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Genetics

Molecular cloning and characterization of the structural gene for protein I, the major outer membrane protein of *Neisseria gonorrhoeae*

(porin/oligonucleotides/phage T7 promoter/vaccine/virulence)

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ABSTRACT **Protein I (P.I) is the major outer membrane protein of *Neisseria gonorrhoeae* and serves as a porin. By using oligonucleotide probes derived from the known amino-terminal sequence of the mature protein, we have cloned the gene encoding the P.I of gonococcal strain FA19 in three overlapping fragments and determined the DNA sequence. The gene sequence predicts a protein with characteristics typical of the porins of other Gram-negative bacteria. A clone expressing P.I in *Escherichia coli* was obtained by removing a portion of the P.I gene promoter and reconstructing the entire P.I gene in a position just downstream from a phage T7 promoter. Expression of P.I was then achieved by introducing this recombinant plasmid into an *E. coli* strain containing an inducible T7 polymerase gene. The clone produced a protein that was identical in size to native P.I and reacted with anti-P.I monoclonal antibodies. Prolonged expression of the protein apparently was lethal for *E. coli*, possibly explaining failures to clone an intact P.I gene with its own promoter.**

The outer membrane of *Neisseria gonorrhoeae* includes a variety of proteins, the most abundant of which is known as protein I (P.I). P.I is a matrix or porin protein (1), forming an aqueous channel for the selective passage of solutes through the outer membrane. Like the porins of other Gram-negative bacteria, gonococcal P.I exists as a trimer in the outer membrane (2) and forms a pore similar in size to that of the major porins of *Escherichia coli* (3). P.I is a major antigen on the gonococcal cell surface and it may have pathogenic properties in addition to its porin activity. It is able to insert directly into artificial bilayer membranes and into human erythrocytes when gonococci are mixed with these cells (4), though the significance of this event and whether it takes place *in vivo* are unknown. Antibodies against P.I are opsonic (5), and P.I has been associated with gonococcal serum resistance (6) and inactivation of polymorphonuclear leukocyte degranulation (4). The universal occurrence of this protein in gonococci and its comparatively conserved nature make it attractive as a component of a gonorrhea vaccine.

There are two immunochemical classes of P.I, termed P.IA and P.IB. Different serovars of P.IA and P.IB are defined by reactions with a set of monoclonal antibodies (7). The P.I proteins of the two serotypes show other structural differences. P.IB proteins have an apparent molecular weight by $NaDodSO_4$/polyacrylamide gel electrophoresis of 34,000–38,000 and are susceptible to cleavage by the proteolytic enzymes trypsin and chymotrypsin, whereas P.IA proteins normally have a slightly lower apparent molecular weight and are resistant to these proteolytic enzymes (8).

Genetic studies of P.I in the gonococcus have been limited. A locus designated *nmp* is known to affect the molecular weight and antigenicity of P.I (9) and is closely linked to loci involved in serum resistance (*sac*) and antibiotic resistance (*penB, str, spc*) (10). It is not known whether the *nmp* locus is the structural gene for P.I or a regulatory gene affecting expression of the structural gene located elsewhere. Cloning of the P.I structural gene has presented problems; a portion of the P.IB gene of gonococcal strain MS11 has apparently been cloned in *E. coli* (11), but a full report of these results has not been published. Reasons for difficulty in cloning this biologically important protein have been unclear.

In this paper, we report the cloning and characterization of the structural gene for the P.IA of gonococcal strain FA19 and its expression under the control of a foreign promoter in *E. coli*.*

MATERIALS AND METHODS

Bacterial Strains and Plasmids. The *N. gonorrhoeae* strain used in this study was FA19 (P.IA) (12). *E. coli* strain HB101 has been described previously (13), and BL21(DE3) (14), a generous gift from F. William Studier (Brookhaven National Laboratory, Upton, NY), is a lysogen in which the phage T7 polymerase gene is present but under the control of the *lacUV5* promoter. Plasmids used were pBR322 (13) and pGEM-2 obtained from Promega Biotec (Madison, WI).

Media. *N. gonorrhoeae* was grown in GC base medium (Difco) containing Kellogg's supplements I and II (15) in a 5% CO_2/95% air atmosphere. *E. coli* strains were grown in Luria medium (13) with the following additions where appropriate: ampicillin (50 μg/ml), tetracycline (10 μg/ml), and isopropyl β-D-thiogalactopyranoside (iPrSGal; 100 μg/ml).

