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Isolation, Cloning, and Expression of a 70-Kilodalton Plasminogen Binding Protein of *Borrelia burgdorferi*

LINDEN T. HU,[1]* STEVEN D. PRATT,[2] GEORGE PERIDES,[1] LEONARD KATZ,[2] RICK A. ROGERS,[3] AND MARK S. KLEMPNER[1]

Tupper Research Institute, Division of Geographic Medicine and Infectious Diseases, Tufts University School of Medicine, New England Medical Center, Boston, Massachusetts 02111[1]; Abbott Laboratories, Abbott Park, Illinois 60064[2]; and Harvard School of Public Health, Boston, Massachusetts 02115[3]

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Surface receptors for plasminogen are expressed by many gram-positive and gram-negative bacteria and may play a role in the dissemination of organisms by binding plasminogen, which upon conversion to plasmin can digest extracellular matrix proteins. Two plasminogen binding proteins have been identified for *Borrelia burgdorferi*, outer surface protein A and a 70-kDa protein (BPBP). We purified BPBP by plasminogen affinity chromatography and obtained its amino acid sequence by Edman degradation of a tryptic digest. The gene coding for BPBP was isolated from a λ-ZAP II genomic library with probes developed from sequenced portions of the protein. This gene was expressed in *Escherichia coli*; the recombinant product was seen by antibody raised against native BPBP and also bound ^{125}I-labeled plasminogen. The experimentally derived amino acid sequences corresponded to the predicted sequence encoded by the BPBP gene. The deduced amino acid sequence for BPBP revealed significant similarity to p30, a 30-kDa protein of *B. burgdorferi* (54% identity and 65% similarity), to a 60-kDa protein in *Borrelia coriaceae* (66% identity and 80% similarity), to oligopeptide binding protein A of *E. coli* (34% identity and 57% similarity), and, more generally, to the periplasmic oligopeptide binding family of proteins.

We have previously shown that *Borrelia burgdorferi*, the causative agent of Lyme disease, binds the human fibrinolytic proteins plasmin(ogen) and urokinase (17). Unlike other bacteria which spread in skin, *B. burgdorferi* does not appear to secrete any endogenous proteases. Thus, acquisition of host plasmin, a serine protease with broad substrate specificity, would be an important strategy for giving the organism a mechanism to digest extracellular matrix. In vitro, plasmin bound to the surface of *B. burgdorferi* enhances the penetration of the organism through endothelial cell monolayers grown on connective tissue substrates compared with that of untreated controls (7). The ability to bind plasmin(ogen) rather than utilize free plasmin is important because free plasmin in serum is quickly inactivated by inhibitors such as α_1- and α_2-antiplasmin. Plasmin bound to the surface of a cell or to fibrin is stabilized and protected against inactivation (9, 21, 24).

Studies of the kinetics of plasminogen binding to *B. burgdorferi* are consistent with the presence of two binding sites: a high-affinity and a low-affinity site (7). After our initial description of plasminogen binding to *B. burgdorferi* (18, 19), outer surface protein A (OspA), a 30-kDa borrelial surface protein, was reported to bind plasminogen (10). Interestingly, studies of OspA expression in mouse models of Lyme disease have shown that expression is down regulated almost immediately after a blood meal by the tick vector and OspA is expressed minimally if at all at the time of early infection in the mouse (28). This would suggest the need for an alternative in vivo plasmin(ogen) binding site, since mutant *B. burgdorferi* devoid of OspA binds amounts of plasminogen equal to those bound by wild-type spirochetes (15).

A reasonable candidate for the physiologic plasminogen binding protein would be the 70-kDa borrelial plasminogen binding protein (BPBP) found in both wild-type and OspA-deficient *B. burgdorferi*. This protein has a higher affinity for plasminogen than does OspA (15). In the present study, we describe the purification of BPBP and determination of the nucleotide sequence of the gene encoding this protein.

MATERIALS AND METHODS

Borrelial isolates and cultivation. *B. burgdorferi* was grown at 32°C in modified Barbour-Stoenner-Kelly (BSK II) medium prepared as previously described (2, 17). *B. burgdorferi* (sensu stricto) strains used in these experiments were NECK (17) and 7x297 (22). 7x297 is a mutant strain of *B. burgdorferi* which is missing the 48-kb plasmid containing the coding sequences for OspA and -B. Non-*B. burgdorferi* (sensu stricto) strains used included the pKo strain of *Borrelia afzelii* (4) and the pBi strain of *Borrelia garinii* (4) and *Borrelia coriaceae* (25).

