**Adherence of *Borrelia burgdorferi***

**IDENTIFICATION OF CRITICAL LYSINE RESIDUES IN DbpA REQUIRED FOR DECORIN BINDING**

(Received for publication, April 15, 1999, and in revised form, June 3, 1999)

Eric L. Brown, Betty P. Guo‡, Pamela O’Neal, and Magnus Höök§

*From the Center for Extracellular Matrix Biology, Albert B. Alkek Institute of Biosciences and Technology, Texas A&M University Health Science Center, Houston, Texas 77030*

---

*Borrelia burgdorferi*, the causative agent of Lyme disease, expresses on its surface two decorin binding adhesins, DbpA and DbpB. Previous studies have demonstrated that vaccination of mice with DbpA provided protection against challenge with heterologous *Borrelia* strains despite considerable sequence variability among DbpA in these strains.

We have now examined the importance of individual amino acid residues in DbpA for decorin binding. We demonstrated that chemical modification of lysine residues resulted in loss of ligand binding activity. Of the 27 lysine residues in native DbpA from strain 297, 6 are present in most and 5 are conserved in all 30 DbpA sequences examined so far. Analysis of recombinant DbpA in which individual lysine residues have been mutated to alanine suggested that three of the conserved residues distributed throughout the DbpA sequence are required for decorin binding. These mutants lost their ability to bind decorin in Western ligand blot assay and bound reduced amounts of decorin in an ELISA. Furthermore, these mutant DbpA proteins did not inhibit the adherence of *B. burgdorferi* to a decorin substrata, and they did not recognize decorin in an extracellular matrix established by human fibroblast cultures. We conclude that the three lysine residues Lys-82, Lys-163, and Lys-170 are crucial for the binding of DbpA to decorin.

*Borrelia burgdorferi*, and other closely related Borreliae are the causative agents of Lyme disease. Lyme disease is transmitted by infected ticks, which during a blood meal can deposit a small number of organisms in the dermis of the host animal (1). This initial skin infection is often accompanied by a local rash (erythema migrans), which can be followed by a general flu-like illness (2, 3). Untreated Lyme borreliosis can develop into a chronic, multisystemic disorder that may affect the joints (Lyme arthritis), skin, heart, and central nervous system (2).

Microbial adhesion to and colonization of host tissue is an early, critical event in an infection process. In the case of Lyme disease, host tissue adherence appears to be of importance during different stages of the disease process. Initially, during an infected tick’s blood meal, a small number of spirochetes are deposited in the dermis of the host, where the bacteria appear to colonize collagen fibers (4, 5). As the infection disseminates to other tissues, bacteria may colonize additional extracellular matrix structures, and host cells may be involved. We previously showed that adherence of *B. burgdorferi* to collagen fibers involved a specific binding of the spirochete to decorin, a dermatan sulfate proteoglycan that is associated with and “decorates” collagen fibers, whereas a direct binding to collagen could not be demonstrated (6–9). A dermal route of entry into the host appears to be important for the development of disease. Spirochetes administered intravenously are rapidly and effectively cleared by Kupffer cells in the liver (10), whereas those inoculated intradermally consistently establish infection (11). Perhaps the initial dermal colonization allows the organism to adapt to *in vivo* conditions before blood stream dissemination.

We and others (8, 12, 13) have recently cloned and sequenced the genes coding for the two decorin-binding proteins (DbpA and DbpB), which are expressed at the surface of the spirochete as lipoproteins and act as adhesins of the microbial surface component-recognizing adhesive matrix molecule family (14). We showed that recombinant forms of DbpA and DbpB are capable of binding to decorin and that DbpA effectively inhibited the adherence of *B. burgdorferi* to a decorin substrate (8).

Active and passive immunization of mice using DbpA and DbpA antiserum, respectively, protected against challenge with *B. burgdorferi* (12, 13, 15, 16). In a recent study (16), DbpA sequences were found to vary significantly among different *Borrelia* strains. Nevertheless, antibodies to one recombinant form of DbpA conferred broad protection against various strains, suggesting that at least some immunoprotective epitopes are conserved.

In this study, we have initiated a detailed analysis of the decorin/DbpA interactions. We report that chemical modification of primary amino groups in DbpA resulted in loss of decorin binding activity. Furthermore, using a panel of site-directed mutants, we identified three specific lysine residues conserved in DbpA sequences among *B. burgdorferi sensu lato* isolates (16) as critical for the ligand binding activity of the protein.

