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Identification and Functional Characterization of the *Neisseria gonorrhoeae lbpB* Gene Product

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We cloned *lbpB*, encoding a predicted 80-kDa lipoprotein, upstream of *lbpA*. A nonpolar mutant (LbpB$^-$ LbpA$^+$) had normal lactoferrin (LF) binding and grew normally with LF as an iron source, whereas LbpB$^-$ LbpA$^-$ and LbpB$^+$ LbpA$^-$ strains had reduced binding of LF and did not grow with LF as an iron source. LbpB bound LF directly in an affinity purification, suggesting that LbpB might play a still-uncharacterized role in the LF iron utilization.

Most organisms use inorganic iron (Fe) as a cofactor in carrying out a variety of metabolic functions (34). In an oxidized environment, ferric iron is highly insoluble and relatively unavailable. Mammals solubilize and sequester extracellular Fe by binding to the glycoproteins transferrin (TF) in serum (34) and lactoferrin (LF) on mucosal surfaces (22). Since there is essentially no free Fe in humans, pathogens have evolved mechanisms to scavenge Fe from host proteins. Many bacteria synthesize and secrete siderophores, low-molecular-weight compounds which bind and transport Fe into the cell through specific siderophore receptors (16). Production of siderophores facilitates infection in several bacterial pathogens (26). *Neisseria gonorrhoeae* and several other gram-negative mucosal pathogens including *Neisseria meningitidis* do not produce siderophores (10, 23) but produce specific receptors for each of the glycoproteins TF and LF, as well as hemoglobin (5, 7, 10, 15, 21).

A receptor for LF, LbpA, was identified and the corresponding *lbpA* gene was cloned and characterized in *N. gonorrhoeae* (3) and *N. meningitidis* (29). Recently, a second LF receptor protein, LbpB, was identified in *N. meningitidis* (6, 20, 28). Here we report the cloning of *lbpB* from *N. gonorrhoeae* and characterization of the role of LbpB in gonococcal LF binding and Fe acquisition.

Cloning of *lbpB*. We previously cloned the entire *lbpA* gene on four overlapping clones (3). pUNCH127 (Fig. 1) included 1,246 bp of DNA upstream of the 5′ end of the *lbpA* open reading frame (ORF). To test if the region upstream of *lbpA* was also involved in LF utilization, we inactivated it by inserting (31) an Ω cassette (32) into an *Ava*I site in pUNCH127, creating pUNCH130. Sequence analysis of pUNCH130 confirmed that the Ω fragment was inserted at the *Ava*I site. pUNCH130 DNA was introduced into wild-type gonococcal strain FA19 by transformation (4), resulting in a mutant strain, FA6839. The presence of the Ω fragment in the *Ava*I site in FA6839 was confirmed by Southern analysis with the use of an *lbpB*-specific oligonucleotide probe (positions 2451 to 2470 in the *lbpB* sequence in GenBank) or the 2-kb Ω fragment (32). The mutant FA6839 had a phenotype similar to that of *lbpA* mutant FA6775 (3), including loss of ability to (i) bind LF in a solid-phase assay, (ii) take up Fe from LF, (iii) utilize LF as an Fe source, and (iv) express LbpA. These results suggest that insertion of Ω upstream of *lbpA* had a polar effect on expression of LbpA.