Reagents. Unless otherwise noted, all reagents were purchased from Sigma Chemical Co. (St. Louis, Mo.). Bacterial culture media other than BSK II were purchased from Gibco BRL (Grand Island, N.Y.). Restriction enzymes were purchased from Gibco BRL.

Purification of BPBP. Detergent phase extracts of *B. burgdorferi* were prepared as previously described (15). Prior to plasminogen affinity purification, detergent phase proteins were separated from Triton X-114 by ammonium sulfate fractionation. The precipitated proteins were resuspended in H_2O and then dialyzed overnight in a molecularporous dialysis membrane (Spectra/Por 1; Spectrum Medical Industries, Inc., Los Angeles, Calif.) against H_2O.

Human glu-plasminogen (American Diagnostica, Greenwich, Conn.) was coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia Biotech, Uppsala, Sweden) according to the manufacturer's instructions. The coupling efficiency of 10 mg of plasminogen to 2 ml of Sepharose was >95%. Detergent phase proteins were batch adsorbed to the plasminogen-Sepharose column for 2 h at 4°C. The column was then washed with 0.4 M phosphate buffer (pH 7.3) until the absorbance reached less than 0.05 at 280 nm. Proteins bound to plasminogen were eluted with 0.2 M ε-aminocaproic acid in phosphate buffer.

Purified protein was checked by ligand blotting for the ability to bind ^{125}I-labeled plasminogen as described previously (15).

Screening of human sera for antibody to BPBP. Sera from patients with Lyme disease were obtained from the Centers for Disease Control National Lyme Disease Reference Serobank. Western blots with either purified BPBP or detergent phase proteins of *B. burgdorferi* run on sodium dodecyl sulfate (SDS)-polyacrylamide gels were performed as described previously (15) with the following changes. Polyvinylidene difluoride strips (Immobilon-P; Millipore, Bedford, Mass.) containing transferred proteins were blocked for 30 min in phosphate-buffered saline (PBS) containing 3% milk. Human serum was diluted 1:1,000 in PBS containing 1% milk prior to incubation with the strips. Alkaline-

* Corresponding author.

phosphatase-conjugated anti-human antibody was obtained from Promega, Madison, Wis.

Preparation of polyclonal antibody to BPBP. One hundred micrograms of purified protein was electrophoresed in a 10 to 15% gradient polyacrylamide gel. The completed gel was stained for 30 min with Coomassie blue and then destained overnight in 20% methanol and 10% acetic acid. The gel was allowed to equilibrate in distilled H_2O at 4°C for 24 h. The BPBP-containing region was cut from the gel and mechanically sheared into >1-mm-square pieces. The BPBP was then injected into a pathogen-free New Zealand White rabbit. A booster vaccination was done after 28 days. Serum was collected from the rabbit prior to immunization and every 2 weeks, beginning two weeks after the booster injection. Immune serum identified a single 70-kDa protein on Western blotting of detergent phase proteins of *B. burgdorferi* which was not identified by preimmune serum. Immune serum also recognized plasminogen-Sepharose affinity-purified BPBP.

Fluorescent surface staining. Surface expression of BPBP was determined by indirect immunofluorescence. Two milliliters of cultured spirochetes (7x297; 10^7 spirochetes) was harvested and then washed three times with Dulbecco's saline solution (DBSS) containing 0.01% bovine serum albumin (BSA). The spirochetes were then fixed in 500 μl of 2% paraformaldehyde for 30 min. The bacteria were spun down, resuspended in DBSS–0.01% BSA, and placed on slides in a cytospin chamber. The slides were allowed to air dry. Antibody to BPBP was added at a dilution of 1:10 and allowed to incubate for 1 h. The area was washed three times with DBSS–0.01% BSA. Fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit antibody was added at a dilution of 1:200 and allowed to incubate for 1 h. The slide was then washed three times with DBSS–0.01% BSA and viewed by confocal laser scanning microscopy as previously described (17).

Amino acid analysis and sequencing. Detergent phase extraction of borrelial membrane proteins (NECK; 10^{10} spirochetes) was performed as described above. Two-dimensional gels were run on the detergent phase proteins of solubilized *B. burgdorferi* by the method described by Celis and Bravo (3) and Hu et al. (15). The completed gels were transferred electrophoretically to polyvinylidene difluoride membranes (Millipore) (31).

Amino acid sequencing and in situ digestion with trypsin were performed by the Harvard Microchemistry Laboratory, Cambridge, Mass., as follows. Protein bands were stained with 0.1% Ponceau S in 1% acetic acid for 60 s followed by destaining with 1% acetic acid. After several washes with H_2O the bands of interest were cut out and subjected to amino acid analysis to determine the amount of protein transferred to the membrane.