**EXPERIMENTAL PROCEDURES**

*Bacterial Strains, Culture, and Materials—Low-passage *B. burgdorferi* strain 297 (passage 7) was used in this study and cultured in Barbour-Stoenner-Kelly II medium at 34 °C (17). Bacterial cultures were incubated in CO₂-enriched atmosphere in a GasPak chamber

---

1 The abbreviations used are: Dbp, decorin-binding protein; Osp, outer surface protein; K-mod, lysine-modified DbpA; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; BB, binding buffer; ELISA, enzyme-linked immunosorbent assay.
(BBL Microbiologica Media, Baltimore, MD) containing BBL GasPak Plus envelopes and a GasPak anaerobic indicator (Beckton Dickinson, Cockeysville, MD) until the cells reached log phase. Cells were harvested by centrifugation at 5000 rpm for 15 min and resuspended in sterile phosphate-buffered saline (PBS) (137 mM NaCl, 3 mM KCl, 4 mM Na₂HPO₄, 1 mM KH₂PO₄, pH 7.4). The density of bacteria was determined using a Petroff-Hauser chamber.

_Escherichia coli_ strain JM101 (Qiagen, Chatsworth, CA) was grown at 37 °C in Lennox broth (Difco) (18) containing the appropriate antibiotics.

Human fibroblast skin cells (ATCC CRL-1475) were cultured on 16-well chamber slides (Nunc, Naperville, IL) in Dulbecco's modified essential medium containing 10% fetal bovine serum at 37 °C as described previously (8).

**Labeling of Decorin**—Decorin from bovine fetal skin was purified as described previously and provided by Dr. Lawrence Rosenberg (8, 19). Decorin was labeled with digoxigenin-3-O-methylcarbonyl-e-aminoacapric acid-N-hydroxysuccinimide ester (digoxigenin) (Roche Molecular Biochemicals) according to the manufacturer's instructions and stored at -20 °C. To label decorin with biotin, 7.5 mg of NHS-LC-biotin (sulfoconjugated)-6-(biotinamido) hexanone (Pierce) was dissolved in 100 μl of dimethyl sulfoxide and combined with 0.5 mg of decorin and 0.5 ml of 0.2 M sodium borate (pH 8.0) in a total reaction volume of 1 ml as described previously (8, 20).

**Construction of Expression Plasmids and Site-directed Mutagenesis**—_B. burgdorferi_ Dbp-Decorin Interactions

| Protein | Forward primer(s) | Reverse primer(s) |
|---------|------------------|-------------------|
| DbpA    | (BN10F2), 5'-GCCGTGATC CAACAATTTACTTAAACTA-3' | (End P) 5'-GCCGTGATCCAGGATTTACACTCCAGGCT-3' |
| Lys-14  | 5'-GCCGTGATCAAGATTTACTTAAACTA-3' | End P |
| Lys-32  | 5'-ACGAGCGCAGCAAGAATTACT-3' | 5'-TTAGTGATGATTAGTTATTACACTCCAGGCT-3' |
| Lys-40  | 5'-AAATTAGACATCATGGGCTTAAACTG-3' | 5'-CTCAATATCCGCTAGGTTACCTAAATT-3' |
| Lys-50  | 5'-AGATGCAATTGCAAAAAAATTAC-3' | 5'-AGACCGCTTTTGAAATGTCATCT-3' |
| Lys-51  | 5'-AGAACCGCAGCAAGAATTACT-3' | 5'-AGATGCAATTGCAAAAAAATTAC-3' |
| Lys-82  | 5'-ATACCTGGAGACGCTAGGCTTCAAG-3' | 5'-TTAGCGAAGCATTCGTAA-3' |
| Lys-91  | 5'-TACCGAGAGCAAGAATTAC-3' | 5'-CCAGTTTTTTATTAGCTAGGCT-3' |
| Lys-102 | 5'-AAGCTAAGGACATCAAAGAATT-3' | 5'-CCAGTTTTTTATTAGCTAGGCT-3' |
| Lys-104 | 5'-AAGCTAAGGACATCAAAGAATT-3' | 5'-AAGCTAAGGACATCAAAGAATT-3' |
| Lys-163 | 5'-AAATAGAAGAAGAATCTTACAAAGAATT-3' | 5'-AAGCTAAGGACATCAAAGAATT-3' |
| Lys-170 | 5'-TCTCAACCGGAGAATGAAATT-3' | 5'-AAGCTAAGGACATCAAAGAATT-3' |

* B10F2 and EndP were also used in the formation of this mutant.