Colony Blot Radioimmunoassay and Immunoblotting. Bacterial colonies were assayed for binding of monoclonal antibodies by the method of Cannon *et al.* (16). The monoclonal antibodies used to detect P.IA were 4A12, 4G5, 2F12, 6D9, 5G9, and 5D1 (7). Immunoblotting was performed according to the method of Burnette (17). After $NaDodSO_4$/polyacrylamide gel electrophoresis of whole cell lysates in a Laemmli buffer system (18), transfer for immunoblotting and P.I detection were carried out as described previously (19).

DNA Techniques. Gonococcal genomic DNA was prepared by the method of Stern *et al.* (20). Preparation of plasmid DNA and transformation into *E. coli* were performed as described by Maniatis *et al.* (13). Restriction endonucleases, DNA polymerase Klenow fragment, T4 DNA ligase, and polynucleotide kinase were obtained from either Bethesda Research Laboratories or New England Biolabs and were used according to the instructions of the manufacturer.

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Abbreviations: P.I, protein I of *N. gonorrhoeae*; iPrSGal, isopropyl β-D-thiogalactopyranoside; bp, base pair(s); kb, kilobase pair(s).

*The sequence reported in this paper is being deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03029).

Oligonucleotide Synthesis. Oligonucleotides were synthesized in an Applied Biosystems (Foster City, CA) DNA synthesizer, model 380A, and purified by electrophoresis on a 20% polyacrylamide/45% urea gel followed by elution of the DNA from a gel slice in 0.5 M ammonium acetate.

Southern Blotting and DNA Hybridization. Restriction endonuclease-digested DNA was transferred from agarose gels onto nitrocellulose by the method of Southern (21). For colony hybridization, bacterial colonies were transferred onto nitrocellulose filters that were prepared for hybridization according to the method of Grunstein and Hogness (22). Oligonucleotides were labeled with [γ-^{32}P]ATP (ICN) using polynucleotide kinase following the method of Maniatis *et al.* (13). Hybridization of labeled oligonucleotides to DNA on nitrocellulose filters was carried out overnight in 4× SSPE (1× SSPE is 0.18 M NaCl/10 mM NaH_2PO_4, pH 7.4/1 mM EDTA)/2× Denhardt's solution (13)/20 mM sodium pyrophosphate/0.2% $NaDodSO_4$ containing salmon sperm DNA at 50 μg/ml and with 10^6 cpm of labeled oligonucleotide per ml of hybridization buffer. Filters were washed with 1× SSC (0.15 M NaCl/0.015 M sodium citrate)/5 mM sodium pyrophosphate/0.1% $NaDodSO_4$. Hybridization and wash temperatures for each oligonucleotide were determined by the formula [2 × (A + T) + 4 × (G + C) − 10]°C (23). Thus, the temperatures used for oligonucleotides 1 and 2 (Fig. 1) were 46°C and 40°C, respectively. Filters were rinsed with 5× SSC, dried, and exposed to Kodak x-ray film.

RESULTS

Design of Synthetic Oligonucleotides and Hybridization with *N. gonorrhoeae* DNA. Using monoclonal antibodies to detect P.I epitopes, attempts to clone P.I in pBR322 or other vector plasmids in HB101 were repeatedly negative, and it was concluded that either gonococcal P.I is lethal for HB101 or the monoclonal antibodies did not recognize the protein when expressed in *E. coli*. Therefore oligonucleotides derived from the N-terminal amino acid sequence of P.I of strain R10 (P.IB) (2) were synthesized for use as hybridization probes to clone P.I gene sequences (Fig. 1). A limited amount of codon usage data for *N. gonorrhoeae* was obtained from the gene sequences of pilin (24) and protein II (J. Cannon, personal communication). Oligonucleotides NC1 and NC2 were designed based on this information, oligonucleotide NC1 as a unique sequence and oligonucleotide(s) NC2 as a mixed population to increase the probability of obtaining the correct sequence. In a colony hybridization assay, both these oligonucleotides hybridized to FA19 but not to HB101 containing the vector plasmids pBR322 or pGEM-2 (data not shown). Southern hybridization of genomic restriction digests of FA19 DNA probed with oligonucleotide NC1 showed hybridization to a single fragment in each case; the sizes of the relevant fragments were *Eco*RI, 10 kilobase pairs (kb); *Sal* I, 5.5 kb; *Sau*3AI, 900 base pairs (bp); *Taq* I, 750 bp (data not shown). Results were identical with oligonucleotides NC1 and NC2.

