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Borrelia burgdorferi BmpA Is a Laminin-Binding Protein[▽]

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The *Borrelia burgdorferi* BmpA outer surface protein plays a significant role in mammalian infection by the Lyme disease spirochete and is an important antigen for the serodiagnosis of human infection. *B. burgdorferi* adheres to host extracellular matrix components, including laminin. The results of our studies indicate that BmpA and its three paralogous proteins, BmpB, BmpC, and BmpD, all bind to mammalian laminin. BmpA did not bind mammalian type I or type IV collagens or fibronectin. BmpA-directed antibodies significantly inhibited the adherence of live *B. burgdorferi* to laminin. The laminin-binding domain of BmpA was mapped to the carboxy-terminal 80 amino acids. Solubilized collagen inhibited BmpA-laminin binding, suggesting interactions through the collagen-binding domains of laminin. These results, together with previous data, indicate that BmpA and its paralogs are targets for the development of preventative and curative therapies for Lyme disease.

Early during the course of Lyme disease, humans frequently produce antibodies directed against a *Borrelia burgdorferi* antigen originally described as "P39" (66). Antibodies recognizing P39 are considered to be specific and diagnostic for Lyme disease spirochete infection (5, 18, 30, 62, 64). The antigenic protein was subsequently identified as BmpA (*Borrelia* membrane protein A) (65). The *bmpA* gene is located on the main borrelial chromosome, adjacent to three paralogous genes named *bmpB*, *bmpC*, and *bmpD*, which together form a complex operon (3, 4, 28, 32, 55, 56, 65). These other Bmp proteins are also often antigenic in infected humans (14). In addition to the serological data described above, examination of *B. burgdorferi* within skin and joint tissues confirmed the production of BmpA protein during mammalian infection (21, 49). BmpA is located in the borrelial outer membrane (46), where it is exposed to the external environment and can be a target of bactericidal antibodies (49, 63; F. Cabello, personal communication). BmpA and its paralogs have been implicated as playing roles in some symptoms of Lyme disease (49, 72). *B. burgdorferi* mutants in which *bmpA* or *bmpB* is specifically deleted are unable to persist in mouse joint tissues (49), indicating an important role for these proteins in the maintenance of mammalian infection. Despite the extensive research conducted on these important antigens, functions for the Bmp proteins had not been determined previously.

B. burgdorferi is an extracellular organism, frequently found associated with its hosts' connective tissues (6–9, 16, 17, 24, 26, 31, 36, 39, 48). In the laboratory, *B. burgdorferi* shows affinity for various host extracellular matrix (ECM) components, such as type I collagen, fibronectin, and decorin (16, 33, 34, 50, 74). We recently determined that *B. burgdorferi* also adheres to mammalian laminin, an important component of many mammalian ECMs (13). Ligand affinity blot analyses of a *B. burgdorferi* cell fraction enriched for outer membrane components revealed that the type strain, B31, can produce several distinct laminin-binding proteins, one of which we previously identified as being the surface-exposed outer membrane lipoprotein ErpX (11, 13, 69). We now present data indicating that BmpA and its paralogs are also laminin-binding proteins.

MATERIALS AND METHODS

Bacteria. An infectious clone of the sequenced culture of *B. burgdorferi* type strain B31, named B31-MI-16, was used for all studies (44). Bacteria were cultured at 34°C in Barbour-Stoenner-Kelly II medium supplemented with 6% rabbit serum (75). After reaching mid-logarithmic phase (10^7 bacteria/ml), bacteria were harvested for either Triton X-114 extraction (see below) or isolation of chromosomal DNA (58).

Cellular fractionation. An outer membrane-enriched fraction of *B. burgdorferi* B31-MI-16 was extracted by Triton X-114 solubilization and phase partitioning as described previously (22, 51, 53). Briefly, cultured bacteria were washed in phosphate-buffered saline (PBS) and then gently extracted in 1% protein-grade Triton X-114 (EMD-Calbiochem, San Diego, CA) at 4°C for 12 h. Protoplasmic cylinders were pelleted by centrifugation at 15,000 × *g* for 10 min, and the supernatant, consisting of periplasmic and outer membrane contents, was retained. The supernatant was warmed to 37°C to induce phase separation, followed by centrifugation for 15 min at 15,000 × *g*. The outer membrane component-enriched detergent phase was extracted two additional times with PBS, and proteins contained in that phase were precipitated with methanol-chloroform.