**Recombinant Proteins**—Recombinant _B. burgdorferi_ strain 297 were expressed in _E. coli_ (JM101) harboring the appropriate plasmid. _E. coli_ was grown in Lennox broth until it reached an _A_600 of 0.6. Isopropyl-β-D-thiogalactopyranoside (Life Technologies, Inc.) was added to a final concentration of 0.2 mM, and the cells were incubated at 37 °C for an additional 4 h. Cells from a 1-liter culture were harvested by centrifugation and resuspended in 10 ml of binding buffer (BB) (20 mM Tris-HCl, 0.5 M NaCl, 15 mM imidazole, pH 8.0) and lysed in a French pressure cell at 11,000 pounds/inch². The lysate was centrifuged at 40,000 × _g_ for 15 min, and the supernatant was filtered through a 0.45 μm filter. A 1 ml imidom acidic acid-Sepharose column (Sigma) was charged with 75 mM NiCl₂-NH₄O and equilibrated with BB. The filtered supernatant was applied to the column and washed with 10 volumes of BB and then 10 volumes of BB containing 60 mM imidazole. The bound proteins were eluted with BB containing 200 mM imidazole, dialyzed against PBS containing 10 mM EDTA, then dialyzed against PBS. The protein concentration was determined by the bicinchoninic acid protein assay (Pierce), and proteins were stored at -20 °C.

**SDS-PAGE, Western Ligand Blots, and Western Blot**—Proteins (purified DbpA, DbpA site-directed mutants and OspC) were subjected to SDS-PAGE (reducing conditions) and probed with rabbit anti-DbpA polyclonal sera (R625) or with digoxigenin-labeled decorin as described previously (8).

**Binding of Decorin to DbpA and DbpA Mutants**—Immuno-1 microtiter plate wells (Dynatech Laboratories, Chantilly, VA) were coated with 0.4 μg of DbpA, DbpA mutants, and K-mod in 50 μl of PBS overnight at 4 °C. The wells were washed and then blocked with 200 μl of Super Block (Pierce) for 1 h. After washing, 0.12 μg/ml of biotin-conjugated decorin in 100 μl of Super Block was added to the wells and incubated for 1 h. After washing, 100 μl of a 1:10,000 dilution of alkaline phosphatase-conjugated streptavidin (Roche Molecular Biochemicals) was added and incubated for 1 h. The wells were washed and incubated for 30 min with 100 μl of a 1 mg/ml Sigma 104 phosphatase substrate (Sigma) dissolved in 1 mM diethanolamine, 0.5 mM MgCl₂, pH 9.8. Plates were washed four times with PBS-0.05% Tween 20 between all steps and all incubations took place at 37 °C unless otherwise specified. All dilutions were made using Super Block. The absorbance at 450 nm was determined in a microplate reader (Molecular Devices, Menlo Park, CA).

**Inhibition of DbpA Decorin Binding by DbpA Mutants**—Immuno-1 microtiter plate wells were coated with 0.4 μg of DbpA in 50 μl of PBS overnight at 4 °C. The wells were washed and then blocked as described above. Biotin-conjugated decorin (100 μl of 0.12 μg/ml solution) was preincubated at 37 °C with various amounts (1.0, 0.5, 0.25, 0.12, and 0.0625 μg/ml) of unlabeled DbpA, DbpA site-specific mutants, or K-mod. After washing, 100 μl of the biotin-conjugated decorin/inhibitor mixture was added to the wells and incubated for 1 h. After washing, the wells were incubated with streptavidin alkaline phosphatase and developed as described above. All dilutions were made using Super Block.

**Attachment of _B. burgdorferi_ to Decorin Substrates and Inhibition by Recombinant Proteins**—Immuno-1 microtiter plate wells were coated with 0.4 μg of DbpA in 50 μl of PBS overnight at 4 °C.