DNA upstream of *lbpA* was cloned in two chromosome walking steps involving the use of oligonucleotide primers L58 and L62 (Fig. 1). Southern hybridization analysis showed that L58 hybridized to a 2.3-kb *Rsa*I fragment (data not shown). We cloned the 2.3-kb *Rsa*I fragment into the *Sma*I site of pUNCH615 (32), creating pUNCH142. Hybridization analysis with probe L62 identified a 1.1-kb *Sau*3A fragment of FA19 DNA. We ligated the 1.1-kb *Sau*3A fragment into the *Bam*HI site of pMCL210 (27) harboring *lacZ*, allowing rapid color screening for inserts. Screening by colony hybridization produced two clones, pUNCH192 and pUNCH193, that differed in size. The clone pUNCH192 in *Escherichia coli* produced colonies on X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) and IPTG (isopropyl-β-D-thiogalactopyranoside) plates that had three phenotypes: slightly blue, white, and blue. Plasmid DNA from a colony belonging to each class was sequenced. Plasmid DNA from a slightly blue colony designated pUNCH192SB (Fig. 1) contained an 1,138-bp *Sau*3AI insert with a complete 5′ end of an apparent 2,189-bp-long ORF (Fig. 1). In contrast, the corresponding plasmid isolated from a white colony was 328 bp shorter than pUNCH192SB. The missing DNA included 21 bp at the 5′ end of the 2,189-bp ORF. Plasmid DNA from the blue colony contained primarily vector DNA. The clone pUNCH193 produced stable white colonies on similar media and was stable on subculture. Interestingly, pUNCH193 lacked 20 bp of DNA that included 5′-GTC GAA TCA ACG CCG ACC GC-3′ at positions 31 to 51 from the ATG codon start site of the 2,189-bp ORF in pUNCH192SB. To verify that the DNA in pUNCH192SB represented wild-type FA19, we PCR amplified and directly sequenced FA19 DNA flanking the deleted 20-bp region. A comparison of the sequences of FA19 DNA in the amplified product and the corresponding DNA in pUNCH192SB showed complete identity, confirming that the clone pUNCH192SB contained wild-type FA19 DNA. Thus, the set of three overlapping clones, pUNCH127, pUNCH142, and pUNCH192SB, represents 2,493 bp of contiguous FA19 DNA upstream of *lbpA*.

Nucleotide sequence analysis. An examination of the sequences in clones pUNCH127, pUNCH142, and pUNCH

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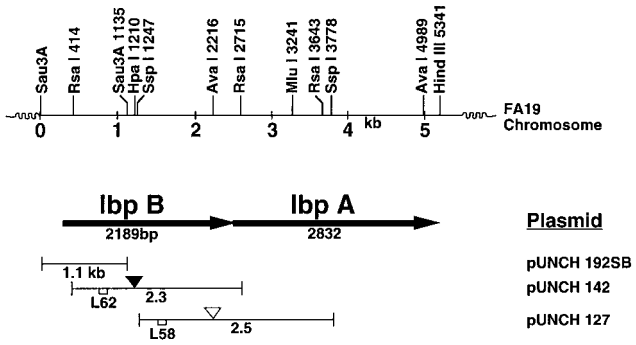

FIG. 1. Physical map of the *N. gonorrhoeae* chromosome showing genetic organization of the *lbpBA* operon region. The top line represents the restriction map of the cloned FA19 chromosome fragment. The organization of *lbpBA* genes including the direction of transcription as indicated by arrows is shown in the next line. Plasmid pUNCH192SB is a 1.1-kb *Sau*3AI fragment. Plasmids pUNCH142 and pUNCH127 contain 2.3-kb *Rsa*I and 2.5-kb *Ssp*I fragments, respectively. The solid inverted triangle denotes the position of the *aphA3* insertion in strain FA6965(pUNCH142), and the open inverted triangle denotes the position of the Ω insertion in strain FA6839(pUNCH127). L58 and L62 refer to the oligonucleotides used as probes in isolating the clones pUNCH142 and pUNCH192SB, respectively.

Region 1

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NG 462 DEEDSGINNGEESEDEEEIAEESEDEVSEDDNGEDEDEIVEEEADEAEEI
       ||||| |.|||||||||     ||  :: .  | | :|||  | |  | :|
NM 453 DEEDSDIDNGEESEDEIGDEEEGTEDAAAGDEGSEEDEATENEDGEEDEA

NG 512 EEEAEEEEPEEESPEE 527
       ||  ||   |
NM 503 EEPEEESSAE..... 512
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Region 2

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NG 689 ............EDIENEVENEADVGEQLEPE  708
                    |:: :|: :||| |||.||
NM 675 EGTENKVEADVDVDVDVDADADV.EQLKPE  705
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FIG. 2. Lineup of negatively charged residues in region one and region two of the gonococcal (NG) and meningococcal (NM) LbpB proteins. The numbers indicate the position in the unprocessed protein of the first amino acid listed. Amino acids identical between the two proteins are denoted by vertical lines; colons indicate highly conservative substitutions, and dots indicate less conservative substitutions.