Two-dimensional electrophoresis. The *B. burgdorferi* outer membrane-enriched Triton X-114 fraction was separated by 2-dimensional electrophoresis using the MultiPhor II system (GE Healthcare, Piscataway, NJ). The detergent-phase pellet was resuspended in ReadyPrep rehydration buffer (Bio-Rad, Hercules, CA) and allowed to rehydrate ReadyStrip immobilized pH gradient strips (pH 3 to 10; Bio-Rad) overnight. Isoelectric focusing was performed for 3,000 V-h (500 V, 6 h, 10°C). After the completion of isoelectric focusing, strips were equilibrated and then separated by conventional sodium dodecyl sulfate-12.5% polyacrylamide gel electrophoresis (SDS-PAGE). Gels were either stained with SYPRO Ruby (Molecular Probes, Eugene, OR) or transferred to nitrocellulose membranes for a laminin immunoaffinity assay. Protein spots of interest were extracted and analyzed by matrix-assisted laser desorption ionization–time-of-flight (MALDI-TOF) mass spectrometry (University of Louisville, Louisville, KY). Spectrometry results were compared with the known sequence of *B. burgdorferi* strain B31 using Mascot (Matrix Science, Boston, MA).

Recombinant proteins. Total chromosomal DNA from *B. burgdorferi* B31-MI-16 was used as a template to PCR amplify wild-type *bmpA*, *bmpB*, *bmpC*,

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TABLE 1. Oligonucleotides used during this work[a]

Primer name	Primer pair sequences (5′ to 3′)
pBmpA	CAC CGG TAA AGG TAG TCT TGG GA CCA TTT CAA TTA TTC AAA CAA AAC CAA TGT
pBmpA-N2	CAC CCC CGA TAT GAA ATA TGC AAT TAT TG CCA TTT CAA TTA TTC AAA CAA AAC CAA TGT
pBmpA-N3	CAC CGG TAG AAG CGT TGC AAC TAG CCA TTT CAA TTA TTC AAA CAA AAC CAA TGT
pBmpA-N4	CAC CGA TGT TGG TAG AGC TTT AAA TAT CCA TTT CAA TTA TTC AAA CAA AAC CAA TGT
pBmpA-I	CAC CCC CGA TAT GAA ATA TGC AAT TAT TG TTA AAT TCC TCC AAG GCC TGC AGC
pBmpA-C2	CAC CGG TAA AGG TAG TCT TGG GA TTA GGT CAT GCC CAC CAA ATT TGC
pBmpB	CAC CTT TAG TAG AAA TGG AAT AGA ATC TAG GCA AAA TCC TCT AAA ACA ACA GAA ATG
pBmpC	CAC CTT TAA ATC TAA TAA AAA GTC TAT TAA ATC TG CCC TTT ACA AAC AAA GCT ATA TTT AAG TAG
pBmpD	CAC CTC TAG CTC TGA TGA TGG CAA GTC G GAA TTA AAA AGA TTT TTC ACA AAT CAG CTC

[a] All amplicons were cloned into pET200, and the resultant plasmids were used to produce recombinant proteins.

bmpD, and amino- and carboxy-terminal truncations of *bmpA*, using the gene-specific primers listed in Table 1. In order to facilitate maximal expression and solubility of recombinant proteins, all amplicons lacked the amino-terminal leader polypeptide- and lipidation site-encoding sequences (29). Amplicons were cloned into pET200 (Invitrogen, Carlsbad, CA), and inserts were completely sequenced on both strands to ensure against the accidental introduction of mutations during cloning processes. Recombinant plasmids thus produced were transformed into *Escherichia coli* Rosetta(DE3)(pLysS) (Novagen, Madison, WI). Expression of polyhistidine-tagged recombinant proteins was induced by the addition of 1 mM isopropyl thiogalactopyranoside to mid-exponential-phase cultures grown at 37°C. Induced *E. coli* bacteria were harvested after 3 h and lysed by sonication, and debris was cleared by centrifugation. Recombinant proteins were purified from cleared lysates using MagneHis nickel-conjugated magnetic beads (Promega, Madison, WI). The purities of recombinant proteins were assessed by separation by SDS-PAGE, followed by staining with Coomassie brilliant blue. Concentrations of protein preparations were determined by a bicinchoninic acid assay (Pierce, Rockford, IL).