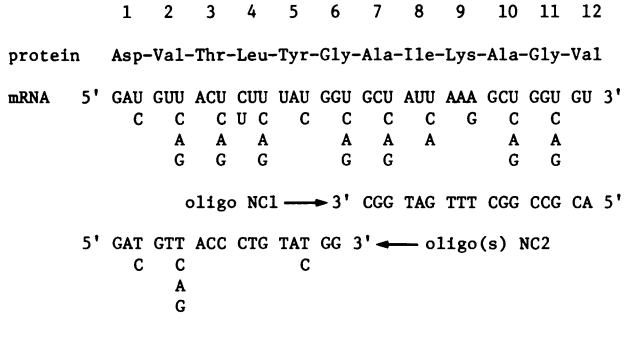

FIG. 1. Amino acid sequence of residues 1–12 of P.I of strain R10 (2), the encoding mRNA sequence including degenerate bases, and the oligonucleotides synthesized. Where degeneracy occurred, sequences were chosen based on codon usage data from other sequenced gonococcal genes (see text).

Cloning and Sequencing of the P.I Gene Fragments of FA19. When libraries of HB101 colonies containing *Eco*RI-digested or *Sal* I-digested FA19 DNA cloned into pBR322 were probed with oligonucleotide NC1 by colony hybridization, no positive colonies were found, further supporting the idea that P.I is lethal for *E. coli*. This suggested that the smaller oligonucleotide NC1-hybridizing fragments in the *Sau*3AI and *Taq* I digests of FA19 DNA, which presumably did not contain a complete P.I gene, would be more suitable candidates for cloning. Total FA19 DNA was digested to completion with *Sau*3AI, ligated with *Bam*HI-digested pGEM-2 DNA and transformed into HB101. Of ≈2000 transformants, one colony hybridized to oligonucleotide NC1. Plasmid DNA was prepared from this clone, digested with *Sau*3AI and probed with oligonucleotide NC1 by Southern hybridization. A single fragment of 900 bp, the same size as the *Sau*3AI genomic fragment of FA19 identified by Southern hybridization, hybridized with the oligonucleotide. This fragment was recovered from an agarose gel and religated into *Bam*HI-digested pGEM-2 to create the recombinant plasmid pUNC3 (Fig. 2). The same strategy was used to clone the 750-bp *Taq* I fragment of FA19 DNA that hybridized with oligonucleotide NC1 into the *Acc* I site of pGEM-2, resulting in formation of the recombinant plasmid pUNC11 (Fig. 2).

The DNA sequence of the 750-bp *Taq* I fragment was determined by first subcloning smaller (<300 bp) restriction fragments derived from the *Taq* I fragment into M13mp18 replicative form DNA (25) and sequencing these by the method of Sanger *et al.* (26). From this sequence a 17-nucleotide oligonucleotide was synthesized corresponding to the sequence just downstream of the *Sau*3AI site in the P.I gene (Fig. 2). This oligonucleotide, designated NC8, was used to identify by Southern hybridization an 850-bp *Sau*3AI fragment of FA19 DNA adjacent to the 900-bp fragment previously cloned, and this 850-bp *Sau*3AI fragment was cloned into pGEM-2 (to give pUNC15, Fig. 2) and sequenced using the strategies described above. The sequence of the P.I gene is shown in Fig. 3. The only large open reading frame in this sequence lies between bases 84 and 1062, which corresponds to a protein of 326 amino acids. From the published N-terminal amino acid sequence of various P.I proteins (27), the first residue of the mature protein presumably is aspartic acid at base 141, giving a mature protein of 307 amino acids and a signal peptide of 19 amino acids. The predicted

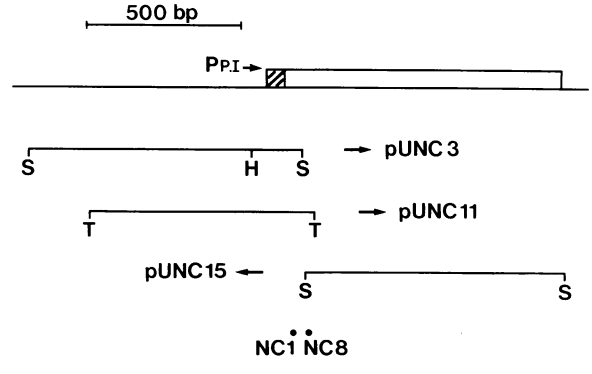

FIG. 2. *Sau*3AI and *Taq* I fragments of FA19 genomic DNA including portions of the P.I gene. Fragments were cloned into the vector plasmid pGEM-2, resulting in recombinant plasmids having the designations shown above. The relative position of the P.I coding region on the genome is indicated by the open box above (the shaded box corresponds to the N-terminal signal sequence) and the relative position of the oligonucleotide probes is shown below the fragments.

