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Journal Title: Antimicrobial Agents and Chemotherapy
Volume: Volume 58, Number 7
Publisher: American Society for Microbiology | 2014-07-01, Pages 4230-4233
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.1128/AAC.03108-14
Permanent URL: https://pid.emory.edu/ark:/25593/pfq8v

Final published version: http://dx.doi.org/10.1128/AAC.03108-14

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Accessed August 24, 2017 6:57 PM EDT
**Phase-Variable Expression of lptA Modulates the Resistance of Neisseria gonorrhoeae to Cationic Antimicrobial Peptides**

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Phosphoethanolamine (PEA) decoration of lipid A produced by *Neisseria gonorrhoeae* has been linked to bacterial resistance to cationic antimicrobial peptides/proteins (CAMPs) and *in vivo* fitness during experimental infection. We now report that the lptA gene, which encodes the PEA transferase responsible for this decoration, is in an operon and that high-frequency mutation in a polynucleotide repeat within lptA can influence gonococcal resistance to CAMPs.

*N. gonorrhoeae* is a strict human pathogen that has caused the sexually transmitted infection termed gonorrhea for thousands of years. Over the millennia, *N. gonorrhoeae* has developed multiple mechanisms to resist innate host defenses, including cationic antimicrobial peptides/proteins (CAMPs) produced by phagocytes and epithelial cells (1). Phosphoethanolamine (PEA) decoration of the lipid A possessed by *N. gonorrhoeae* and *N. meningitidis* has been shown to contribute to their resistance to CAMPs by a mechanism that likely involves a reduction in ionic interactions of CAMPs with the bacterial surface (1–6). Resistance of *N. gonorrhoeae* to complement-mediated killing by normal human serum (3,4), *N. gonorrhoeae* fitness during experimental infection in mice and humans (5, 7), and the proinflammatory potential of *N. gonorrhoeae* (7, 8). Most commensal *Neisseria* do not encode *lptA* (8), but *N. gonorrhoeae* and *N. meningitidis* (2, 3, 8) typically contain *lptA* and produce multiple isoforms of lipid A that differ in PEA decoration at the 4′ position. The *lptA* gene, which encodes a putative lipid A phosphoethanolamine transferase, *NGO1282* encodes a hypothetical gene, NGO1282 encodes a putative phosphoserylaminotransferase, NGO1282 codes for a putative nitroreductase. The locations of the *serC* (undefined) and *lptA* (defined in the Fig. 2B legend) promoters are depicted with bent arrows. The *lptA* transcriptional start point, −10, and −35 promoter elements are shown below the illustration. The approximate sites of annealing for oligonucleotide primers used in RT-PCR experiments (Table 1) are shown with arrows.

**FIG 1** Genetic context of *lptA* in *N. gonorrhoeae* FA19. The 3.8-kb region of the FA19 genome shown corresponds to nucleotides 1236150 to 1232381 in *N. gonorrhoeae* FA 1090 (http://www.genome.ou.edu/gono.html and GenBank accession number AE004969.1). *serC* encodes a putative phosphoserine aminotransferase, *NGO1282* encodes a putative nitroreductase. The *lptA* (undefined) and *lptA* (defined in the Fig. 2B legend) promoters are depicted with bent arrows. The approximate sites of annealing for oligonucleotide primers used in RT-PCR experiments (Table 1) are shown with arrows.
ing of seven Ts (T-7), which would result in production of a truncated LptA enzyme due to a new translational stop codon (Fig. 3). However, our independent sequencing of a PCR product containing the lptA gene from FA 1090 as well as from strain FA19 showed the presence of a T-8 tract (data not presented and Fig. 3A), which would result in production of a full-length LptA enzyme (Fig. 3B). Moreover, analysis of the online (http://www.broadinstitute.org/annotation/genome/neisseria_gonorrhoeae/GenomesIndex.html) whole-genome sequences of 13 other gonococcal strains indicated that their lptA gene contains the T-8 tract (data not presented). In addition, the genome sequence for 73 N. gonorrhoeae clinical isolates from patients with symptomatic gonorrhoea was determined using Illumina technology; the details of this genome shotgun sequencing effort will be published separately. The nucleotide sequence of the FA19 lptA gene was searched against a BLAST database of all the whole-genome of N. gonorrhoeae hypersusceptible to this model CAMP (3, 5, 7). After replica plating approximately 3,000 colonies of strain FA 1090 (T-8 tract and PMB MIC of 100 μg/ml) onto gonococcal base (GCB) agar plates with or without PMB selection, we identified (approximate frequency of 3.3 × 10^{-4}) a colony (strain BB22) that was unable to grow on GCB agar plates containing 10 μg/ml PMB. Moreover, the absence of PMB selection procedure since loss of lptA genes were then extracted and screened for the presence of a T-8 tract on both the forward and reverse strands of the gene using pattern matching. The results showed that all strains contained a T-8 tract and a full-length lptA sequence with 100% nucleotide identity to FA 1090 (data not presented). Accordingly, we propose that possession of an in-frame lptA gene is a common feature of N. gonorrhoeae isolates.