For in situ digestion, the polyvinylidene difluoride membrane was cut into 1- by 2-mm pieces and placed in a solution containing 100 μl of 10% acetonitrile, 1% Triton X-100, 100 mM Tris-HCl (pH 8.0), and 0.5 μg of trypsin previously reduced and methylated to prevent autolytic digestion and treated with *N*-tosyl-L-phenylalanine chloromethyl ketone to remove any chymotrypsin-like activity (Promega). The incubation at 37°C was allowed to proceed for 24 h. The resulting peptide mixture was separated by narrow-bore high-pressure liquid chromatography (HPLC) with a 2.1- by 150-mm Vydac C-18 reverse-phase column on a Hewlett-Packard 1090 HPLC with 1040 diode array detector. Separation of the peptides was achieved by HPLC with a gradient of 5 to 90% acetonitrile in H_2O with 0.06% trifluoracetic acid over a period of 90 min. Optimum fractions were chosen based on symmetry, resolution, and UV absorbance and submitted to automated Edman degradation on an Applied Biosystems Model 477A protein sequencer. Strategies for peak selection, reverse-phase separation, and protein microsequencing have been described previously (20). All sequenator reagents and solvents were from Applied Biosystems, Inc.

Preparation of *B. burgdorferi* (NECK) libraries. Borrelial DNA was prepared as described previously (29). The genomic library was prepared commercially (Stratagene, La Jolla, Calif.). Two hundred micrograms of DNA (*B. burgdorferi* NECK) was mechanically sheared to generate fragments approximately 6 to 10 kb in length. The DNA was then blunted with S1 nuclease, and the *Eco*RI sites on the fragments were methylated with *Eco*RI methylase. *Eco*RI linkers were ligated to the ends of the DNA, and the fragments were digested with *Eco*RI. Following purification through a sucrose gradient, the fragments were packaged into the λ-ZAP II vector with Gigapack II Gold packaging extract (Stratagene).

Size-restricted minilibraries were prepared by digesting genomic *B. burgdorferi* (NECK) DNA with various restriction enzymes. After electrophoresis on 1% agarose gels and transfer to nylon membranes, fragments of interest were identified by hybridization with ^{32}P-labeled probes. An area including 1 kb above and below the identified fragment was cut from low-melting-point 1% agarose gels, and the DNA was extracted with a QiaexII gel extraction kit (Qiagen, Chatsworth, Calif.). The extracted DNA was ligated into an appropriately digested and dephosphorylated Bluescript II KS− vector with DNA ligase (Pharmacia, Piscataway, N.J.) as described previously (26).

PCR preparation of BPBP DNA probe. Using amino acid sequences we obtained for BPBP as described above, we designed degenerate oligonucleotide primers containing restriction sites for *Eco*RI and *Bam*HI (sense primer, 5′-GGCGAATTCAAYGCNGARGARTAYTTYGAYGGNAAR, with a degeneracy of 2,048-fold; antisense primer, 5′-GCGGATCCYTTNCCRTANSWRTARTCRTCRAA, with a degeneracy of 1,536-fold). Hot-start PCR amplification was done with *Taq* polymerase (Gibco BRL) and *B. burgdorferi* (NECK) genomic DNA as the template. The amplification profile for the first five cycles was 94°C for 30 s, 49°C for 30 s, and 72°C for 45 s. The following 34 cycles were carried out with denaturation at 94°C for 30 s, annealing at 62°C for 30 s, and extension at 72°C for 45 s. A final cycle at 72°C for 5 min completed the amplification.

The PCR product was ligated into a pNoTA/T7 plasmid (5 Prime→3 Prime, Inc., Boulder, Colo.). Extraction of plasmid DNA containing the insert was performed with the QIAprep spin plasmid miniprep kit (Qiagen) according to the manufacturer's instructions. The DNA insert was digested with *Eco*RI and *Bam*HI (10 U each; New England Biolabs, Beverly, Mass.) and isolated from a 1% low-melting-point agarose gel.

^{32}P labeling of the PCR product was done with the Megaprime 1604 DNA labeling system (Amersham, Arlington Heights, Ill.) according to the manufacturer's instructions. The final product was centrifuged at 1,100 × *g* in a Sephadex G-50 spin column (Boehringer-Mannheim, Indianapolis, Ind.) to remove unincorporated deoxynucleoside triphosphates.

Screening of the *B. burgdorferi* library. Plating of the λ-ZAP II library with *Escherichia coli* XL1-Blue cells (Stratagene) and transfer to nylon membranes was done according to standard procedures (26).

