SUPPLEMENTAL MATERIALS

Diversity of innate immune recognition mechanism for bacterial polymeric *meso*-diaminopimelic acid-type peptidoglycan in insects

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This file includes supplemental Table 1 and Figures S1 –S4.

Table 1. Quantitative amino acid composition of the DAP-type PGs from *E. coli* and *B. subtilis*

Amino acid	Amino acid quantities / 1 μg DAP-type PG (pmol)	Normalized to (Alanine=2.0)
	E. coli / B. subtilis	*E. coli / B. subtilis*
Diaminopimelic acid	90.4 / 90.2	1.1 / 1.1
Muramic acid	43.1 / 43.2	0.5 / 0.5
Glucosamine	25.3 / 25.1	0.3 / 0.3
Phenylalanine	2.5 / 2.1	0.0 / 0.0
Cystenine	0.2 / 0.1	0.0 / 0.0
Leucine	5.1 / 5.0	0.1 / 0.1
Isoleucine	2.8 / 2.9	0.0 / 0.0
Lysine	9.0 / 9.2	0.1 / 0.1
Methionine	0 / 0	0.0 / 0.0
Valine	3.6 / 3.8	0.0 / 0.0
Tyrosine	1.6 / 1.1	0.0 / 0.0
Glutamic acid	96.6 / 96.2	1.2 / 1.2
Aspartic acid	7.6 / 7.1	0.1 / 0.1
Alanine	160.9 / 161.1	2.0 / 2.0
Proline	1.8 / 2.1	0.0 / 0.0
Threonine	2.4 / 2.5	0.0 / 0.0
Glycine	8.2 / 8.1	0.1 / 0.1
Serine	6.5 / 6.3	0.1 / 0.1
Histidine	0.8 / 0.6	0.0 / 0.0
Arginine	7.4 / 7.1	0.1 / 0.1

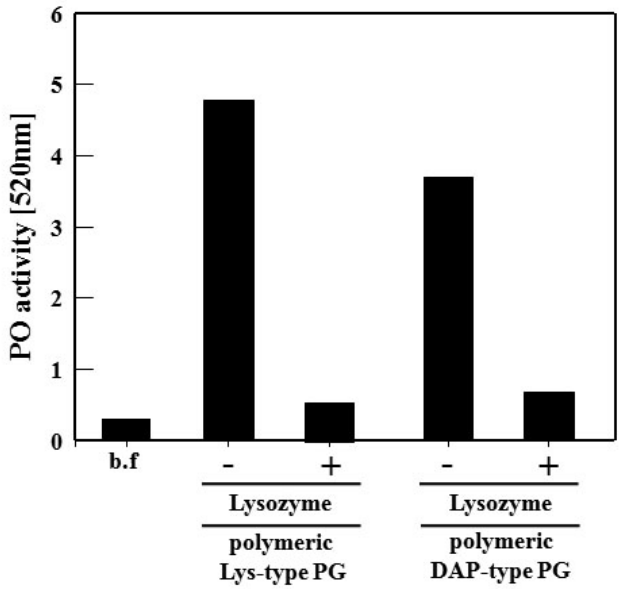

Figure S1. Measurements of PO activities by lysozyme treated-polymeric Lys-type and DAP-type-PGs in *Tenebrio* hemolymph. To measure PO activity, 30 µl of crude hemolymph (150 µg of proteins) was preincubated in 85 µl of 20 mM Tris-HCl buffer (pH 8.0) containing 1 µg of polymeric PGs or lysozyme-treated PGs for 10 min at 30°C, and then 400 µl of substrate solution (1 mM 4-methylcatechol, 2 mM 4-hydroxyproline ethylester in 20 mM Tris-HCl buffer, pH 8.0, containing 5 mM $CaCl_2$) was added to the reaction mixture. After incubation at 30°C for 10 min, the increase in absorbance at 520 nm was measured using a Shimadzu spectrophotometer. One unit of PO activity was defined as the amount of enzyme causing an increase in absorbance of 0.1 at 520 nm per 10-min incubation (A_{520}/10 min).