**Fig 2** Transcription of the lptA coding sequence. (A) Transcriptional linkage between serC, NGO1282, lptA, and nfnB. All RT-PCRs were performed on purified RNA harvested (RNeasy minikit; Qiagen) from a log-phase culture of strain FA19 grown as described previously (9). First-strand cDNA was generated using SuperScript II reverse transcriptase (Invitrogen) and a gene-specific reverse primer (LptA8) that binds in the nfnB gene and primes elongation of a single-stranded cDNA toward serC. PCR was then performed to confirm transcriptional linkage between pairs of genes. Sections A, B, and C of the gel are grouped by forward- and reverse-primer locations and separated by 1-kb PLUS DNA ladders (Invitrogen). Lane 1, FA19 cDNA; lane 2, −RT negative control (RT omitted); lane 3, FA19 genomic DNA positive control; lane 4, no-template negative control. Section A, “serC” = serC_F; “NGO1282” = NGEG_02068_R. Section B, “serC” = serC_F; “…lptA” = lptArev. Section C, “…lptA” = LptA6; “nfnB” = LptA8. (B) Primer extension of the lptA transcript. Primer extension analysis was performed as described previously (9) using 20 μg of FA19 total RNA as the template and a radioactively ([γ-32P]ATP) labeled reverse primer (LptA7_R) that anneals 67 bp downstream of the lptA start codon. RNA was purified and cDNA generated as described for panel A. Radioactive single-stranded cDNA products were separated on a polyacrylamide gel alongside sequencing reactions that used the same reverse primer (LptA11 was the forward primer used for generation of the sequenced lptA promoter template). The TSP corresponds to the band labeled with an asterisk and is 4 bp downstream of a consensus σ70-type −10 element. A second band appeared running approximately 4 to 5 nucleotides shorter than the proposed +1 TSP band and could be due to a degraded mRNA transcript.

**Table 1** Oligonucleotide primers used in this study

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<tr>
<th>Primer name</th>
<th>Primer sequence</th>
<th>Purpose</th>
</tr>
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<tbody>
<tr>
<td>LptA6</td>
<td>5′-GGGTTTTGATGATGGATCGATT-3′</td>
<td>Transcriptional linkage</td>
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<tr>
<td>LptA7</td>
<td>5′-GGCTTTCTCTTTCCTGTATTCTT-3′</td>
<td>Sequencing of the poly-T tract</td>
</tr>
<tr>
<td>LptA7_R</td>
<td>5′-AGAATACGGGAAAGAAGG-3′</td>
<td>Primer extension</td>
</tr>
<tr>
<td>LptA8</td>
<td>5′-ACGGTCTAACGGCTACCGG-3′</td>
<td>Transcriptional linkage</td>
</tr>
<tr>
<td>LptA11</td>
<td>5′-CGCGCTGGAATTTTGCTTACG-3′</td>
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<td>LptADelL</td>
<td>5′-TGCGAGGTACATGAAATTAGAG-3′</td>
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<td>5′-TAAGAATCTTTCTCTTAATCCGGAT-3′</td>
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<tr>
<td>lptArev</td>
<td>5′-GGCTCAGGTTCCGTTTATAC-3′</td>
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<tr>
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<td>5′-TCTAGAATACGGGAAAGAAGG-3′</td>
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</tr>
<tr>
<td>NGEG_02068_R</td>
<td>5′-GGGTTTTGATGATGGATCGATT-3′</td>
<td>Transcriptional linkage</td>
</tr>
<tr>
<td>serC_F</td>
<td>5′-GGACTACGGGACTTTACG-3′</td>
<td>Transcriptional linkage</td>
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of PMB. The PMB MIC against BB22 was 0.2 μg/ml (Fig. 3A), which is similar to the PMB MIC against an lptA deletion mutant of FA 1090 described previously (5). DNA sequence analysis of the lptA sequence of BB22 revealed that it possessed a T-9 tract that would result in premature truncation of LptA (Fig. 3). We then selected for spontaneous variants of BB22 that would grow on GCB agar containing 10 μg/ml of PMB. In four separate experiments, spontaneous PMB-resistant variants arose at a frequency of approximately 10−3; in contrast, spontaneous erythromycin-resistant mutants (selected at 1 μg/ml) were recovered at a frequency of 10−8 (data not presented). The PMB MIC against PMB-resistant mutants of BB22 was, like that seen with parental strain FA 1090, 100 μg/ml (see strain BB22R data in Fig. 3A). DNA sequencing of the lptA PV tract from sixteen randomly picked PMB-resistant revertants of BB22 showed that all possessed a wild-type T-8 tract (see BB22R in Fig. 3A) and would produce a frameshift mutation, which would result in premature stop codon shortly after this PV tract in the lptA mRNA transcript (B). Impact of the PV poly-T tract on LptA protein length. When in the phase-on state, the lptA poly-T tract (hatched boxes) has 8 nucleotides and lptA encodes a protein 544 amino acids long. Frameshift of the lptA open reading frame (ORF) due to an insertion of a single T nucleotide within this poly-T tract would result in a premature stop codon and subsequent truncation of the LptA nascent polypeptide at just 61 amino acids. Phase-off variants are not predicted to translate the C-terminal sulfatase domain of the LptA protein (13). DUF, domain of unknown function.

**ACKNOWLEDGMENTS**

We thank Jo-Anne Dillon, Carlos del Rio, and Magnus Unemo for providing N. gonorrhoeae strains and DNA used in whole-genome sequencing (WGS).

This work was supported by NIH grants U19 AI031496 (to P. F. Spurting, University of North Carolina-Chapel Hill), R37 AI021150-29 (W.M.S.), R43 AI09768 (T.D.R.), and R01 AI42053 (A.E.J.) and a VA Merit Award from the Medical Research Service of the Department of Veterans Affairs to W.M.S. W.M.S. was supported by a Senior Research Career Scientist Award from the Medical Research Service of the Department of Veterans Affairs.

We declare that we have no conflicts of interest.

**REFERENCES**