Prehybridization of membranes was carried out for 2 h at 42°C in a solution containing 6× SSC buffer (0.9 M sodium chloride, 0.09 M sodium citrate [pH 7.0]), 20 mM NaH_2PO_4, 0.4% (wt/vol) SDS, 5× Denhardt's reagent, and denatured sonicated salmon sperm DNA (500 μg/ml). Hybridization with the ^{32}P-labeled PCR product was carried out overnight at 65°C in a solution of 6× SSC buffer, 20 mM NaH_2PO_4, 0.4% (wt/vol) SDS, 10 μM EDTA, and denatured sonicated salmon sperm DNA (500 μg/ml). Membranes were washed twice in 2× SSC with 0.1% SDS at 37°C followed by two washes at 60°C. They were then wrapped in plastic wrap and exposed to film (X-Omat AR; Eastman Kodak Co., Rochester, N.Y.) overnight at −70°C with intensifying screens. Reactive plaques were isolated and purified through one additional screening.

DNA sequencing. DNA sequencing was performed by dideoxy chain termination with the 70770 Sequenase kit version 2.0 (Amersham) with [α-^{33}P]dATP according to the manufacturer's instructions. Sequences were determined by using the universal forward primer supplied with the kit, and sequence-specific oligonucleotides were synthesized on a DNA synthesizer.

Expression cloning of BPBP. The gene for BPBP was cloned by first designing primers for PCR containing bp 1 to 25 of the open reading frame (ORF) with an *Nde*I site on the sense primer and bp 1546 to 1571 of the ORF with an *Eco*RI site on the antisense primer. The PCR was done with a GeneAmp XL PCR kit (Perkin-Elmer, Branchburg, N.J.) with the following temperature-cycling conditions: one cycle of denaturation at 94°C for 2 min followed by four cycles of denaturation at 94°C for 15 s, annealing at 47°C for 20 s, and extension at 72°C for 80 s; then 28 cycles of denaturation at 94°C for 15 s and annealing and extension at 67°C for 70 s; and finally one cycle of extension at 72°C for 3 min. The product was ligated into the plasmid pET28c(+) (Novagen, Madison, Wis.). This plasmid was designated pHPR-2 and was cotransformed into *E. coli* BL21(DE3) (Novagen) along with pOM61, a pACYC184 derivative containing the *argU* gene, which encodes a tRNA for arginine that reads the rare codons AGA and AGG. The amplified regions were sequenced to ensure that no mutations were introduced during PCR.

Immunoblot analysis of BPBP. Detergent phase proteins of various strains of *B. burgdorferi* and *B. coriaceae* or Triton X-114-solubilized proteins of *E. coli* (XL1-Blue), both with and without the BPBP insert, were electrophoresed in preparative 10% polyacrylamide gels. The separated proteins were transferred electrophoretically to polyvinylidene difluoride membranes (Millipore). The membranes were blocked with 3% milk–PBS and then incubated in sera raised against BPBP diluted 1:100 in 1% milk–PBS. After 1 h, the strips were washed three times with 1% milk–PBS. They were subsequently incubated for 1 h with alkaline phosphatase-conjugated anti-rabbit immunoglobulin (Promega). After being washed three times with 1% milk–PBS, the strips were developed with a 5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium reagent (Vector Laboratories, Inc., Burlingame, Calif.).

Nucleotide sequence accession number. The complete nucleotide sequence of the BPBP gene has been submitted to the GenBank database with the accession no. AF005657.

RESULTS

Purification of BPBP. The focus of this study was to isolate and characterize the 70-kDa plasminogen binding protein of *B. burgdorferi*. Due to the existence of a second plasminogen binding protein, OspA, we chose to use the OspA-deficient mutant 7x297 as the starting material for the isolation. By plasminogen-Sepharose affinity purification of membrane proteins which had been separated from *B. burgdorferi* by Triton X-114 detergent phase extraction followed by an ammonium sulfate precipitation, we were able to obtain BPBP which was >90% pure by scanning densitometry (Bio-Rad GS-700 imaging densitometer) of Coomassie blue-stained gels (Fig. 1). The

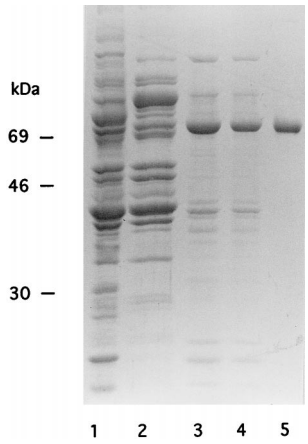

FIG. 1. Plasminogen-Sepharose affinity purification of BPBP. Lane 1, whole-cell lysates of *B. burgdorferi*; lane 2, total proteins of the spirochetes solubilized in Triton X-114; lane 3, the same proteins after detergent phase separation; lane 4, product of lane 3 subjected to ammonium sulfate precipitation to separate proteins from Triton X-114; lane 5, purified protein after ε-aminocaproic acid elution of adsorbed ammonium sulfate-precipitated proteins from a plasminogen-Sepharose affinity column.

purified protein also bound ^{125}I-labeled plasminogen as shown by ligand blotting (data not shown).