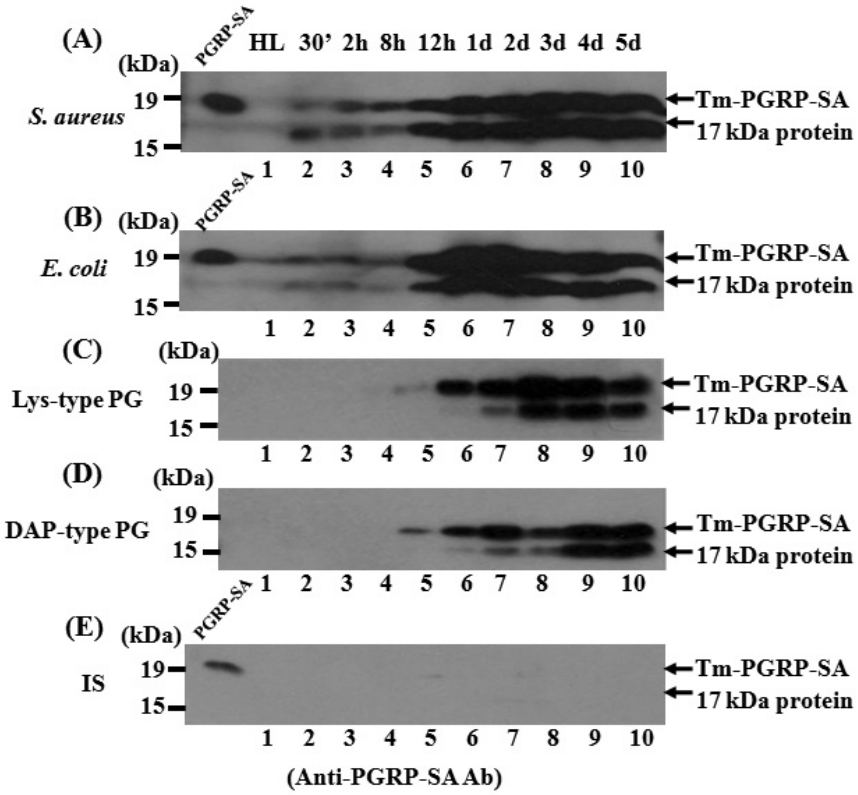

Figure S2. The amounts of *Tenebrio* PGRP-SA and 17-kDa protein in the hemolymph increased after injection of bacteria and PGs.

(A), (B), (C), (D) and (E) represent the Western blot analyses by using anti-PGRP-SA antibody after injection of *S. aureus* ($2x10^6$ cells/larva), *E. coli* ($2x10^6$ cells/larva), polymeric *S. aureus* Lys-type PG (100 ng/larva), polymeric *E. coli* DAP-type PG (100 ng/larva) and insect saline (IS) (4 μl), respectively. On indicated times, hemolymph was collected and then a portion (50 μg of protein) of each sample was analyzed by immunoblotting using affinity-purified anti-*Tenebrio* PGRP-SA antibody.

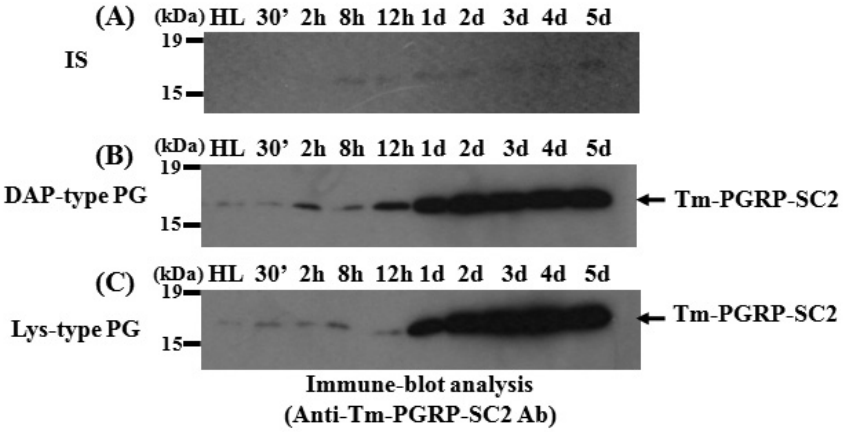

Figure S3. The amounts of *Tenebrio* PGRP-SC2 in the hemolymph increased after injection of polymeric PGs.