192SB revealed a 2,189-bp ORF. The last 4 bp (ATGA) of the 2,189-bp ORF partially overlapped with the ATG initiation codon of the *lbpA* ORF. We designated the 2,189-bp ORF located upstream of *lbpA* as *lbpB*. Further upstream, we found 170 bp that were 56% identical to the 3′ end of an *E. coli era* gene that encodes a GTP binding protein (1). Immediately downstream of the *era* homolog there was a potential hairpin loop structure at positions 180 to 212. This structure has a ΔG (25°C) value of −20.8 kcal (33) and probably corresponds to a transcription terminator. In addition, a 10-bp inverted repeat at positions 184 to 208 and another 10-bp sequence at positions 1902 to 1911 matched gonococcal uptake sequence (12, 14).

Translation of the *lbpB* coding region predicted a protein of 728 amino acids with a molecular mass of 80 kDa and a pI value of 4.53. Analysis of the deduced N-terminal amino acid sequence of LbpB revealed an 18-amino-acid signal sequence with the lipoprotein modification consensus sequence LSAC at positions 16 to 19 (17). These data suggested that LbpB was a lipoprotein. Based on fluorographic analysis and palmitic acid labeling, Lewis et al. concluded that LbpB in *N. meningitidis* is a lipoprotein (20). LbpB was 31% identical to TbpB of the same gonococcal strain and 81% identical to the recently reported meningococcal LbpB (6, 28). Comparison of the predicted protein sequence of FA19 LbpB with that of meningococcal LbpB (6, 28) showed very similar features that differentiate each from the neisserial TbpB family. Both gonococcal and meningococcal LbpB proteins contained two stretches of negatively charged residues that were not present in TbpB family members: region one, residues 462 to 527, and region two, residues 689 to 708 (Fig. 2). These regions also exhibit considerable variation between meningococcal and gonococcal LbpB proteins. There was a deletion in region one in meningococcal LbpB relative to the gonococcal LbpB, and in region two, there was a deletion in gonococcal LbpB. Overall, these two charged domains were only about 50% identical, compared to 81% for the whole proteins. Each of the LbpB proteins contained an identical 21-amino-acid C terminus that was absent in all neisserial TbpB family members.

***lbpB* gene product.** To study the function of *lbpB*, we disrupted *lbpB* by inserting a nonpolar *aphA3* cassette (24) into an *Hpa*I site in pUNCH142, creating pUNCH195 (Fig. 1; Table 1) and exchanged this mutant allele into the gonococcal strain FA19 as described above, creating FA6965. Sequence analysis confirmed that the cassette was inserted in the correct orientation and translational frame into pUNCH195. Southern hybridization with the *lbpB*-specific probe L66 (positions 1156 to 1176) or the 0.9-kb *aphA3* fragment (24) confirmed the expected insertion in *lbpB* in FA6965. The *lbpB* gene product was identified with the use of polyclonal, affinity-purified antiserum raised against a synthetic 16-mer peptide, RTRDNGINLSGNGSTN, from the predicted C terminus of LbpB. We used isogenic strains FA6815, FA6985, and FA6986 (Table 1) lacking TbpB and TbpA in order to avoid potential problems due to production of proteins similar in size and structure to LbpB and LbpA. A Western blot probed with LbpB antibodies reacted with a protein of about 95 kDa in the parent strain FA6815 (Fig. 3A, lane 1). A protein of the same size also was produced by FA6985 (*lbpA*::mTn*3* Cm) (Fig. 3A, lane 3) but not FA6986 (*lbpB*::*aphA3*) (Fig. 3A, lane 4). The latter strain did produce the 103-kDa LbpA, as expected (Fig. 3B, lane 4). FA6839 (*lbpB*::Ω) produced neither LbpA nor LbpB (Fig. 3, lanes 2). These data showed that the 95-kDa protein was the *lbpB* gene product and also provided experimental evidence that *lbpB* and *lbpA* were part of a single transcriptional unit.