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                                    -35          -10          RBS          Met Lys Lys Ser Leu Ile Ala Leu Thr
AGCCCCTCGGCGGTAAATGCAAAGCTAAGCGGCTTGGAAAACCCGGCCTGCTTAAATTTCTTAACCAAAAAAGGAATACAGCA ATG AAA AAA TCC CTG ATT GCC CTG ACT  110
                                    HaeIII

Leu Ala Ala Leu Pro Val Ala Ala Met Ala Asp Val Thr Leu Tyr Gly Thr Ile Lys Ala Gly Val Glu Thr Ser Arg Ser Val Ala His
TTG GCA GCC CTT CCT GTT GCA GCA ATG GCT GAC GTT ACC CTG TAC GGC ACC ATC AAA GCC GGC GTA GAA ACT TCC CGC TCC GTA GCT CAC  200
—signal peptide ————————————

His Gly Ala Gln Ala Asp Arg Val Lys Thr Ala Thr Glu Ile Ala Asp Leu Gly Ser Lys Ile Gly Phe Lys Gly Gln Glu Asp Leu Gly
CAT GGA GCT CAG GCG GAT CGC GTT AAA ACC GCT ACC GAA ATC GCT GAT TTG GGT TCG AAA ATC GGC TTT AAA GGC CAA GAA GAC CTC GGC  290
          Sau3AI                                                TaqI

Asn Gly Leu Lys Ala Ile Trp Gln Leu Glu Gln Lys Ala Tyr Val Ser Gly Thr Asp Thr Gly Trp Gly Asn Arg Gln Ser Phe Ile Gly
AAC GGC CTG AAA GCC ATT TGG CAG TTG GAA CAA AAA GCC TAC GTC AGC GGT ACT GAC ACA GGC TGG GGC AAC CGC CAA TCC TTC ATC GGT  380

Leu Lys Gly Gly Phe Gly Lys Val Arg Val Gly Arg Leu Asn Ser Val Leu Lys Asp Thr Gly Gly Phe Asn Pro Trp Glu Gly Lys Ser
TTG AAA GGC GGC TTC GGT AAA GTG CGC GTC GGC CGT TTG AAC AGC GTC CTG AAA GAC ACC GGC GGC TTC AAT CCT TGG GAG GGT AAA AGC  470

Tyr Tyr Leu Gly Leu Ser Asn Ile Ala Gln Pro Glu Glu Arg His Val Ser Val Arg Tyr Asp Ser Pro Glu Phe Ala Gly Phe Arg Ala
TAC TAT TTG GGT TTA AGC AAC ATT GCC CAA CCC GAA GAA CGC CAC GTT TCC GTA CGC TAC GAT TCT CCC GAA TTT GCC GGC TTC AGG GCA  560

Val Gln Tyr Val Pro Asn Asp Asn Ser Gly Lys Asn His Ser Glu Ser Tyr His Ala Gly Phe Asn Tyr Lys Asn Ser Gly Phe Phe Val
GTG CAA TAC GTG CCT AAC GAC AAT TCG GGC AAA AAT CAC AGC GAA TCT TAC CAT GCA GGC TTC AAC TAC AAA AAC AGC GGC TTC TTC GTG  650

Gln Tyr Ala Gly Phe Tyr Lys Arg His Ser Tyr Thr Thr Glu Lys His Gln Val His Arg Leu Val Gly Gly Tyr Asp His Asp Ala Leu
CAA TAT GCC GGC TTC TAT AAA AGA CAT AGT TAC ACG ACT GAG AAA CAC CAG GTT CAC CGT TTG GTC GGC GGT TAC GAC CAT GAT GCC CTG  740

Tyr Ala Ser Val Ala Val Gln Gln Gln Asp Ala Lys Leu Thr Trp Arg Asn Asp Asn Ser His Asn Ser Gln Thr Glu Val Ala Ala Thr
TAC GCT TCC GTA GCC GTA CAG CAA CAA GAC GCG AAA TTG ACT TGG CGC AAC GAT AAT TCG CAC AAC TCT CAA ACC GAA GTT GCC GCT ACC  830

Ala Ala Tyr Arg Phe Gly Asn Val Thr Pro Arg Val Ser Tyr Ala His Gly Phe Lys Gly Ser Val Tyr Asp Ala Asp Asn Asp Asn Thr
GCG GCA TAC CGC TTC GGC AAC GTA ACG CCC CGC GTT TCT TAC GCC CAC GGC TTC AAA GGT TCG GTT TAT GAT GCA GAT AAC GAC AAT ACT  920

Tyr Asp Gln Val Val Val Gly Ala Glu Tyr Asp Phe Ser Lys Arg Thr Ser Ala Leu Val Ser Ala Gly Trp Leu Gln Arg Gly Lys Gly
TAC GAC CAA GTG GTT GTC GGT GCG GAA TAC GAC TTC TCC AAA CGC ACT TCT GCC TTG GTT TCT GCC GGT TGG TTG CAA AGA GGC AAA GGC 1010