Assays of protein binding to laminin. Ligand affinity blot analyses were performed essentially as described previously (13, 70). Briefly, recombinant proteins were separated by SDS-PAGE, electrotransferred to nitrocellulose membranes, and then blocked overnight at 4°C with 5% bovine serum albumin (BSA) in Tris-buffered saline–Tween 20 (TBS-T; 20 mM Tris [pH 7.5], 150 mM NaCl, 0.05% Tween 20). Membranes were washed with TBS-T and then incubated for 1 h at room temperature with 13 mg/ml Engelbreth-Holm-Swarm (EHS) mouse sarcoma laminin (Sigma-Aldrich) in TBS-T. This source of laminin is particularly relevant to investigations of *B. burgdorferi*, in that mice and other rodents are natural reservoir hosts of the Lyme disease spirochete, particularly in the northeastern United States, where strain B31 was isolated (42). After extensive washing with TBS-T, membranes were incubated for 1 h at room temperature with an affinity-isolated rabbit anti-EHS laminin polyclonal antiserum (Sigma-Aldrich), diluted 1:5,000 with TBS-T. Following a wash with TBS-T, membranes were incubated for 1 h at room temperature with horseradish peroxidase-conjugated protein G (Invitrogen), diluted 1:20,000 in TBS-T. Membranes were then developed with the SuperSignal West Pico enhanced chemiluminescence substrate (Pierce), and bands were visualized with BioMax Light film (Kodak).

For enzyme-linked immunosorbent assay (ELISA)-based binding assays, wells of Maxisorp 96-well plates (Nalge-Nunc, Rochester, NY) were coated overnight with 13 μg/ml EHS laminin in 50 mM $NaCO_3$ (pH 9.6) at 4°C. Plates were brought to room temperature and washed three times with PBS plus 0.5% Tween 20 (PBS-T). Wells were blocked for 2 h at room temperature with 2% BSA in PBS-T and then washed three times with PBS-T. Wells were incubated for 1 h at 37°C with various concentrations of recombinant BmpA (rBmpA) in PBS-T. Following three washes with PBS-T, wells were incubated for 1 h at 37°C with a 1:500-diluted BmpA-specific rabbit polyclonal antiserum (gifts of Tom Schwan and Felipe Cabello). Plates were washed three times with PBS-T, and then wells were incubated for 1 h at room temperature with horseradish peroxidase-conjugated protein G, diluted 1:5,000. Wells were again washed three times with PBS-T; 100 μl/well ready-to-use 3,3′,5,5′-tetramethyl benzidine substrate solution (1-Step Turbo TMB-ELISA; Thermo Scientific, Rockford, IL) was added; then reactions were stopped by addition of 2 N H_2SO_4 at 50 μl/well. Absorbance was read at 450 nm with a Spectramax plate reader using SoftMax Pro (Molecular Devices, Sunnyvale, CA). Statistical analyses were performed using Student's *t* test by assuming unequal variances.

Essentially the same ELISA protocol was followed to test the binding of other components of mammalian extracellular matrices. Human fibronectin (Sigma-Aldrich), murine collagen I (Sigma-Aldrich), and murine collagen IV (Santa Cruz Biotechnologies, Santa Cruz, CA) were tested, all at 20 μg/ml. Wells were coated with 2 μg/ml rBmpA. Binding of human proteins to rBmpA was assessed using appropriate specific primary antibodies (all from Sigma-Aldrich).