We noticed that the nonpolar mutant FA6986 (*lbpB*::*aphA3*) expressed reduced amounts of LbpA (Fig. 3B, lane 4) compared to the parent strain FA6815 ($lbpB^+$ $lbpA^+$) (Fig. 3B, lane 1). We confirmed this by comparing the intensities of the LbpA protein bands in linear range of detection in Western blots. We estimated that the reduction was approximately fivefold from the level for the parent strain (data not shown). We concluded that the *aphA3* insertion in *lbpB* in FA6986 caused reduced production of the downstream *lbpA* gene product. The reason for reduced expression of LbpA in the *lbpB*::*aphA3* mutant is not understood. Insertion of the *aphA3* cassette in *lbpB* clearly did allow transcriptional read-through into *lbpA* but may have attenuated transcription downstream of the insertion.

LF binding. To determine whether LbpB participated in LF binding, we performed dot blot assays of LF binding to whole cells. Bound LF was detected with horseradish peroxidase-conjugated anti-human LF antibody (Accurate Chemical & Scientific Corp., Westbury, N.Y.) (3). The results demonstrated that the $LbpB^-$ $LbpA^+$ mutant FA6986 bound approximately as much LF as the $LbpB^+$ $LbpA^+$ parent strain FA6815 (data not shown). In contrast, no binding was detected

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant characteristics	Source or reference(s)
N. gonorrhoeae strains		
FA19	LF^+ TF^+	5, 23
FA1090	LF^- TF^+	8
FA6775	LF^- Cm^r (*lbpA*::mTn*3* Cm)	3
FA6815	TF^- Str^r Spc^r (*tbpB*::Ω)	2
FA6839	LF^- TF^+ Str^r Spc^r (*lbpB*::Ω); transformant, pUNCH130→FA19	This study
FA6965	LF^+ Kan^r (*lbpB*::*aphA3*); transformant, pUNCH195→FA19	This study
FA6985	LF^- (*lbpA*::mTn*3* Cm) TF^- (*tbpB*::Ω), Cm^r Str^r Spc^r; transformant, FA6775→FA6815	This study
FA6986	LF^+ (*lbpB*::*aphA3*) TF^- (*tbpB*::Ω) Kan^r Str^r Spc^r; transformant, FA6965→FA6815	This study
Plasmids		
pHP45Ω	Source for the Ω fragment (Str^r Spc^r)	32
pMCL210	Cloning vector, low copy; *lacZ*α Cm^r	27
pUC18K	Source for the *aphA3* cassette (Kan^r)	24
pUNCH127	Ap^r Tet^r; 2.5-kb FA19 *lbpAB* *Ssp*I fragment cloned into *Ssp*I site in pBR322	3
pUNCH128	Ap^r; FA19 insert in pUNCH127 cloned into *Bam*HI site in pUNCH615	3
pUNCH130	Str^r Spc^r; Ω cassette inserted into *Ava*I site in pUNCH128	This study
pUNCH142	Ap^r; 2.3-kb FA19 *Rsa*I fragment cloned into *Bam*HI site in pUNCH615	This study
pUNCH192	Like pUNCH192SB but unstable	
pUNCH192SB	Cm^r; 1.1-kb FA19 *Sau*3A fragment cloned into *Bam*HI site in pMCL210	This study
pUNCH193	Like pUNCH192SB but deleted	This study
pUNCH195	Kan^r; *aphA3* cassette inserted into *Hpa*I site in pUNCH142	This study
pUNCH615	Ap^r; pBluescript II KS(−) with *Pst*I site replaced with *Mlu*I	32

for cells lacking LbpA, regardless of whether LbpB was present. Therefore, the solid-phase binding assay failed to detect LF binding by gonococci expressing LbpB alone. LbpB-specific binding was not detected under slightly different conditions (6, 28) for the solid-phase assay (data not shown).