**TABLE I**

**Proteins used to construct DbpA 549 and the DbpA lysine mutants**

**FIG. 1.** SDS-PAGE and Western ligand blot analysis of DbpA and chemically modified DbpA (K-mod). Purified DbpA and K-mod were subjected to SDS-PAGE (12%) under reducing conditions and stained with Coomassie Brilliant Blue (A) or transferred to a nitrocellulose membrane (B). After blocking additional protein-binding sites, proteins on the membrane were probed with digoxigenin-labeled decorin and visualized by alkaline phosphatase reactivity.
with 1.0 µg of decorin in 50 µl of PBS overnight at 4 °C. After washing and blocking as described above, 0.5 µg of inhibitor protein (DbpA, DbpA site-directed mutants, K-mod, or OspC) was added per well in 50 µl of Super Block and incubated for 1 h. Subsequently, 4 x 10⁷ Borreliae were added to each well in 50 µl of Super Block, and the bacteria were allowed to attach to the substrate for 1 h at 37 °C as described previously (8). After washing, 100 µl of a 1:500 dilution of monoclonal mouse anti-OspA was added and incubated for 1 h. The wells were washed and incubated with 100 µl of a 1:3000 dilution of alkaline phosphatase-conjugated goat anti-mouse IgG for 1 h. After washing, the wells were developed as described above. All incubations took place at 37 °C unless otherwise specified. All dilutions were made using Super Block.

Immunofluorescence—Human skin fibroblasts were cultured and fixed as described previously (8). Cells were plated onto 16-well chamber slides (Nunc) at a density of 2.5 x 10⁴ cells/ml, grown for 2–3 days, and then fixed with –20 °C acetone and washed twice in PBS. All subsequent washes were done by immersing the slides in a staining dish filled with PBS three times, 10 min each wash unless otherwise specified. All incubations were done at room temperature. Unoccupied protein-binding sites on the slides were blocked with 80 µl of PBS (pH 7.5) twice each (23). After washing, 100 µl of a 1:50 dilution of rhodamine-conjugated goat anti-rabbit IgG was added and incubated for 20 min. After washing, 60 µl of a 1:500 dilution of rabbit anti-DbpA was added for 1 h. After washing, a final incubation with 60 µl of a 1:50 dilution of rhodamine-conjugated goat anti-rabbit IgG was added for 30 min in the dark. The slides were examined after washing using fluorescence microscopy. Photographs were taken at x20 magnification using Fuji Film 1600 ASA slide film. Anti-DbpA and rhodamine-conjugated goat anti-rabbit IgG antibodies were adsorbed against CRL-1475 cells prior to use. An equal mixture of 1 x 10⁵ sonicated and whole CRL 1475 fibroblasts in 300 µl of PBS were incubated end over end at 37 °C for 1 h with an equal volume of each antibody. Adsorbed antiserum was collected after centrifugation of each tube at 5000 rpm for 5 min.

All antibody and protein dilutions were made in blocking buffer. Controls included (a) incubations with secondary antibody only, (b) anti-DbpA + secondary antibody, and (c) DbpA or osteopontin + secondary antibody.

Chemical Modification of Lysine and Arginine Residues—Chemical modification of lysine residues was performed by incubating 700 µl of DbpA (1 mg/ml) with 5.4 mg of Nα-glyceraldehyde and 37.8 mg of sodium cyanoborohydride for 1.5 h at room temperature followed by overnight dialysis at 4 °C in 2 liters of 50 mM ammonium bicarbonate and 2 liters of PBS (pH 7.5) twice each (23).

Chemical modification of arginine residues on DbpA was performed as described (24). Briefly, 1 mg of DbpA (2 ml) was dialyzed against 0.2 M sodium borate (pH 9.0) overnight at 4 °C. The dialyzed protein was transferred to an Eppendorf tube and incubated in the dark at 37 °C with 0.05 ¥ cyclohexanedione. DbpA was then passed through a 0.02 µm sodium borate-primed PD-10 column (Amersham Pharmacia Biotech), and four 0.5-ml aliquots were collected (25).