Humoral immune response to BPBP in Lyme disease patients. We evaluated sera from 25 patients with well-characterized Lyme disease from a panel of patients submitted to the National Lyme Disease Reference Serobank. Eight percent (1 of 12) of the patients with acute Lyme disease and 38% (5 of 12) of the patients with chronic Lyme disease (arthritis or neuroborreliosis) had immunoglobulin G (IgG) antibodies to BPBP. By comparison, 0% (0 of 13) and 31% (4 of 13) of these same patients with acute and chronic Lyme disease, respectively, had IgG antibodies to OspA. Serum from non-Lyme disease patients and reactions with secondary antibody alone did not show reactivity with either BPBP or OspA.

Surface staining of *B. burgdorferi* for BPBP. Organisms incubated with antibody raised against BPBP showed labeling over the entire length of the organism, which was not seen in organisms incubated with preimmune serum (Fig. 2). This finding confirms that BPBP is expressed on the surface of the spirochete.

DNA sequencing and characterization of the BPBP gene. Amino acid sequences were obtained for four peptides generated by tryptic digest of BPBP from the strain NECK (NAQEYFDET, YGENWTNPENIVVSGAYK, IRDDYYS GLK, and FDDYSYGK). Degenerate oligonucleotide primers for the NAQEYFDET and FDDYSYGK peptides were synthesized (see Materials and Methods). PCR was performed with these primers and DNA from strain NECK as a template, which produced a single 615-bp product.

To locate the complete gene, the PCR product was used as a probe to screen a λ-ZAP II *B. burgdorferi* (NECK) genomic library as described in Materials and Methods. Using this strategy, we selected clones containing the partial gene for sequencing. The sequence encoding the carboxyl terminus of the protein was not found in any of the clones. The remainder of the sequence was identified by digesting genomic *B. burgdorferi* (NECK) DNA with *Eco*RV. After electrophosesis on a 1% agarose gel, the product was hybridized to ^{32}P-labeled probe specific for previously identified portions of the sequence. Hybridization to a 1.6-kb DNA fragment was seen. This fragment was excised from the low-melting-point agarose gel and ligated into a Bluescript plasmid vector. The ligation products were used to transform chemically competent *E. coli*. Screening of transformed *E. coli* on agar plates was performed as described in Materials and Methods with the same ^{32}P-labeled DNA probe. Plasmids from colonies hybridizing to the probe were extracted and sequenced. A schematic of the ORFs is shown in Fig. 3. DNA sequences matching the experimentally derived amino acid sequences were found between bp 402 and 429, 567 and 621, 811 and 840, and 993 and 1017 of the ORFs. Continued downstream sequencing from the BPBP gene showed an ORF with a 91% identity with *p30*, a previously reported chromosomal gene of *B. burgdorferi* (8).

Recombinant BPBP was expressed in *E. coli*. Recombinant BPBP was shown by ligand blotting to bind ^{125}I-labeled plasminogen and was recognized by antibody raised against native BPBP (Fig. 4). The predicted molecular mass for BPBP is ~60 kDa. Both the native and recombinant proteins, however, have molecular masses of 69 to 70 kDa on SDS-polyacrylamide gels. This discrepancy may be due to lipidation, glycosylation, phos-

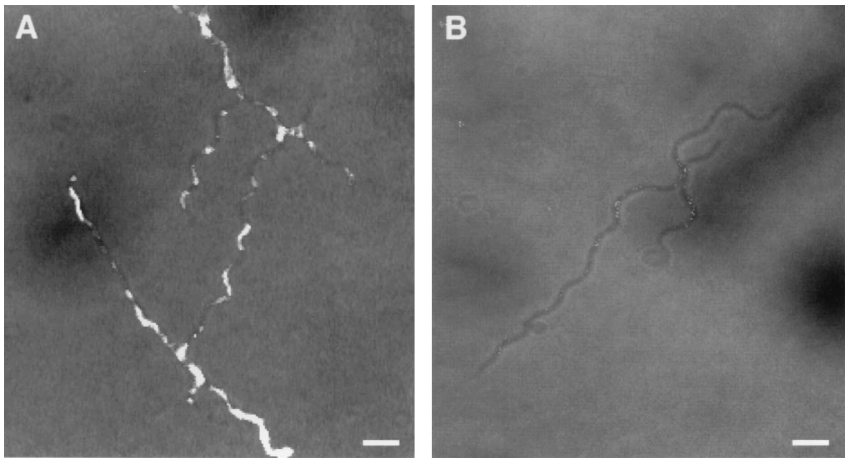

FIG. 2. Localization of BPBP by indirect immunofluorescence of spirochetes. (A) Spirochetes incubated sequentially with rabbit polyclonal anti-BPBP antibody followed by FITC-labeled goat anti-rabbit IgG antibody. (B) Spirochetes incubated sequentially with preimmune rabbit serum followed by FITC-labeled goat anti-rabbit IgG antibody. Bars, 2 μm.