To better quantitate LF binding, we measured the amount of radiolabeled LF bound by living cells in an equilibrium-phase binding assay developed by Cornelissen and Sparling (9). The specific activity of iodinated LF was 2.9×10^5 cpm/μg of LF. Approximately 1×10^7 to 5×10^7 CFU of gonococcal cells grown in chelexed defined medium with no added Fe (CDM-0) to induce Fe stress (5) were mixed with 2 to 100 nM ^{125}I-LF in the presence of 1% bovine serum albumin and 5 μM unlabeled LF in individual wells of a Multi-Screen microtiter dish (0.45-μm-pore-size filter; MAHV N45; Millipore, Cambridge, Mass.). After being allowed to bind for 20 min at room temperature, unbound LF was removed by filtration, followed by five washes with CDM-0. Filters were dried, punched out, and counted. Specific binding was the difference between total binding without cold LF and binding that occurred in the presence of at least a 50-fold-excess concentration of cold LF. All four strains studied exhibited specific, saturable LF binding (Fig. 4). The wild-type strain bound significantly more LF than did each of the other strains. An apparent trend toward an intermediate level of LF binding in FA6965 ($LbpB^-$ $LbpA^+$) was noted, but the differences were not statistically significant by the *t* test in conjunction with the Bonferroni procedure (19).

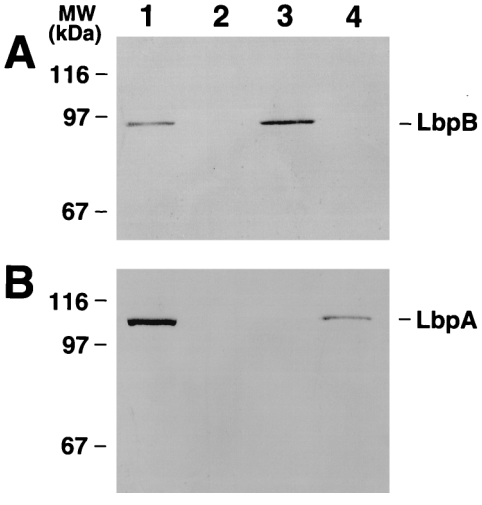

FIG. 3. Expression of LbpB and LbpA in *N. gonorrhoeae*. Shown are Western blots containing total membranes prepared from Fe-starved cells probed with affinity-purified LbpB polyclonal antibody (A) and monoclonal LbpA antibody (B) (3). Lanes: 1, parent strain FA6815 ($LbpB^+$ $LbpA^+$); 2, FA6839 ($LbpB^-$ $LbpA^-$); 3, FA6985 ($LbpB^+$ $LbpA^-$); 4, FA6986 ($LbpB^-$ $LbpA^+$).

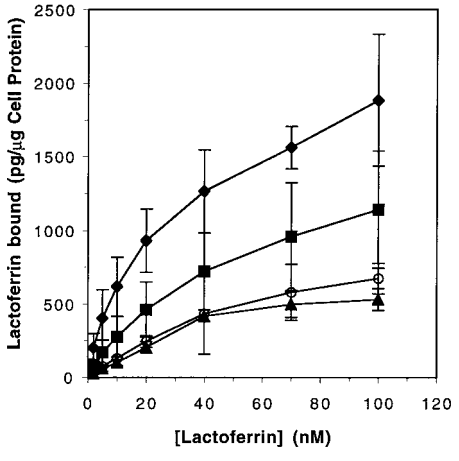

FIG. 4. Isotherms for binding of LF to *lbp* mutants. The curves represent the amount of specifically bound LF as a function of LF concentration. Each point represents the mean of four individual experiments. Symbols: ◆, FA19 ($LbpB^+$ $LbpA^+$); ■, FA6965 ($LbpB^-$ $LbpA^+$); ○, FA6775 ($LbpB^+$ $LbpA^-$); ▲, FA6839 ($LbpB^-$ $LbpA^-$).

TABLE 2. K_d and copy number estimates for LF receptors expressed by isogenic mutants

Strain	Phenotype	K_d (nM)	Copy number (M)
FA19	LbpA$^+$ LbpB$^+$	4.8 (site 1)	9.3×10^{-12} (N1)
		550 (site 2)	6.9×10^{-11} (N2)
FA6965	LbpA$^+$ LbpB$^-$	5.0 (site 1)	2.3×10^{-12} (N1)
		95 (site 2)	2.0×10^{-11} (N2)
FA6775	LbpA$^-$ LbpB$^+$	45 (site 1)	1×10^{-11} (N1)
FA6839	LbpA$^-$ LbpB$^-$	45 (site 1)	1×10^{-11} (N1)