Circular Dichroism Spectroscopy—Purified recombinant proteins were dialyzed against PBS (pH 7.4) at a concentration of 50 µg per ml. CD spectroscopy measurements were performed using a Jasco J720 spectropolarimeter calibrated with a 0.06% (w/v) 10-D-camphorsulfonic acid ammonium salt solution. Measurements were taken at room temperature in a 0.2-mm-path length quartz cell. All far-UV (250–190 nm) spectra were acquired with a time constant of 1 s, a scan rate of 20 nm/min, and a scan accumulation of 100. measurements were taken at room temperature in a 0.2-mm-path length quartz cell. All far-UV (250–190 nm) spectra were acquired with a time constant of 1 s, a scan rate of 20 nm/min, and a scan accumulation of 100.

RESULTS

Lysine Residues in DbpA Participate in Decorin Binding—We previously demonstrated that B. burgdorferi expressed two adhesins in the 20-kDa molecular mass range.

![Image](image-url)
(DbpA and DbpB) of the microbial surface component-recognizing adhesive matrix molecule family that mediated the attachment of the spirochete to decorin and bind the proteoglycan with an estimated $K_D$ of approximately $10^{-2}$ M (8, 20). DbpA bound decorin in both ELISA and Western ligand blot analysis. In addition, DbpA recognized decorin in an organized extracellular matrix produced by cultured skin fibroblasts and mediated attachment of DbpA-coated beads to fibroblast cultures (8, 20). To further characterize this interaction, we now seek to identify residues within DbpA that are necessary for binding to decorin.

In initial experiments, we found that biotin conjugation to primary amino groups in DbpA resulted in loss of decorin binding activity, suggesting a role for lysine residues in this interaction. To substantiate this hypothesis, we decided to chemically modify the side chains of lysine residues and examine the binding properties of the resulting protein. Chemical substitution of the primary amino groups resulted in a DbpA form that migrated as a somewhat larger protein compared with unmodified DbpA when analyzed by SDS-PAGE (Fig. 1A). Both forms of DbpA migrated as two bands, monomers at 20 and 21 kDa and dimers at 40 and 42 kDa for DbpA and chemically modified DbpA (K-mod), respectively. The basis for the dimer formation is not entirely clear, although it is in part cysteine-mediated (8). The decorin binding activity of the DbpA and lysine-modified DbpA was examined in a Western ligand blot assay (Fig. 1B). Substitution of primary amino groups in DbpA resulted in loss of decorin binding, whereas unmodified DbpA transferred to a supporting membrane bound digoxigenin-labeled decorin. Chemical modification of arginine residues did not affect the ability of DbpA to bind decorin (data not shown).

The Importance of Individual Lysine Residues for Decorin Binding—The results described above suggested that one or several lysine residues are critical for the decorin binding activity of DbpA. To evaluate the importance of individual lysine residues, we decided to change selected residues to alanine and analyze the decorin binding activity of the resulting mutants. Of the 27 lysine residues present in the native DbpA protein sequence, 3 are present in the leader sequence. The recombinant form of DbpA used in this study contained part of the leader sequence, including one of the lysine residues (Lys-14). By comparing the DbpA sequences of various B. burgdorferi sensu lato strains (26) to the DbpA sequence of B. burgdorferi strain 297 (8) (Fig. 2), we identified five lysine residues that were conserved in all 30 sequences examined (Lys-32, Lys-82, Lys-104, Lys-163, and Lys-170) and six that were conserved in most DbpA sequences (Lys-14, Lys-40, Lys-50, Lys-51, Lys-91, and Lys-102). These residues, shown in Fig. 2, were individually targeted for mutational analysis of recombinant DbpA of B. burgdorferi strain 297 using extension overlap polymerase chain reaction (Fig. 2) (8). DbpA and all mutant proteins were expressed as N-terminal polyhistidine (His tag) fusions and purified using nickel-chelating chromatography (9).

SDS-PAGE analysis of DbpA site-directed mutants (Lys-14, Lys-32, Lys-40, and Lys-163 are not shown) revealed no significant differences when compared with native DbpA (Fig. 3A). CD spectroscopy showed that the overall secondary structure of DbpA and all site-directed mutants were nearly identical (data not shown), indicating that the mutations did not grossly alter the structure of the protein. Western ligand blot analysis, however, demonstrated that, although all mutants were still recognized by anti-DbpA polyclonal antiserum (data not shown), proteins with mutations of Lys-82, Lys-163 (not shown), and Lys-170 lost their ability to bind decorin (Fig. 3B). The protein mutated at Lys-51 showed reduced decorin binding activity, and a recombinant, unrelated His-tag protein OspC did not bind decorin (Fig. 3B).

