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The outer surface protein A of the spirochete *Borrelia burgdorferi* is a plasmin(ogen) receptor

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ABSTRACT **The spirochete *Borrelia burgdorferi* is the causative agent of Lyme borreliosis (Lyme disease) and is transmitted to mammalian hosts by tick vectors. In humans, the bacteria induce a complex disease, which involves the skin, joints, heart, and nervous system. However, the pathogenic principles of this multisystem illness are far from being understood. To disseminate from the site of the tick bite and invade multiple organ sites, spirochetes have to penetrate normal tissue barriers, such as vascular basement membranes and other organized extracellular matrices. Substantial evidence from other invasive bacterial infections suggest that spirochetes may use endogenous or host-derived enzymes—in particular, proteinases—for this purpose. Here we show that *B. burgdorferi* binds human plasmin(ogen)—mainly via its outer cell surface lipoprotein A. Binding of plasminogen to spirochetal receptor leads to an accelerated formation of active plasmin in the presence of host-derived plasminogen activator. The cell-surface-associated plasmin cannot be regulated by the serum inhibitor α_2-antiplasmin and degrades high-molecular-weight glycoproteins, such as fibronectin. It is suggested that the acquisition of host-derived proteinase plasmin(ogen) contributes to the pathogenicity of *B. burgdorferi*.**

In humans, the spirochete *Borrelia burgdorferi* induces a multisystem disorder (Lyme borreliosis), which includes skin manifestations, such as erythema chronicum migrans, and acrodermatitis chronica atrophicans, as well as arthritis, carditis, and meningoencephalitis (1). Little is known about the mechanisms underlying spirochetal invasion. However, microorganisms have been recovered from skin lesions, blood, and cerebrospinal fluid and have been seen in small numbers in tissue sections of myocardium, retina, muscle, bone, spleen, liver, meninges, and brain (1, 2). Studies on *B. burgdorferi* infection in animal models—in particular, in mice—demonstrate that viable spirochetes are required for induction of the disease (3). Furthermore, spirochetes have been reported to adhere to and penetrate through endothelial cell layers *in vitro* (4, 5). These data indicate that *B. burgdorferi* organisms play an active role in the development of organ pathology. It is therefore of interest to examine features of the spirochetes that may be responsible for their potential to cross tissue barriers and to colonize distant organ sites.

A prerequisite for bacteria to invade normal tissues is the degradation of matrix proteins. There is ample evidence that plasmin, a trypsin-like serine proteinase, is involved in this process (6, 7). *In situ*, plasmin is generated by limited proteolysis from its serum-derived zymogen precursor plasminogen by the urokinase-type plasminogen activator (uPA) or tissue-type plasminogen activator (tPA) (8, 9). The latter enzymes (uPA, tPA) are produced and secreted by a variety of eukaryotic cells—including endothelial cells, in particular—under conditions of inflammation (10, 11). The activation of plasminogen by uPA and tPA is favored by the attachment of the individual reactants to appropriate high-molecular matrices, including bacterial cell surfaces (12, 13). Active plasmin has a broad substrate specificity and is able to dissolve blood clots, to degrade constituents of extracellular matrices and basement membranes such as fibronectin, and to activate latent collagenases (14). Thus plasmin seems to play a role in pericellular proteolysis of both eukaryotic (11) and prokaryotic (6, 7, 15) cells.

A number of bacteria, like *Yersinia pestis*, produce plasminogen activators that activate host-derived plasminogen (16). Alternatively, bacteria such as *Escherichia coli*, group A, C, and G streptococci, and *Neisseria gonorrhoeae* express surface receptors for plasmin(ogen), which bind plasminogen and facilitate its activation by host-derived plasminogen activators (6, 7, 17–19). We have asked whether the spirochete *B. burgdorferi* uses one of these two pathways.

MATERIALS AND METHODS

Materials. We used the plasmin-specific substrate D-valyl-L-leucyl-L-lysylparanitroanilide dihydrochloride (S-2251) and plasminogen (21.7 units/mg) (Chromogenix, Mölndal, Sweden); high-molecular-mass (54-kDa) uPA (Ukidan; Serono Laboratories, Freiburg, Germany); two-chain tPA (Paesel and Lorei, Frankfurt); and α_2-antiplasmin, tranexamic acid, and aprotinin (Sigma).

Bacteria and Recombinant Outer Surface Protein A (OspA). If not otherwise indicated, the pathogenic *B. burgdorferi* strain ZS7, grown in BSK medium, was used (3). Moreover, the following strains were used: Z37, NE58, NE11H, NE4 (L. Gern, Neuchatel, Switzerland), ZQ1 (3), and GeHo (20). Bacteria were washed three times in phosphate-buffered saline (PBS); if required, the second washing step was done with PBS/50 mM tranexamic acid to remove bound plasmin(ogen). Recombinant OspA was prepared as described (21).