The data obtained in the liquid-phase LF-binding experiments were analyzed with the computer program Receptor Fit Saturation Two-Site (Lundon Software, Inc., Cleveland Heights, Ohio). This program generates K_d and copy number estimates based on the best fit of observed data to progressively more complex models. Table 2 contains the best estimates of K_d and copy numbers generated by this program. Considerable variability was observed for LF binding to all strains; thus, these estimates are best considered comparisons of LF binding to isogenic strains rather than absolute indicators of intrinsic receptor affinity or copy number. Isogenic strains expressing LbpA exhibited complex binding phenomena consistent with the presence of at least two populations of binding sites, with K_ds ranging from 5 to 500 nM. In contrast, the isogenic mutants that did not express LbpA bound LF to a more homogeneous population of receptors, with a single apparent K_d of approximately 45 nM. This was true of both LbpB$^+$ and LbpB$^-$ strains, suggesting that LbpB played no detectable role in LF binding under the conditions employed in this assay. Somewhat surprisingly, the mutant that did not express either of the two identified LF-binding proteins (LbpB and LbpA) retained the ability to bind LF. This observation suggested that there were uncharacterized LF-binding components on the gonococcal cell surface.

LF affinity isolation of LbpB. To determine whether LbpB could bind LF in the absence of LbpA, total membranes prepared from Fe-starved FA6815 (LbpB$^+$ LbpA$^+$) and its isogenic derivatives, FA6985 (LbpB$^+$ LbpA$^-$) and FA6986 (LbpB$^-$ LbpA$^+$), were subjected to LF-agarose affinity purification (7, 11). Total membrane proteins were dissolved in 2% Zwittergent 3, 14 (Calbiochem) and rocked with a 50% slurry of LF-agarose overnight at 4°C. The unbound material was removed by centrifugation, and the LF-binding protein was eluted in a Laemmli sample buffer for analytical sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting. Results (Fig. 5) showed an excellent recovery of LbpB in the absence of LbpA, indicating that LbpB was capable of binding LF under these conditions. LbpA also could be isolated by this procedure from total membranes in the absence of LbpB. The amount of LbpA in membrane preparations from the two strains was roughly the same (lanes A and C), but less LbpA was purified from the LbpB$^-$ LbpA$^+$ strain than from wild type (lanes D and F). This suggested that LbpA alone did not bind to LF as avidly as it did in the presence of LbpB.

LF utilization. To assess the contribution of LbpB in utilization of Fe from LF, we compared growth in CDM supplemented with LF of strains that carried either intact *lbpB* or inactivated *lbpB*. On agar plates or in CDM broth culture (5), the nonpolar *lbpB* mutant FA6965 (LbpB$^-$ LbpA$^+$) failed to show a growth defect, growing to a level equal to that of wild-type FA19 (data not shown).

^{55}Fe-LF uptake. To detect smaller effects of LbpB in LF utilization, we measured the amount of ^{55}Fe-LF taken up by the use of established procedures (2, 5). Results indicated that the nonpolar *lbpB*::*aphA3* mutant FA6965 (LbpB$^-$ LbpA$^+$) acquired ^{55}Fe from LF at about 60% of wild-type levels ($P < 0.03$) (data not shown). In contrast, LbpA$^-$ mutants, FA6775 and FA6839, failed to take up a significant amount of ^{55}Fe from LF ($P < 0.01$). We could not determine whether reduced Fe uptake from LF in FA6965 was due to loss of LbpB or to decreased expression of LbpA.