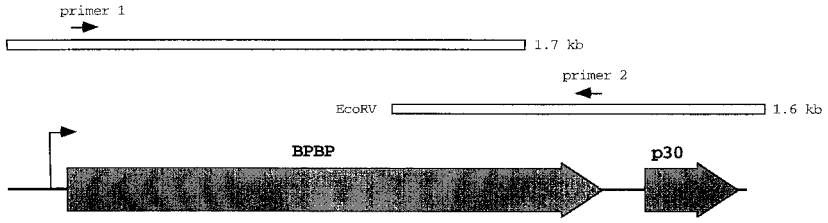

FIG. 3. ORF map of sequenced clones. White boxes show the overlapping clones from which the final sequence was obtained. Large arrows show ORFs of the final sequence. Small arrows show the location of the primers used in constructing recombinant BPBP. The bent arrow shows the location of a promoter region.

phorylation, or conformational factors which may alter the electrophoretic mobility of the protein.

Sequence homologies. The deduced amino acid sequence of BPBP was compared with deduced amino acid sequences for genes entered in the National Center for Biotechnology Information (Bethesda, Md.) nonredundant protein database (May 1997) by using the BLAST program based on the algorithm described previously (1). Alignments with the three highest-scoring sequences are shown in Fig. 5. BPBP was found to have 66% identity and 80% similarity to the reported sequence for the 60-kDa *B. coriaceae* protein (GenBank S75873). No function has yet been described for this protein. Polyclonal antibody specific for BPBP weakly recognized a 67-kDa protein from detergent phase proteins from *B. coriaceae* by Western blotting, and this 67-kDa protein bound ^{125}I-labeled plasminogen as shown by ligand blotting (data not shown). The deduced sequence for BPBP was also found to have 54% identity and 65% similarity to p30 (GenBank U29143), a 30-kDa chromosomal protein of *B. burgdorferi* with no identified function (8). By Western blotting, polyclonal antibody for BPBP did not recognize any 30-kDa protein detergent phase extracts from the *B. burgdorferi* strain in which p30 was described (N40). Although plasminogen does bind to a protein at 30 kDa, this binding disappears in mutants devoid of the 48-kb plasmid containing the OspA gene (15). The periplasmic oligopeptide binding proteins (OppA) of *Salmonella typhimurium* (GenBank P06202), *Bacillus subtilis* (GenBank P24141), and *E. coli* (GenBank P23843) exhibited the next-greatest homology, with ~34% identity and ~57% similarity to BPBP. Other high-scoring sequences included other substrate binding proteins, such as the dipeptide binding protein of *B. subtilis* and the heme binding protein of *Haemophilus influenzae.*

A search for known binding or membrane attachment motifs revealed a prokaryotic membrane lipoprotein lipid attachment site (amino acids 3 to 17) shared by many other bacterial proteins, including the outer surface proteins A and B of *B. burgdorferi* (16, 32, 33). This area of conserved sequence is recognized by a peptidase which cuts upstream of a cysteine residue to which a glyceride-fatty acid lipid is attached. The presence of this motif further suggests that BPBP is likely to be a membrane lipoprotein.

DISCUSSION

The relationship of *B. burgdorferi* to the clotting and thrombolytic pathways of its hosts is intricate and is just beginning to be explored. In addition to binding plasmin(ogen) and its activator, urokinase, *B. burgdorferi* has been shown to bind to the integrin $\alpha_{IIB}\beta_3$ on platelets (5). The ability to bind enzymes such as plasmin, which are capable of digesting clots and extracellular matrix proteins, and to bind platelets, which could act as carriers for the organism, may play a role in both the ability of the organism to disseminate from its inoculation site and its localization to particular organ systems where the clotting cascade is likely to be active (e.g., joints and heart). Activators of the thrombolytic pathway have been firmly established as important determinants in the virulence of bacterial species, such as streptococci and *Yersinia pestis* in animal models, as well as in the metastases of tumors through the extracellular matrix (14, 27, 30). We have previously shown that plasminogen bound to *B. burgdorferi* can be converted to the active enzyme plasmin in the presence of urokinase (17). In vitro studies have shown that plasmin bound to the surface of *B. burgdorferi* enhances the penetration of the organism through an artificial extracellular matrix (5). Plasminogen also appears to play an important role in dissemination of the organism within the tick. The dissemination of the spirochete from the midgut to the salivary glands of the tick vector during feeding is important in the transmission cycle of Lyme disease. Coleman et al. have shown that *B. burgdorferi* organisms resid-

