged (50000 × g, 30 min, 4 °C) and the supernatant collected was supplemented with 20 mM imidazole before incubating with 3 ml of Ni Sepharose 6 Fast Flow resin (GE Healthcare) for 1 h. The resin was then collected and washed with 6 column volumes (CV) buffer A (20 mM Tris-HCl, 500 mM NaCl, 0.1% reduced Triton X-100, 20 mM imidazole, pH 8.5). ΔTMPBP2A was then eluted from the resin by 6 CV of elution buffer (20 mM Tris-HCl, 500 mM NaCl, 0.1% reduced Triton X-100, 500 mM imidazole, pH 7.8). The fractions containing ΔTMPBP2A were collected and concentrated using an Amicon Ultra Centrifugal

Filter Device (MW 100 kDa cutoff) (Millipore). After performing gel filtration on Superdex 200 10/300 GL (GE Healthcare) with running buffer (20 mM Tris-HCl, 150 mM NaCl, 0.1% reduced Triton X-100, pH 8.5) to remove protein aggregates, the protein was concentrated and the yield was estimated to be 10 mg/L by *Dc* protein assay (BioRad).

PGT assays:

In a typical PGT assay, 8 μM heptaprenyl-[^{14}C]-Lipid II **1** was mixed with reaction buffer and PGT was added to initiate polymerization. Reaction buffers were optimized for each enzyme. For *E. coli* PBP1A and *E. faecalis* PBP2A: 50 mM HEPES pH 7.5, 10 mM $CaCl_2$, 1000 units/mL penicillin G and 20% v/v DMSO. For *E. coli* PBP1B: 50 mM HEPES pH 8, 10 mM $CaCl_2$, 0.2 mM decyl PEG, 1000 units/mL penicillin G and 20% v/v DMSO. For *S. aureus* PBP2: 50 mM CHES, HEPES, AcOH and MES pH 5, 10 mM $CaCl_2$, 1000 units/mL penicillin G and 20% v/v DMSO. The reaction mixtures were incubated at room temperature for the times specified (see Fig 2, 3 and S1) until being quenched by mixing with 0.1 μL of 1 mM moenomycin A (DMSO solution). The mixtures were placed on ice and dried by vacuum centrifugation prior to SDS-PAGE. The SDS-PAGE assays were performed by using 9 % acrylamide gels and detected as described.[6,7]

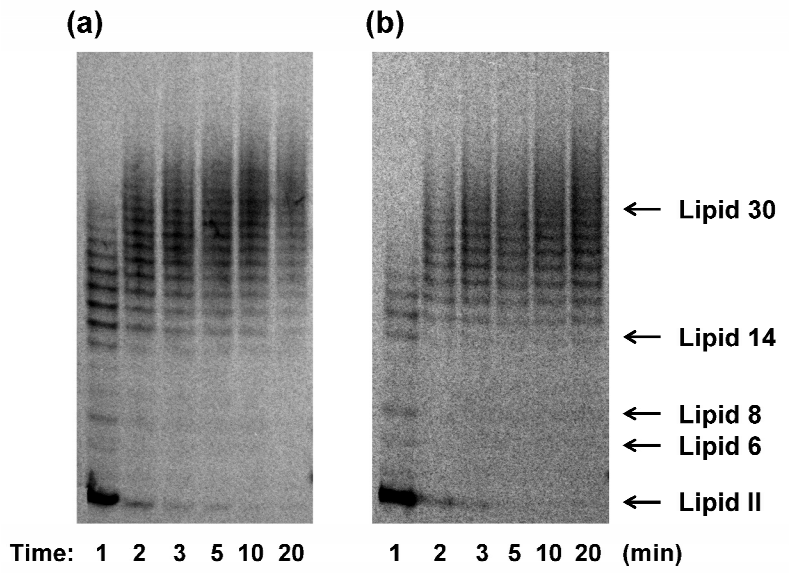

Figure S1. Time course of heptaprenyl-[^{14}C]-Lipid II **1** polymerization by (a) full-length and (b) truncated ΔTM *E. faecalis* PBP2A. The reactions were quenched after 1, 2, 3, 5, 10 and 20 minutes. The results were shown that the transmembrane anchor does not affect the final length distribution.

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