We also examined the ability of biotin-conjugated decorin to bind to different forms of DbpA-coated microtiter wells (Fig. 4).
Inhibition of Borrelia attachment to decorin by DbpA mutants. Decorin-coated microtiter wells were incubated with 0.5 μg of DbpA, lysine mutants, K-mod, or OspC for 1 h prior to the addition of 4 × 10^6 Borrelia (strain 297, passage 7). Wells were probed with a monoclonal anti-OspA antibody. The data are expressed as A_405 ± S.E. of triplicate wells minus the substrate control.

The chemically modified DbpA (K-mod) was essentially unable to support the binding of decorin. Proteins mutated at the single lysines Lys-82, Lys-163, and Lys-170 bound significantly less decorin compared with the wild-type DbpA, and the binding of decorin to Lys-51 was marginally reduced. Because we have not determined the efficiency of binding to the wells for the different DbpA forms, a direct comparison between the different proteins decorin binding capacity from these results should be made with caution. However, a similar trend was observed in this ELISA-type assay as in the Western ligand blot assay.

To directly compare the decorin binding potential of the different forms of DbpA, we designed an inhibition ELISA-type assay (Fig. 5). In this assay, labeled decorin was first incubated with increasing concentrations of soluble inhibitor protein, and the residual DbpA binding activity of decorin was then assessed by ELISA. All proteins tested as potential inhibitors caused a concentration-dependent reduction in decorin binding to DbpA. Unmodified DbpA and the Lys-91 mutant were equally effective in inhibiting the decorin-DbpA interaction. The Lys-14 (not shown), Lys-50, Lys-102, and Lys-104 mutants were also efficient inhibitors whereas Lys-51, Lys-82, Lys-163, and Lys-170 were significantly less effective, suggesting an impaired decorin binding function for these proteins. The chemically modified DbpA protein, K-mod, was the weakest inhibitor and caused less than 50% inhibition at the highest concentration tested.

Inhibition of B. burgdorferi Attachment to Decorin by DbpA Proteins—We have previously demonstrated that recombinant DbpA inhibited the adherence of B. burgdorferi to a decorin substrate (8). We have now compared the abilities of different DbpA mutants to interfere with bacterial attachment (Fig. 6). At the concentration of inhibitor protein examined (0.5 μg/well), mutations of Lys-104 and Lys-91 inhibited B. burgdorferi attachment as well as wild-type DbpA. Mutations of Lys-14 (not shown) Lys-50, Lys-51, and Lys-102 also caused a significant reduction in the number of bacteria adhering to the decorin substrate whereas mutations of Lys-82, Lys-163 (not shown), and Lys-170 did not inhibit bacterial attachment. Likewise, the chemically modified DbpA protein K-mod and the unrelated His-tag protein OspC, did not affect bacterial attachment. Thus the ability of the DbpA mutants to interfere with the adherence of B. burgdorferi to decorin mimics their relative affinity for the proteoglycan.

Binding of DbpA Mutants to a Fibroblast Matrix—In the studies described above, we have used decorin extracted from bovine fetal skin under denaturing conditions as the ligand for DbpA (8). The conformation of decorin is probably different from the mature form of decorin that is found deposited in an extracellular matrix associated with other extracellular matrix components (27). We have previously shown that DbpA can be used in immunofluorescent staining of an extracellular matrix produced by cultured skin fibroblasts, demonstrating that extracellular matrix-incorporated decorin has binding sites available for DbpA (8). Similar fibrillar patterns were seen when DbpA or an antibody to decorin was used in these experiments (9). We have now examined the ability of the different DbpA proteins to bind to the fibroblast matrix using the immunofluorescent staining technique (Fig. 7). An extensive fibrillar extracellular matrix staining was observed when the cells were incubated with wild-type DbpA, Lys-51, or Lys-102 (Fig. 7, A, C, and F, respectively) or with Lys-14, Lys-32, Lys-40, Lys-91, or Lys-104 (not shown). Intermediate staining was seen when fibroblasts were incubated with Lys-50 (Fig. 7B), and matrix staining was not detected using Lys-82 or K-mod (Fig. 7, D and E, respectively) or Lys-163, Lys-170, or osteopontin (not shown). Incubation of the matrix with primary and secondary antibodies alone or secondary antibody resulted in no detectable binding (not shown). These results suggested that the different DbpA mutants have the same relative affinity for decorin in a fibroblast matrix compared with extracted, isolated decorin.