(A), (B) and (C) represent the Western blot analyses by using anti-PGRP-SC2 antibody after injection of insect saline (IS, 4 μl), polymeric *S. aureus* Lys-type PG (100 ng/larva), and polymeric *E. coli* DAP-type PG (100 ng/larva), respectively. On indicated times, hemolymph was collected and then a portion (50 μg protein) of each sample was analyzed by immunoblotting using affinity-purified anti-*Tenebrio* PGRP-SC2 antibody.

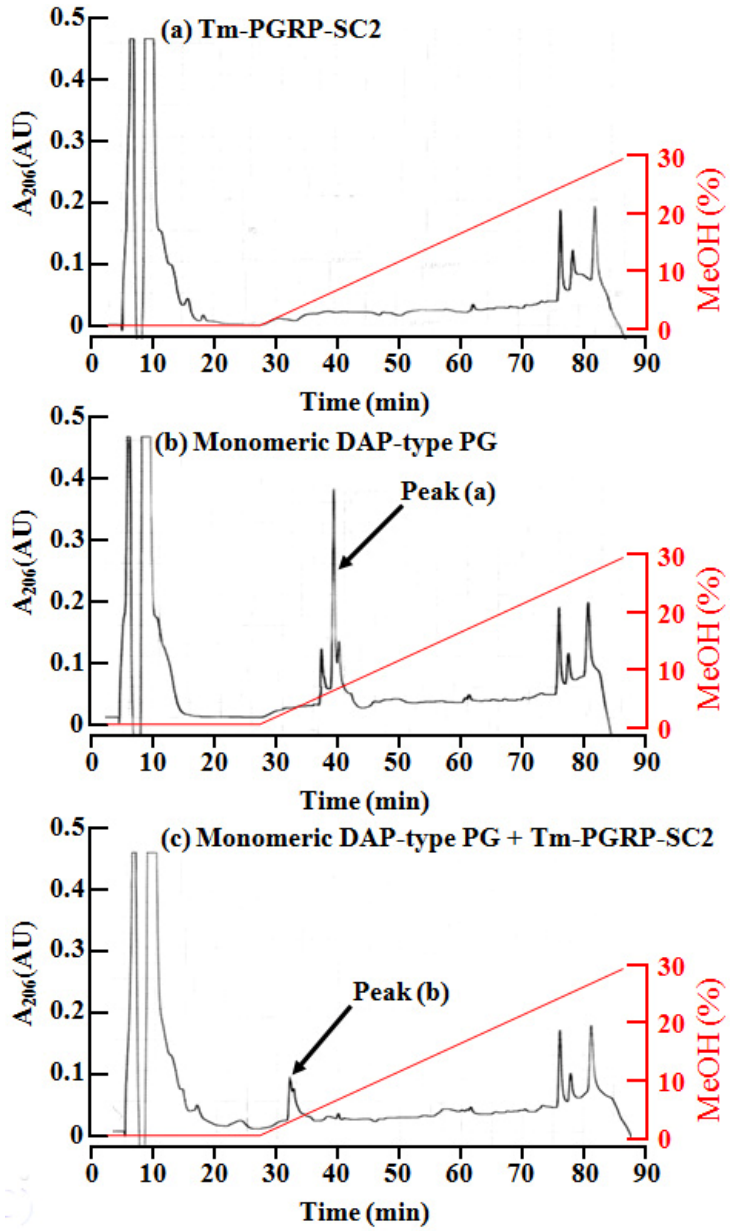

Figure S4. HPLC elution patterns of lysozyme-treated DAP-type PG and PGRP-SC2-treated monomeric DAP-type PG. (A), (B) and (C) represent the elution profile after injection of *Tenebrio* PGRP-SC2, the reaction mixture of lysozyme-treated DAP-type and the reaction mixture of PGRP-SC2 and peak (a), respectively. Polymeric DAP-type PG (600 μg) and lysozyme (100 μg) were incubated for 16 h at 37°C and then the supernatant was fractionated using a Hydrosphere C18 (4.6 x 250 mm I.D.) column with methanol gradient (shown on the *right-hand side*). The major peak (peak a) was again incubated with recombinant PGRP-SC2 (10 μg) for 6 h at 30°C. The mixture was again injected to the same HPLC column and then peak (peak b) was collected and then analyzed by Edman reaction on the automatic amino acid sequencer.