The ability of heparin (Sigma-Aldrich) or solubilized collagen (gelatin; Difco, Becton-Dickinson, Sparks, MD) to compete for the binding of laminin to BmpA was assayed essentially as previously described (35). rBmpA was immobilized to 96-well ELISA plates (100 ng/well) and then incubated with 13 μg/ml EHS laminin plus varying concentrations of either heparin (0 to 50 μM) or solubilized collagen (0 to 100 μg) for 1 h at 37°C. Assay wells were washed three times with PBS-T and were then incubated with a laminin-specific polyclonal antiserum (1:2,500) for 1 h at 37°C. Reaction products were developed and analyzed as described above.

Assays of the adherence of live bacteria to laminin. The effects of BmpA-directed antibodies or soluble rBmpA protein on laminin binding by live *B. burgdorferi* bacteria were examined microscopically. For antibody inhibition studies, Fab fragments of immunoglobulin G were generated using Fab preparation kits (Pierce).

Glass microscope slides were first thoroughly washed with deionized water and then coated by overnight incubation with 5 μg/ml EHS laminin (Sigma-Aldrich) in PBS. Control slides were similarly coated with BSA. The following day, slides were washed three times with PBS and then blocked by incubation with 3% (mass/vol) BSA for 2 h at room temperature, followed by another three washes with PBS. Cultured B31-MI-16 (10^7 bacteria per ml; mid-exponential phase) was harvested by centrifugation, washed three times with PBS, and resuspended in PBS to a final concentration of 2×10^6 bacteria/ml. For antibody inhibition studies, bacteria on slides were incubated for 30 min with purified Fab fragments added to a final concentration of 2.7 μg/ml. For rBmpA inhibition studies, laminin-coated slides were incubated with 50 μg/ml recombinant protein for 30 min prior to the application of bacteria. Slides were covered with suspended bacteria, incubated at 37°C for 2 h, and then gently washed 10 times with PBS. Bacteria were visualized by dark-field microscopy. Numbers of adherent bacteria observed in 10 fields per slide (magnification, ×200) were counted, with two slides per condition. Statistical analyses were performed using Student's *t* test assuming unequal variances.

RESULTS

We recently determined that the ErpX protein of *B. burgdorferi* type strain B31 specifically binds laminin and that cultured *B. burgdorferi* produces additional proteins that can also bind to laminin (13). Proteomic tools were applied to help identify those other borrelial laminin-binding proteins. An outer membrane-enriched fraction of cultured strain B31-MI-16 was subjected to 2-dimensional electrophoresis and was then either stained with SYPRO Ruby (Fig. 1A) or transferred to nitrocellulose membranes. Transferred proteins were examined for laminin-binding activity by ligand affinity blot analysis.