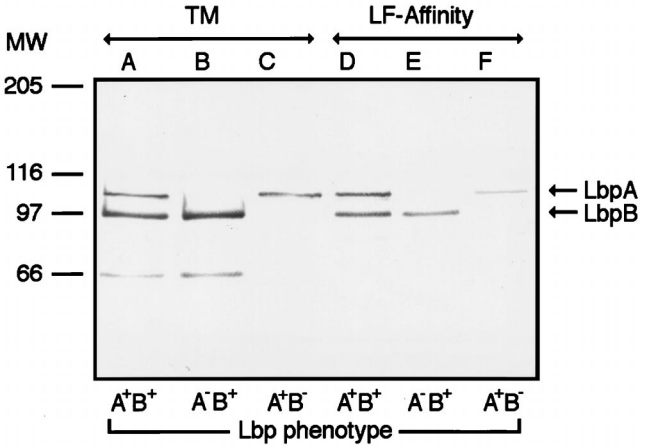

FIG. 5. Western blot of LF-agarose affinity-purified LbpB and LbpA from total membranes of Fe-starved FA6815 (LbpB$^+$ LbpA$^+$) (lanes A and D), FA6985 (LbpB$^+$ LbpA$^-$) (lanes B and E), and FA6986 (LbpB$^-$ LbpA$^+$) (lanes C and F). The blot was probed with antibodies to LbpA and then LbpB. The anti-LbpB antibody used here was raised against an LbpB His-tagged fusion protein. The 66-kDa band present in lanes A and B was recognized by antiserum elicited against full-length recombinant LbpB but not against an LbpB-specific peptide (Fig. 3 and data not shown). This protein band probably represents a breakdown product of holo-LbpB. Lanes A to C, total membranes (TM); lanes D to F, LF-agarose affinity-purified proteins. MW, molecular weight in thousands.

Conclusions. We cloned an *N. gonorrhoeae lbpB* gene, providing genetic evidence for the existence of a second LF receptor protein in the gonococcus, similar to that described recently for meningococci (6, 20, 28). Despite difficulties encountered in isolating the 5′ end of the gonococcal *lbpB* gene, we succeeded in isolating a clone (pUNCH192SB) with an intact 5′ end of *lbpB*. Attempts to clone the 5′ end of the *lbpB* gene in *N. meningitidis* were unsuccessful (6, 20, 28).

A two-gene *lbpB lbpA* transcriptional unit was implicated by the observations that the 3′ end of *lbpB* overlapped the 5′ end of *lbpA*, and creation of a polar mutation in *lbpB* resulted in simultaneous loss of expression of the *lbpB* and *lbpA* genes. Recent experiments by Lewis et al. (20) showed that the *lbpB* and *lbpA* genes in meningococci are organized in a single transcriptional unit.

Both the gonococcal and meningococcal LbpB proteins contain two strongly acidic domains, notably absent in TbpB proteins, which are rich in aspartic acid and glutamic acid. This suggests that these regions could be involved in binding of LF, which is highly cationic (34). These regions are quite dissimilar in primary sequence in the known examples in gonococci and meningococci, suggesting that these also could be antigenic domains recognized by the host immune response. Future experiments hopefully will answer whether these domains are involved in binding LF and are immunogenic.

The role of LbpB in mediating Fe acquisition from LF is unclear. The LbpB$^-$ mutant (LbpB$^-$ LbpA$^+$) retained the ability to bind LF, and this was reflected in its capacity to utilize LF as an Fe source. We demonstrated that LbpB, in the

absence of LbpA, could be isolated by LF-agarose affinity, which is the best evidence that LbpB is an LF-binding protein.

We found that the LbpB$^-$ LbpA$^+$ mutant used LF as an Fe source approximately as well as did the parent, as evidenced by similar growth patterns. The growth patterns of similar meningococcal LbpB$^-$ LbpA$^+$ mutants varied, but all mutants grew to some extent (28). Differences in growth exhibited by strains in these reports might be explained on the basis of varying levels of LbpA expression in the LbpB$^-$ LbpA$^+$ strain. For example, negligible growth noted by Lewis et al. might have resulted from a more than 10-fold reduction in LbpA expression (20). Near-normal growth exhibited by the mutant used by Pettersson et al. was correlated with LbpA expression similar to that of LbpB (28). The general conclusion is that LbpA is essential for LF-Fe utilization, whereas LbpB is not essential.

The question of how important LF is to pathogenesis remains. Many pathogens and nonpathogens can survive on the mucosal surface without being able to bind LF or to use LF as a source of Fe. For example, all *Haemophilus influenzae* strains are LF$^-$ (18, 30), and about one-half of gonococci are LF$^-$ (3, 25). Indeed, gonococcal strain FA1090 is LF$^-$ due to a large *lbpBA* deletion (references 13 and 20 and data not shown), yet it causes urethritis readily in male human volunteers (8). Future work is required to clarify the role of LF in bacterial pathogenesis and the roles of LbpB, in particular in utilization of LF.

Nucleotide sequence accession number. The GenBank accession number for the 2,189-bp ORF is AF072890.

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