DISCUSSION

B. burgdorferi colonization of the skin during the initial stages of infection may be mediated in part by surface-exposed DbpA and DbpB that bind the collagen-associated proteoglycan.
decorin. These adhesins have recently been identified as vaccine candidates in various studies (12, 13, 16) and in fact may be more effective than OspA-based vaccines (15, 16). We have initiated a detailed analysis of the interaction of DbpA with decorin. We demonstrated here that lysine, which makes up 13% of the residues in recombinant DbpA (8), is involved in decorin binding.

Alignment of DbpA sequences from various B. burgdorferi genotypes (26) to the sequence of DbpA from strain 297, used previously in our laboratory (8), allowed us to identify six lysine residues conserved in most DbpA sequences and five residues conserved in all of them. These lysine residues were individually mutated to alanine and examined by various assays for decorin binding activity.

Analysis of Lys-14-Lys-170 mutants revealed that Lys-82, Lys-163, and Lys-170 were not recognized by decorin in a Western ligand blot and bound reduced amounts of decorin in an ELISA. Lys-51 bound decorin weakly compared with Lys-50 and Lys-91-Lys-104 in Western ligand analysis, but in the ELISA, Lys-51 behaved similarly to DbpA and the other decorin-binding mutant proteins. In the Western ligand blot assay no decorin binding to Lys-82, Lys-163, or Lys-170 was observed, whereas in the ELISA-type assay, these mutants bound reduced but significant amounts of decorin. This difference may be explained by a different conformational status of the DbpA in the two assays. In the Western ligand blots, the DbpA may be at least partly denatured (following SDS-PAGE) when the bacterial protein encounters decorin, whereas a properly folded form of DbpA may be the target for decorin binding in the ELISA-type assay. Consistent with previous results, we found that recombinant DbpA inhibited the adherence of Borrelia to decorin-coated microtiter plates. Most of the lysine mutants had effects similar to that of DbpA. However, Lys-82, Lys-163, and Lys-170 had no effect on Borrelia adherence, and Lys-51 had an intermediate effect.

The binding of DbpA to a decorin-containing matrix established by human fibroblasts provided an assay for analyzing the interaction of the bacterial protein with a “native” form of decorin, as the proteoglycan is associated with other extracellular matrix molecules. In this assay, DbpA and most lysine mutants, including Lys-51, bound to the extracellular matrix and revealed a fibrillar-like distribution on immunohistological

**Fig. 7.** Binding of DbpA, lysine mutants, or modified protein to a fibroblast extracellular matrix. Human skin fibroblasts were incubated with either 0.05 μg DbpA 549 (A), Lys-50 (B), Lys-51 (C), Lys-82 (D), K-mod (E), or Lys-102 (F). Cells were washed, incubated with polyclonal rabbit anti-DbpA, washed again, and incubated with a rhodamine-conjugated antibody against rabbit IgG. Magnification is × 20.
examination. However, Lys-82, Lys-163, Lys-170, or the unrelated protein osteopontin did not bind to the matrix.

Taken together, these results suggested that Lys-82, Lys-163, and Lys-170 are critical lysine residues involved in DbpA-decorin interactions. In addition to these three lysine residues, Lys-32 and Lys-104 are also conserved in all DbpA sequences examined so far. However, mutations of these residues did not alter the decorin binding capability of these recombinant proteins. Perhaps these two lysine residues are of importance in an aspect different from decorin binding. The K51A mutation appeared to result in a protein with somewhat reduced decorin binding activity, although the effect was not as dramatic as that observed for mutations of Lys-82, Lys-163, and Lys-170. Because K51A seems to be a relatively common natural variant (Fig. 2), it would be of interest to see whether the Borrelia strains carrying this variation at position 51 demonstrate a reduced adherence to decorin substrates.