Thr Glu Lys Phe Val Ala Thr Val Gly Gly Val Gly Leu Arg His Lys Phe ---
ACA GAA AAA TTC GTA GCG ACT GTC GGC GGT GTC GGT CTG CGC CAC AAA TTC TAA TCTGCAAAGATC 1076
                                                                    Sau3AI
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FIG. 3. DNA sequence of the P.I gene of FA19. The predicted amino acid sequence is shown above the DNA sequence, with the signal peptide indicated. The putative promoter sequences (−35 and −10) and the ribosome binding site (RBS) are shown, as are the *Taq* I and *Sau*3AI sites and the *Hae* III site used in the construction of pUNC7 (see Fig. 5). The last base number of each line is shown on the right.

molecular weight of the protein is 33,786, which is close to the apparent molecular weight of 34,000 for P.I in FA19. The signal peptide appears to have the common characteristics associated with such sequences (28), with a stretch of hydrophobic amino acids, an abundance of alanine residues, and an Ala-Xaa-Ala cleavage site. There are also putative −35 and −10 promoter sequences, which are close in sequence and separation to the consensus for these sequences in *E. coli* (29) and a Shine–Dalgarno ribosomal binding site (30) just upstream of the first residue of the signal sequence. The predicted N-terminal amino acid sequence matches that determined by amino acid sequencing of a putative P.IA protein (27) and is similiar to that of the P.IB protein of R10 (2). The hydropathy profile of the predicted protein is typical of that of an outer membrane porin protein and compares favorably with the major porins of *E. coli*, OmpF and OmpC (Fig. 4), characterized by long hydrophilic regions without any substantial hydrophobic stretches. There is little correlation with the hydropathy profiles of other sequenced gonococcal outer membrane proteins (Fig. 4).

Construction of a P.I Gene Clone and Its Expression in *E. coli*. Attempts to recover an intact P.I gene clone by ligating together the two portions of the gene on the *Sau*3AI fragments of pUNC3 and pUNC15 failed repeatedly, and it was concluded that the expression of gonococcal P.I is lethal for *E. coli*. Therefore, a recombinant plasmid, pUNC7, was constructed such that a portion of the P.I gene promoter was removed and the P.I gene was positioned downstream of the phage T7 promoter on pGEM-2 (Fig. 5). *E. coli* cells do not normally contain T7 polymerase, which transcribes genes downstream from T7 promoters, and so such a plasmid should be stably maintained in *E. coli* without expression of the P.I gene. HB101 containing pUNC7 expressed no detectable P.I in a colony radioimmunoassay. Plasmid pUNC7 was transformed into *E. coli* strain BL21(DE3), a lysogen in which the phage T7 polymerase gene is present but under control of the *lac*UV5 promoter (14). When grown without iPrSGal, BL21(DE3) harboring plasmid pUNC7 produced no detectable P.I but, when T7 polymerase production was induced by growth on medium containing iPrSGal, expression of P.I was detected by the P.IA monoclonal antibodies in a colony blot radioimmunoassay (data not shown). The P.I produced by this *E. coli* strain showed the same immunological reaction as that of FA19 P.I, in that it reacted with all six P.IA monoclonal antibodies singly in colony radioim-

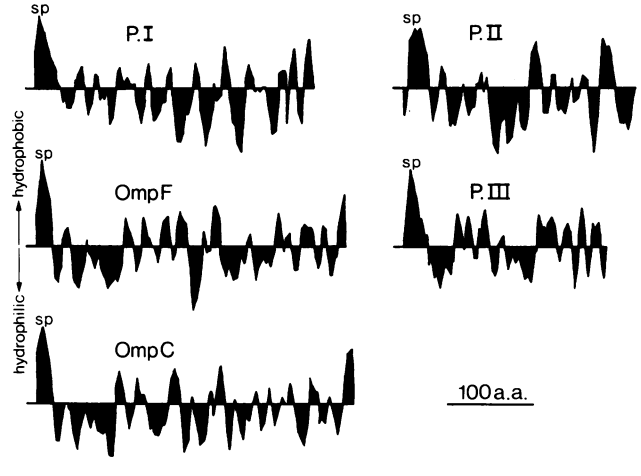

FIG. 4. Hydropathy patterns of the major outer membrane proteins of *N. gonorrhoeae*, P.I, P.II (J. Cannon, personal communication), and P.III (31) and of the *E. coli* porins OmpF and OmpC (32). sp, Signal peptide; a.a., amino acid residues.