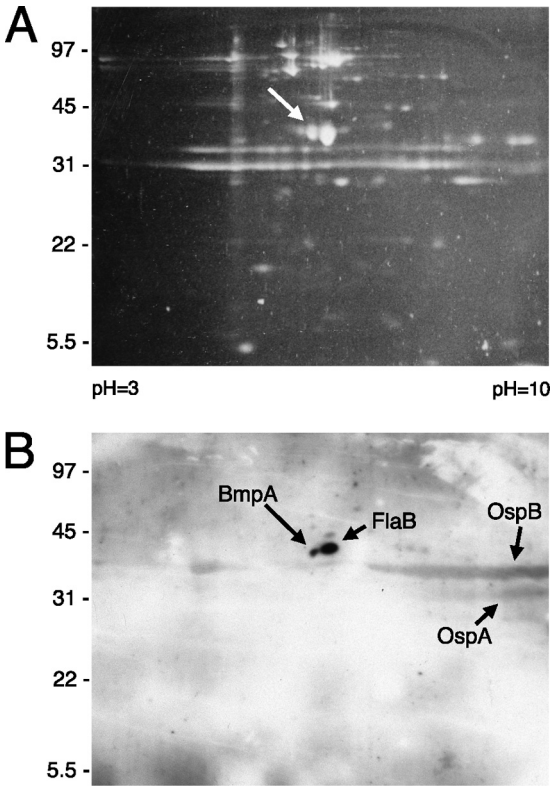

FIG. 1. Two-dimensional electrophoretic analysis of an outer membrane-enriched fraction of cultured *B. burgdorferi* B31-MI-16. (A) Polyacrylamide gel stained with the fluorescent dye SYPRO Ruby. The arrow indicates the protein spot that was determined to correspond with BmpA. (B) A second gel was run simultaneously to that shown in panel A; proteins were transferred to a nitrocellulose membrane and then examined for laminin-binding activities through ligand affinity blot analysis. Strong signals that were determined to correspond with BmpA and FlaB are indicated. Relatively weaker signals were produced from OspA and OspB, both of which often form broad smears across 2-dimensional gels (47).

Two relatively intense spots were observed, both with apparent molecular masses of approximately 40 kDa (Fig. 1B). The ligand affinity blot was aligned with the stained gel, and the two corresponding proteins were extracted and then subjected to mass spectrometric analysis. Note that these procedures served only to identify candidate proteins. Note also that the bacterial fraction analyzed included proteins that are not surface localized in intact borreliae. For those reasons, subsequent analyses of purified proteins that are known to be surface exposed in live *B. burgdorferi* were used to confirm whether or not candidates were able to bind to laminin (see below).

The smaller protein spot yielded the highest-probability match to the outer membrane lipoprotein BmpA, with six polypeptides spanning approximately 30% of that protein (data not shown). The location of this protein on our 2-dimensional gels is consistent with previous results (46, 47). Analysis of the larger spot suggested that it was the FlaB component of the borrelial endoflagella. Since spirochete flagella are localized within the periplasm and are completely shielded from the external environment by the outer membrane (38), interactions between FlaB and laminin were not studied further. Appreciably weaker interactions between laminin and OspB and OspA were also observed; however, since neither of those two borrelial proteins are produced at significant levels during normal mammalian infection (27, 45, 60, 61, 73), those proteins also were not studied further. This particular culture of *B. burgdorferi* did not produce appreciable amounts of ErpX, as has been observed previously (68), so no signal corresponding to the mass and pI of that lipoprotein was detected.

The tentative identification of BmpA as a laminin-binding protein was confirmed through studies using purified recombinant protein (rBmpA) (Fig. 2A). First, ligand affinity blot analyses were performed, in which rBmpA was subjected to SDS-PAGE, transferred to nitrocellulose membranes, and incubated with soluble laminin, and then bound laminin was detected by use of specific antibodies and appropriate secondary antibodies. This method indicated that BmpA is indeed a laminin-binding protein (Fig. 2B). ELISA further supported that conclusion, demonstrating saturable binding and indicating an apparent dissociation constant (K_d) for rBmpA-laminin binding of approximately 0.1 μM (Fig. 2C).

We then examined rBmpA for the ability to bind additional mammalian ECM components: fibronectin, collagen type I, and collagen type IV. Only laminin yielded ELISA signals that