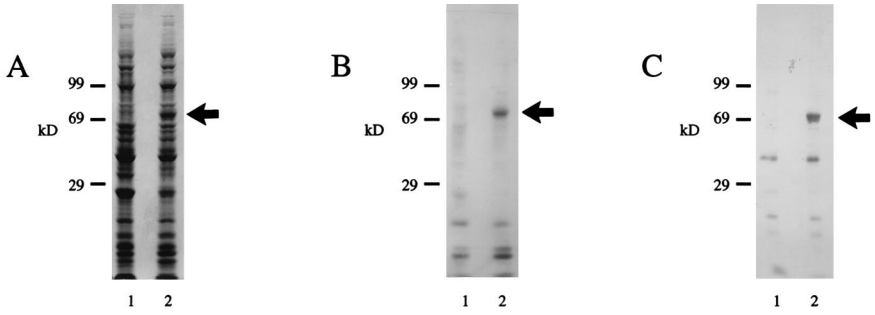

FIG. 4. Expression of recombinant BPBP. Lanes 1, proteins of IPTG (isopropyl-β-D-thiogalactopyranoside)-induced *E. coli* without the BPBP gene; lanes 2, proteins of IPTG-induced *E. coli* containing the BPBP gene. (A) Proteins after Coomassie blue staining; (B) proteins autoradiographed after ^{125}I-labeled plasminogen ligand blotting; (C) proteins after Western blotting with polyclonal rabbit anti-BPBP which had first been purified by adsorption to membranes containing *E. coli* phage lysate (Stratagene). The arrows point to the induced gene products.