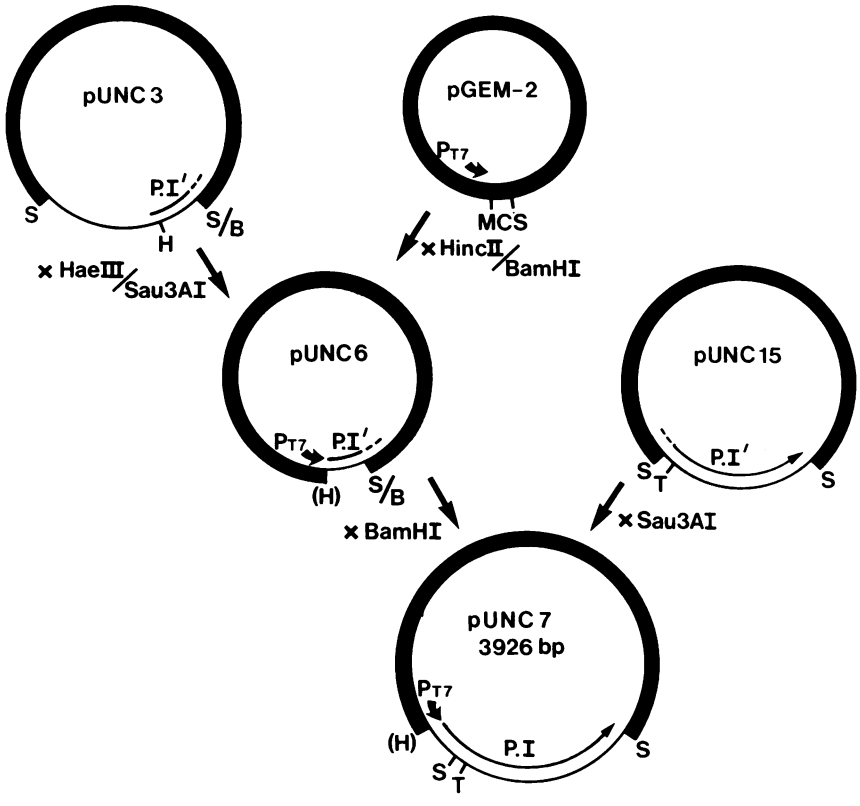

FIG. 5. Scheme for construction of pUNC7. A convenient *Hae* III site between the −35 and −10 regions of the P.I gene promoter (see Fig. 3) allowed removal of the −35 region and the upstream sequence, followed by reconstruction of the P.I gene under the control of the phage T7 promoter on the vector plasmid pGEM-2. The thick line represents pGEM-2 DNA and the thin line represents FA19 DNA. P.I', portion of the P.I gene (the dotted line indicates the direction of the missing segment); P_{T7}, phage T7 promoter; MCS, multiple cloning site; restriction enzyme sites: S, *Sau*3AI; H, *Hae* III; B, *Bam*HI; (H), *Hae* III/*Hin*cII junction (no site); T, *Taq* I.

munoassays. The protein presumably was efficiently exported through the inner membrane of the *E. coli* clone since it was detectable by colony radioimmunoassay without lysis of the cells. The protein was of equivalent apparent molecular weight to FA19 P.I as detected by $NaDodSO_4$/polyacrylamide gel electrophoresis (Fig. 6*A*) and immunoblotting (Fig. 6*B*) and apparently was the most abundant protein produced by the *E. coli* clone after overnight growth in the presence of iPrSGal (Fig. 6*A*). However, the production of this protein during overnight growth was lethal for this *E. coli* strain,