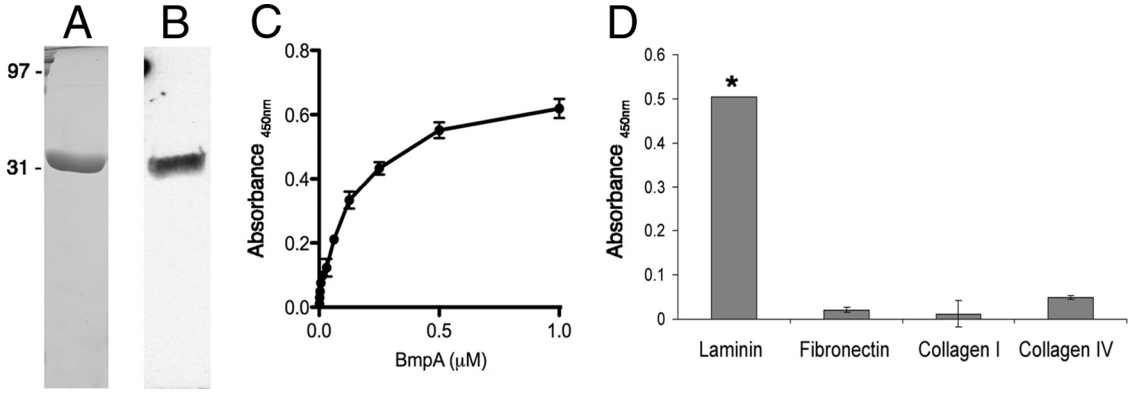

FIG. 2. BmpA binds laminin. (A) Purified rBmpA, subjected to SDS-PAGE and stained with Coomassie brilliant blue. (B) Ligand affinity blot analysis, indicating the adherence of laminin to rBmpA. (C) ELISA of varying concentrations of rBmpA binding to purified laminin affixed to wells. Saturated binding was observed, with an apparent K_d of 0.1 μM. (D) ELISA results indicating that neither mammalian fibronectin, collagen I, nor collagen IV detectably bound to immobilized BmpA. Error bars indicate 1 standard deviation from the mean. The asterisk indicates ligand binding to rBmpA that was significantly different from binding to the control protein BSA ($P < 0.0001$).

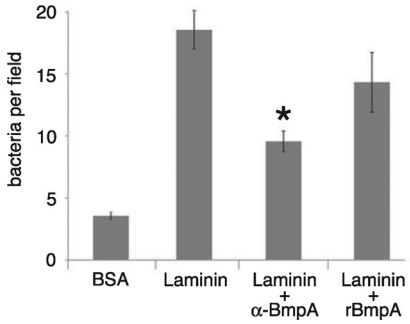

FIG. 3. Microscopic enumeration of bacteria adhering to slides coated with BSA or laminin, showing the effects of BmpA-specific antibodies or soluble rBmpA on adherence. Error bars indicate 1 standard deviation from the mean. Neither antibodies nor recombinant protein was added to control assay mixtures. The asterisk indicates a statistically significant difference from the results of control assays. BmpA-specific antibody Fab fragments significantly inhibited borrelial adherence ($P < 0.04$). The reduced adherence due to the addition of rBmpA was not statistically significant ($P < 0.13$).

were significantly greater than those obtained for the control protein, BSA (Fig. 2D).

The biological significance of BmpA as a laminin-binding protein was investigated by examining the effects of BmpA-specific antibody Fab fragments or rBmpA protein on the adherence of intact, live *B. burgdorferi* bacteria to laminin. As assessed by microscopic examinations, anti-BmpA antibodies significantly inhibited the adherence of intact *B. burgdorferi* to the laminin substrate (Fig. 3). rBmpA also reduced borrelial adhesion, although the changes were not statistically significant. Considering that the cultured *B. burgdorferi* produced additional laminin-binding outer surface proteins, including OspA and OspB, these results indicate that BmpA plays a substantial role in borrelial adherence to laminin.

The laminin-binding domain of BmpA. Mutant rBmpA proteins that lacked large regions of the wild-type protein were produced (Fig. 4A). Each truncated recombinant protein was assessed for laminin-binding activity by ligand affinity blot analyses. Three successively shorter amino-terminally truncated mutant proteins, the 233-residue rBmpA-N2, the 141-residue rBmpA-N3, and the 80-residue rBmpA-N4, all bound laminin (Fig. 4B). In contrast, neither the 112-residue amino-terminal fragment rBmpA-C2 nor the centrally located 117-residue rBmpA-I bound the host protein (Fig. 4B). Even with extended film exposure times (>15 min), no signals indicative of laminin binding by rBmpA-I or rBmpA-C2 were ever detected. Taken together, these data suggest that the carboxy-terminal 80 amino acids of BmpA contain all of the residues necessary for binding to laminin.

Involvement of laminin collagen-binding domains. Laminins are large, multimeric proteins containing distinctive domains through which they interact with other tissue components (20, 59). Competition assays were performed to examine whether BmpA binds laminin through either of two major domain types: those that bind collagen (gelatin) and those that bind heparin. Addition of solubilized collagen led to dose-dependent inhibition of laminin-BmpA binding, suggesting that BmpA and collagen bind to overlapping regions of laminin (Fig. 5A). Addition of 3.12 μM heparin had a small but significant effect ($P < 0.05$) on BmpA-laminin binding, but increasing concentrations of heparin had no additional effects (Fig. 5B).