The three lysine residues (Lys-82, Lys-163, and Lys-170) in DbpA identified in this study as critical for decorin binding are distributed throughout the protein. It is unclear whether these residues come together to form a binding pocket in the properly folded protein. We have previously shown that effective binding of decorin to DbpA involved an intact proteoglycan and that isolated glycosaminoglycan chains or core protein did not interfere with the decorin/DbpA binding. This observation might indicate the presence of two sites in DbpA that are engaged in decorin binding and that the critical lysine residues identified in this study could be involved in forming each of the hypothetical sites.

Acknowledgment—We thank Dr. Steve LaBrenz for technical assistance with the CD experiments and helpful discussions.

REFERENCES

1. Steere, A. C., Green, J., Schoen, R. T., Taylor, E., Hutchinson, G. J., Rahn, D. W., and Malawista, S. E. (1985) N. Engl. J. Med. 312, 869–874
2. Steere, A. C. (1989) N. Engl. J. Med. 9, 586–596
3. Szczepanski, A., and Benach, J. L. (1991) Microbiol. Rev. 55, 21–34
4. VanMierlo, P., Jacob, W., and Docks, P. (1993) Dermatology 186, 306–310
5. Duray, P. H. (1992) in Lyme Disease: Molecular and Immunologic Approaches (Schutzer, S. E., ed) pp. 11–30, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
6. Iozzo, R. V. (1997) Crit. Rev. Bioch. Mol. Biol. 32, 141–174
7. Iozzo, R. V. (1998) Annu. Rev. Biochem 67, 609–652
8. Guo, B. P., Brown, E. L., Durward, D. W., Rosenberg, L. C., and Hook, M. (1998) Mol. Microbiol. 30, 711–723
9. Hooking, A. M., Shinomura, T., and McQuillan, D. J. (1998) Matrix Biol. 17, 1–19
10. Sambri, V., Aldini, R., Massaria, F., Montagnani, M., Casanova, S., and Cevenini, R. (1996) Infect. Immun. 64, 1858–1861
11. Barthold, S. W., de Sousa, M. S. D., Janotka, J. L., Smith, A. L., and Persing, D. H. (1993) Am. J. Pathol. 143, 599–971
12. Feng, S., Hodzie, E., Stevenson, B., and Barthold, S. W. (1998) Infect. Immun. 66, 2827–2835
13. Hagman, K. E., Lahdenne, P., Popova, T. G., Porcella, S. F., Akins, D. R., Radolf, J. D., and Norgard, M. V. (1998) Infect. Immun. 66, 2674–2683
14. Patti, J. M., Allen, B. L., McGavin, M. J., and Hook, M. (1994) Annu. Rev. Microbiol. 48, 585–617
15. Cassatt, D. R., Patel, N. K., Ulbrantd, N. D., and Hanson, M. S. (1996) Infect. Immun. 66, 5379–5387
16. Hanson, M. S., Cassatt, D. R., Guo, B. P., Patel, N. K., McCarthy, M. P., Doward, D. W., and Hook, M. (1998) Infect. Immun. 66, 2143–2153
17. Barbour, A. G. (1984) Yale J. Biol. Med. 57, 521–525
18. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1989) Molecular Cloning: A Laboratory Manual, p. A.3, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
19. Choi, H. U., Johnson, T. L., Pal, S., Tang, L. H., Rosenberg, L. C., and Neame, P. J. (1989) J. Biol. Chem. 264, 2876–2884
20. Guo, B., Norris, S. J., Rosenberg, L. C., and Hook, M. (1995) Infect. Immun. 63, 3467–3472
21. Ho, S. N., Hunt, H. D., Horton, R. M., Pullen, J. K., and Pease, L. R. (1989) Gene 77, 51–59
22. McFarland, R. J., Garza, S., Butler, W. T., and Hook, M. (1995) Ann. N. Y. Acad. Sci. 760, 327–331
23. Acharya, A. S., Sussman, L. G., and Marjula, B. N. (1984) J. Chromatogr. 327–331
24. Patthy, L., and Smith, E. L. (1975) J. Biol. Chem. 250, 557–564
25. Takahashi, K. (1968) J. Biol. Chem. 243, 6171–6179
26. Roberts, W. C., Mullikin, B. A., Lathigra, R., and Hanson, M. S. (1998) Infect. Immun. 66, 5275–5285
27. Ramamurthy, P., Hocking, A. M., and McQuillan, D. J. (1996) J. Biol. Chem. 271, 19578–19584