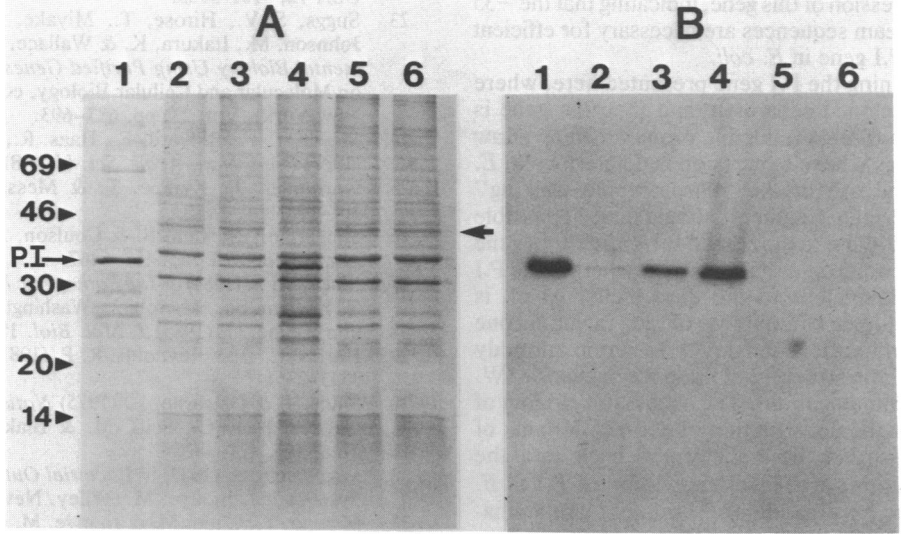

FIG. 6. Expression of the P.I gene on pUNC7 in BL21(DE3). (*A*) $NaDodSO_4$/polyacrylamide gel electrophoresis of whole cell lysates on a 15% gel, stained with Coomassie blue. Sizes of marker proteins (bovine serum albumin, 69 kDa; ovalbumin, 46 kDa; carbonic anhydrase, 30 kDa; chymotrypsin, 20 kDa; lysozyme, 14 kDa) are shown to the left. P.I is indicated, and the arrow in the center indicates a protein that is apparently missing in lane 4. (*B*) Autoradiograph of an immunoblot of the gel in *A* probed with six P.IA monoclonal antibodies. Lanes: 1, FA19; 2, BL21(DE3)pUNC7 grown 16 hr without iPrSGal; 3, BL21(DE3)pUNC7 grown 3 hr with iPrSGal; 4, BL21(DE3)pUNC7 grown 16 hr with iPrSGal; 5, BL21(DE3)pGEM-2 grown 16 hr without iPrSGal; 6, BL21(DE3)pGEM-2 grown 16 hr with iPrSGal.

since no viable cells were recovered from this culture, confirming the assumption that expression of P.I in *E. coli* could not be stably maintained. The expression of one of the major *E. coli* proteins apparently was greatly reduced in this clone after overnight growth under inducing conditions (Fig. 6*A*). If this protein is a membrane protein, this effect may reflect competition for sites in the membrane or a change in the osmotic conditions for the cells that alters the membrane protein profile.

DISCUSSION

The sequence of the gene encoding the P.I of *N. gonorrhoeae* strain FA19 predicts a protein having characteristics typical of Gram-negative bacterial outer membrane porin proteins. Prolonged expression of P.I apparently was a lethal event for the *E. coli* strain used in this study, though the reason for this is unclear. The effect may be due to osmotic destabilization of the *E. coli* cells, either by the activity of the gonococcal porin itself or by the displacement of the *E. coli* porins, or it may be due to a change in the structural integrity of the outer membrane complex when an abundance of the foreign protein is present. The latter seems especially possible since *E. coli* is known to regulate the total amount of its major porins, OmpF and OmpC, in its outer membrane (33). It is possible that the expression of P.I would not be lethal for an *E. coli* strain lacking its own major outer membrane porins, but this possibility has not been tested. In other experiments, it was observed that subclones of the entire P.IA insert from pUNC7 into another plasmid vector, pHSS6 (34), resulted in mucoid "sick" colonies of HB101, presumably because of weak expression of P.IA from a promoter on this vector.

The amino acid sequences of FA19 P.IA, deduced from the DNA sequence here, and of P.IB from R10 (27, 35) are similar through the first 18 residues at the N-terminus but are different after residue 18. This suggests a common function for the N terminus of the protein. Using similar methods to clone and sequence other gonococcal P.I genes, one could compare the structure of P.IA to those of P.IB and of P.IA/P.IB hybrids constructed by genetic transformation (19) and thus perhaps understand more clearly the structure and function of this protein. It is interesting to note the specificity of the gonococcal promoter sequences recognized in *E. coli*. Removal of just the −35 region of the P.I gene promoter resulted in complete lack of expression of this gene, indicating that the −35 region or other upstream sequences are necessary for efficient transcription of the P.I gene in *E. coli*.

The method of cloning the P.I gene presented here, where gene fragments are cloned separately and then the gene is reconstructed with a foreign inducible promoter, may allow cloning of other genes whose expression is deleterious to *E. coli*. In addition, the use of the "chromosome walking" method of cloning adjacent fragments should make it possible to identify genes proximate to the cloned P.IA structural gene on the gonococcal genome and thus establish whether the P.I structural gene is equivalent to the *nmp* locus, which is known to be closely linked to a number of loci, including one (*sac-3*) that is known to affect sensitivity to serum antibody and complement and the structure of lipopolysaccharide (W. Shafer, personal communication). The successful cloning of a P.I gene will allow the construction of defined mutants of the gene, which can then be transformed back into the gonococcus. In addition, high-level expression of P.I in *E. coli* strains will allow purification of P.I without contamination by other gonococcal proteins, which may be important for a variety of studies, including development of a vaccine based on P.I.