BmpB, BmpC, and BmpD also bind laminin. The main chromosome of Lyme disease spirochetes contains three additional genes encoding paralogs of BmpA. Overall predicted amino acid similarities among the Bmp proteins of *B. burgdorferi* strain B31 range from 36 to 64% (Fig. 6A) (56). Since BmpB, BmpC, and BmpD are also produced during mammalian infection, we produced recombinant forms of all three proteins and assessed their abilities to bind laminin. Ligand affinity blot analyses demonstrated that all four members of the Bmp family bound laminin (Fig. 6B).

DISCUSSION

Humans and other mammals infected with Lyme disease spirochetes mount rapid and strong antibody responses directed against the BmpA outer surface protein. Such immune

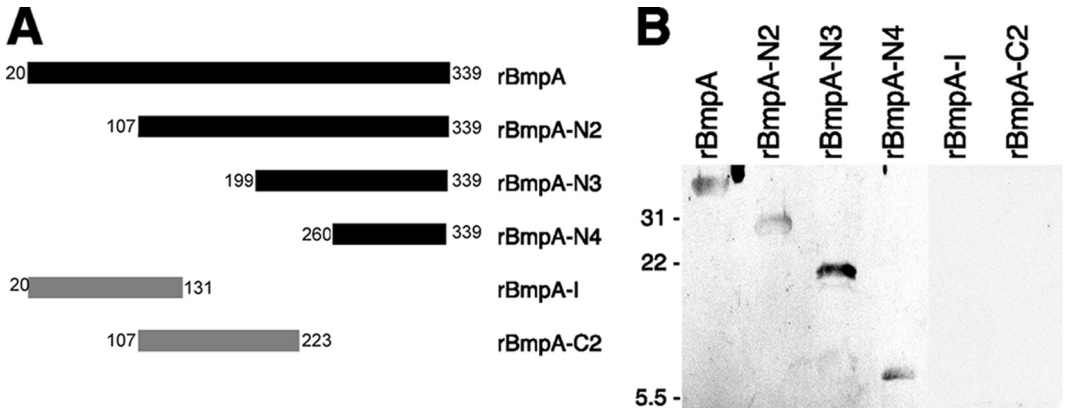

FIG. 4. Laminin-binding analyses of truncated BmpA proteins. (A) Schematic representation of wild-type rBmpA and its truncated mutants. Numbers at either end correspond to the residue numbers of the immature (uncleaved, nonlipidated) wild-type protein. The sequence of rBmpA begins with the first residue downstream of the lipidation site, i.e., residue 20. Filled bars represent recombinant proteins that detectably bound laminin, while shaded bars represent proteins that did not bind laminin. (B) Ligand affinity blot analyses of laminin binding by recombinant wild-type and mutant BmpA proteins. The mobilities of molecular mass markers (in kilodaltons) are given on the left.

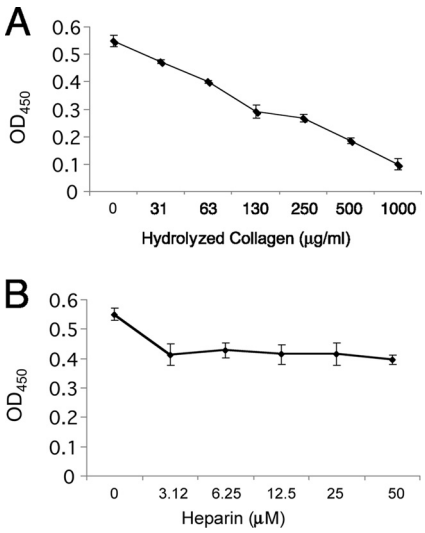

FIG. 5. BmpA and collagen compete for binding to laminin. Shown are ELISA results for the adherence of laminin to rBmpA in the presence of increasing concentrations of solubilized collagen (gelatin) (A) or the sulfated glycosaminoglycan heparin (B).