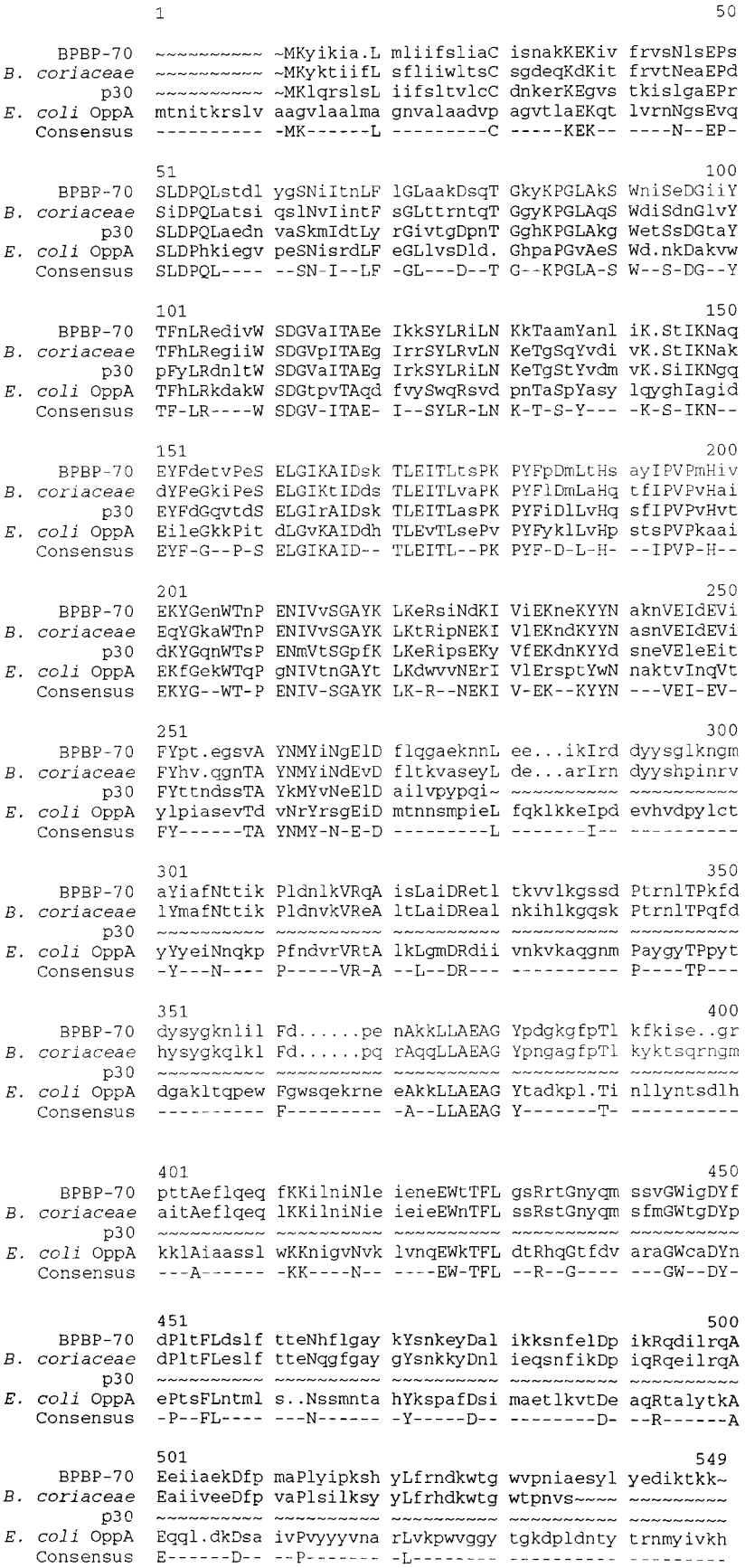

FIG. 5. Comparison of deduced amino acid sequences of BPBP, p30, a 60-kDa protein of *B. coriaceae*, and OppA of *E. coli*. The bottom line (Consensus) denotes sequence homology between the proteins; an uppercase letter indicates identical amino acids in at least three proteins. −, alignments with two or fewer identical amino acids.

ing in the midguts of ticks fed on mice deficient in plasminogen have decreased ability to disseminate to the tick salivary glands during feeding compared with that of *B. burgdorferi* organisms from ticks fed on wild-type mice (6).

In this study, we have isolated and purified one of the plasminogen binding proteins of *B. burgdorferi*, BPBP. We have shown that this protein is antigenic and that humans with Lyme disease develop antibodies to BPBP. We have identified and cloned a 1,569-bp gene coding for BPBP. The expression product of this gene bound plasminogen and was recognized by antibody raised against purified BPBP.

The gene for BPBP is located just upstream of a previously reported *B. burgdorferi* chromosomal gene, *p30*, to which it bears substantial similarity (8). There does not appear to be a termination sequence between the BPBP gene and *p30*, and *p30* does not appear to have its own promoter sequence, suggesting that the genes coding for these two proteins may be within a single operon. The existence of two similar proteins, one with function as a plasminogen binding protein and one without, may give the organism a mechanism by which to regulate plasminogen binding. Nothing is currently known about the in vivo expression or regulation of either BPBP or p30.

Both BPBP and p30 show significant homology to the ABC transporter protein OppA. The oligopeptide permeases of *S. typhimurium* and *E. coli* require five gene products (OppA BCDF) to function (23). OppB and -C are hydrophobic integral membrane domains (12); OppD and -F are believed to couple ATP hydrolysis to the transport process (11). OppA is a periplasmic protein which binds substrates for transport. Members of the OppA family from gram-negative bacteria usually range from 540 to 550 amino acids in length. OppA plays an important nutritional role for the bacteria, transporting peptides containing from two to six amino acid residues into the organism for utilization as carbon and nitrogen sources. The peptide transport systems of prokaryotes are usually rather nonspecific for the amino acid composition of the peptides they transport but exhibit relative specificity for the length of the peptides carried (13). OppABCDF requires its peptide substrates to be α-linked and to possess an ionizable primary or secondary N-terminal group. It is possible that the binding of plasmin, a serine protease with broad substrate specificity, to a permease may result in a higher local level of peptides suitable for transport by the permease. Comparisons of deduced amino acid sequences for the OppABCDF proteins from a variety of organisms reveal a conserved family of proteins. While the sequence of BPBP appears to have significant similarity to those of this family of proteins, the permease function of BPBP, if any, remains to be elucidated.

The only other prokaryotic plasmin(ogen) binding proteins which have been sequenced have been those of group A streptococci, which produce several proteins capable of binding plasmin(ogen), including streptokinase; PAM, a 43-kDa M protein; and Plr, a protein with significant similarity to GAPDH, which binds plasmin but not plasminogen. The sequences for streptokinase, PAM, and Plr do not show any homology with that of BPBP.

In summary, we have identified and isolated a gene for a plasminogen binding protein of *B. burgdorferi*. This protein was found to have significant homology with the family of substrate binding proteins of various bacterial species. Further studies are warranted to investigate the role of BPBP in the virulence and nutritional functions of the organism.

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