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1. Douglas, J. T., Lee, M. D. & Nikaido, H. (1981) *FEMS Microbiol. Lett.* **12,** 305–309.
2. Blake, M. S. & Gotschlich, E. C. (1982) *Infect. Immun.* **36,** 277–285.
3. Hancock, R. E. W. (1987) in *Bacterial Outer Membranes as Model Systems*, ed. Inouye, M. (Wiley, New York), pp. 187–225.
4. Blake, M. S. & Gotschlich, E. C. (1987) in *Bacterial Outer Membranes as Model Systems*, ed. Inouye, M. (Wiley, New York), pp. 377–400.
5. Sarafian, S. K., Tam, M. R. & Morse, S. A. (1983) *J. Infect. Dis.* **148,** 1025–1032.
6. Buchanan, T. M. & Hildebrandt, J. F. (1981) *Infect. Immun.* **32,** 985–994.
7. Tam, M. R., Buchanan, T. M., Sandstrom, E. G., Holmes, K. K., Knapp, J. S., Siadak, A. W. & Nowinski, R. C. (1982) *Infect. Immun.* **36,** 1042–1053.
8. Blake, M. S., Gotschlich, E. C. & Swanson, J. (1981) *Infect. Immun.* **33,** 212–222.
9. Cannon, J. G., Klapper, D. G., Blackman, E. Y. & Sparling, P. F. (1980) *J. Bacteriol.* **143,** 847–851.
10. Cannon, J. G. & Sparling, P. F. (1984) *Annu. Rev. Microbiol.* **38,** 111–133.
11. Ganss, M. T., Buchanan, T. M., Kohl, P. K. & Meyer, T. F. (1985) in *The Pathogenic Neisseriae*, ed. Schoolnik, G. K. (Am. Soc. Microbiol., Washington, DC), pp. 130–131.
12. Maness, M. J. & Sparling, P. F. (1973) *J. Infect. Dis.* **128,** 321–330.
13. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
14. Studier, F. W. & Moffatt, B. A. (1986) *J. Mol. Biol.* **189,** 113–130.
15. Kellogg, D. S., Jr., Peacock, W. L., Jr., Deacon, W. E., Brown, L. & Pirkle, C. I. (1963) *J. Bacteriol.* **85,** 1274–1279.
16. Cannon, J. G., Black, W. J., Nachamkin, I. & Stewart, P. W. (1984) *Infect. Immun.* **43,** 994–999.
17. Burnette, W. N. (1981) *Anal. Biochem.* **112,** 195–203.
18. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
19. Danielsson, D., Faruki, H., Dyer, D. & Sparling, P. F. (1986) *Infect. Immun.* **52,** 529–533.
20. Stern, A., Nickel, P., Meyer, T. F. & So, M. (1984) *Cell* **37,** 447–456.
21. Southern, E. (1975) *J. Mol. Biol.* **98,** 503–517.
22. Grunstein, M. & Hogness, D. S. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3961–3965.
23. Suggs, S. V., Hirose, T., Miyake, T., Kawashima, E. H., Johnson, M., Itakura, K. & Wallace, R. B. (1981) in *Developmental Biology Using Purified Genes*, ICN–UCLA Symposia on Molecular and Cellular Biology, ed. Brown, D. (Academic, New York), Vol. 23, pp. 683–693.
24. Meyer, T. F., Billyard, E., Haas, R., Storzbach, S. & So, M. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6110–6114.
25. Norrander, J., Kempe, T. & Messing, J. (1983) *Gene* **26,** 101–106.
26. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
27. Blake, M. S. (1985) in *The Pathogenic Neisseriae*, ed. Schoolnik, G. K. (Am. Soc. Microbiol., Washington, DC), pp. 251–258.
28. von Heijne, G. (1985) *J. Mol. Biol.* **184,** 99–105.
29. Harley, C. B. & Reynolds, R. P. (1987) *Nucleic Acids Res.* **15,** 2343–2361.
30. Shine, J. & Dalgarno, L. (1975) *Nature (London)* **254,** 34–38.
31. Gotschlich, E. C., Seiff, M. & Blake, M. S. (1987) *J. Exp. Med.* **165,** 471–482.
32. Mizushima, S. (1987) in *Bacterial Outer Membranes as Model Systems*, ed. Inouye, M. (Wiley, New York), pp. 163–185.
33. Mizuno, T., Chou, M. & Inouye, M. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1966–1970.
34. Seifert, H. S., So, M. & Heffron, F. (1986) in *Genetic Engineering, Principles and Methods*, eds. Setlow, J. K. & Hollaender, A. (Plenum, New York), Vol. 8, pp. 123–134.
35. Mietzner, T. A. & Morse, S. A. (1985) in *The Pathogenic Neisseriae*, ed. Schoolnik, G. K. (Am. Soc. Microbiol., Washington, DC), pp. 406–414.