responses are so reproducible that the presence of BmpA-directed antibodies in patient serum is a reliable marker of *B. burgdorferi* infection (5, 18, 30, 62, 64). Despite the apparent ubiquity of BmpA among Lyme disease spirochetes and its known expression during mammalian infection, no function had been defined for the protein prior to this work. We identified BmpA and its three paralogs, BmpB, BmpC, and BmpD, as laminin-binding proteins. This characterization may explain the results from a previous study, which found that a recombinant bacteriophage expressing a fragment of BmpD on its capsid tended to adhere to mouse tissues (2). BmpA and BmpB both play important roles during mammalian infection, as evidenced by the fact that mutants defective in either gene are unable to persistently infect mouse joints (49). The relative importance of BmpC and BmpD has yet to be evaluated. Since those mutant bacteria retained their other *bmp* paralogs plus the gene for the other infection-associated laminin-binding protein, ErpX (13, 49, 52), those results raise the possibility that borrelial laminin-binding proteins are produced at different levels in different tissues and/or that colonization of some host tissues requires synergy between adhesins. Transcription of the *bmp* locus involves multiple promoters and terminators (28, 55, 56), suggestive of complex, potentially independent expression patterns for each gene.

The carboxy-terminal 80 amino acids of BmpA were determined to be sufficient for adhesion to laminin. Competition studies point toward the involvement of collagen-binding domains of laminin in its interactions with BmpA. The function of the remainder of the 39-kDa BmpA lipoprotein remains to be determined. BmpA is predicted to fold in a manner similar to that of the *Treponema pallidum* TmpC (PnrA) lipoprotein, although the two proteins share approximately 30% identical amino acids. TmpC is hypothesized to be involved in purine transport (25), so it is possible that BmpA may perform such a role for *B. burgdorferi*. Dual-function outer surface proteins, such as the *B. burgdorferi* p66 porin/integrin adhesin, the subgroup of *B. burgdorferi* Erp proteins that bind host plasminogen and complement factor H, and the *Neisseria gonorrhoeae* PorB porin/adhesin, are not uncommon in bacteria (1, 12, 23, 37, 40, 43, 54, 57, 67, 71).

Redundancy of function appears to be the norm for the Lyme disease spirochete. In addition to the four laminin-binding proteins identified in the current work, the *B. burgdorferi* type strain also produces the unrelated ErpX laminin-binding protein (13). As additional examples, two decorin-binding pro-

FIG. 6. All four members of the *B. burgdorferi* Bmp protein family bind laminin. (A) Alignment of BmpA, BmpB, BmpC, and BmpD predicted protein sequences from the *B. burgdorferi* type strain, B31. Identical residues are boxed and shaded. Spaces introduced to maximize alignment are indicated by dashes. The amino-terminal cysteine residue modified by lipidation in each mature protein is indicated by an arrowhead. The carboxy-terminal region of BmpA that is sufficient for adherence to laminin is indicated by a horizontal line over the sequence. (B) Equal masses of recombinant BmpB, BmpC, and BmpD were subjected to SDS-PAGE, transferred to a nitrocellulose membrane, and then assessed for the ability to adhere to laminin by ligand affinity blot analysis. The mobilities of molecular mass markers (in kilodaltons) are given on the left. rBmpC consistently migrated more slowly on SDS-PAGE gels than the other rBmp proteins, perhaps reflecting differences in the charges of the proteins (pI values, 5.6 for rBmpB, 5.8 for rBmpD, and 8.7 for rBmpC).

teins, three fibronectin-binding proteins, five complement factor H-binding-proteins, and several plasminogen-binding proteins have been identified in the type strain, B31 (10, 12, 19, 34, 41, 50). For some of those examples, it has been shown that different borrelial proteins that adhere to the same host ligand are produced at different times during the spirochete's mammal-tick infectious cycle (15). It remains to be seen whether that is also the case with the multiple borrelial laminin-binding proteins, or if each binding protein is produced only in certain host tissues. Studies of *bmpA* and *bmpB* mutants indicate that both encoded proteins play important roles in at least some aspects of mammalian infection. Thus, the Lyme disease spirochete laminin-binding proteins are compelling targets for the development of therapies that disrupt infection processes.

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