Immunocytological Staining of Intact Bacteria. Bacteria (3×10^6) in 3 μl of PBS were coated onto microscopic glass slides by air-drying, blocked with PBS/2% bovine serum albumin (BSA) (15 min), and incubated for 1 hr at room temperature with human serum (serum/PBS, 1:2). After being washed three times in PBS/2% BSA the spirochetes were incubated for 1 hr at room temperature with polyclonal goat anti-human plasminogen IgG (no. 3104808; Behringwerke) and then incubated with fluorescein-labeled swine anti-goat IgG antibody (1:20, 30 min, room temperature; no. F205; Dakopatts, Glostrup, Denmark). Slides were mounted in glycerin/gelatine and analyzed by fluorescence microscopy.

Binding of Fluorescence-Labeled Plasminogen to Intact Bacteria. Plasminogen was labeled with the fluorochrome 5(6)-carboxyfluorescein-*N*-hydroxysuccinimide ester (FLUOS;

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Abbreviations: BSA, bovine serum albumin; mAb, monoclonal antibody; OspA, outer surface protein A; tPA, tissue-type plasminogen activator; uPA, urokinase-type plasminogen activator.

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no. 1386 093; Boehringer Mannheim). The bacteria were fixed onto microscopic glass slides (see above). After incubation in PBS/2% BSA for 15 min at room temperature, the spirochetes were incubated with FLUOS-plasminogen (20 μg/ml; 1 hr, room temperature) plus or minus tranexamic acid. After being washed the slides were examined by fluorescence microscopy.

Chromogenic Substrate Assays for Plasmin and Plasminogen Activators. Assay buffer was 30 mM Tris·HCl/60 mM NaCl, pH 7.4. If not otherwise indicated, intact *B. burgdorferi* organisms (20 μg/ml) were incubated with plasminogen (5×10^{-1} units/ml) plus or minus 50 mM tranexamic acid for 20 min at 37°C in 96-well flat-bottom microtiter plates. Then either 54 kDa uPA (10 ng/ml) or two-chain tPA (20 ng/ml) as well as the plasmin substrate S-2251 was added (0.4 mg/ml). The absorbance change at 405 nm was followed directly in the plate.

Interaction of Soluble and Bacteria Cell-Surface-Bound Plasmin with α_2-Antiplasmin: Functional Assay. Whole *B. burgdorferi* organisms were incubated with 5×10^{-2} units of plasmin per ml for 1 hr at 37°C and then centrifuged at 10,000 × *g* for 20 min and washed in PBS. The amount of cell-bound plasmin was then determined by adding the plasmin substrate S-2251. For comparison, an equivalent amount of soluble plasmin (4×10^{-2} units/ml) was tested. α_2-Antiplasmin (0.01–100 μg/ml) was added to the plasmin-loaded bacteria and to the soluble plasmin and allowed to react for 20 min at room temperature. The residual plasmin activity was then determined by addition of substrate S-2251.

Complex Formation Between ^{125}I-Labeled Plasmin and α_2-Antiplasmin. For this study intact bacteria were incubated with ^{125}I-labeled plasminogen (75 nM; 60 min; 37°C) plus or minus 50 mM tranexamic acid and then washed three times in PBS. High-molecular-weight uPA (1 μM) was then added to convert plasminogen into plasmin. After 30 min, α_2-antiplasmin was added to a final concentration of 7 μg/ml. After 30 min, preparations were centrifuged (30 min; 10,000 × *g*), and supernatants were removed (unbound ligand). The pellet (bacteria-bound ligand) was washed in PBS. Bacteria-bound and soluble (unbound) ligand were analyzed by SDS/PAGE (7.5% acrylamide gel, nonreducing conditions) and autoradiography.

Degradation of ^{125}I-Labeled Fibronectin. Intact bacteria were incubated for 1.5 hr at 37°C with 5×10^{-2} units of plasmin per ml plus or minus 50 mM tranexamic acid, washed three times in PBS to remove unbound plasmin, and then resuspended in assay buffer (see above). ^{125}I-Labeled fibronectin (8 mg/ml; 10^4 cpm) was added with or without aprotinin (1000 units/ml) for 8 hr at 37°C. The degradation was analyzed by SDS/PAGE (6.5% acrylamide, reducing conditions) and autoradiography.

SDS/PAGE. SDS/PAGE (15–20 μg of protein per lane) was done according to ref. 22. Separated proteins were silver-stained (23) or electrophoretically transferred to Immobilon-P membranes (Millipore). The membranes were then blocked with PBS/2% (wt/vol) BSA (Sigma). For immunoblotting (overnight, 4°C) polyclonal rabbit anti-recombinant OspA (ZS7) immunoglobulin [1:200 in Tris buffered saline/0.5% (wt/vol) BSA] or a mixture of monoclonal anti-OspA antibodies [1:100 in Tris buffered saline/0.5% (wt/vol) BSA] were used. For visualization a peroxidase-labeled goat anti-rabbit IgG antibody (no. 111.3646; Dianova, Hamburg, Germany) or goat anti-mouse IgG antibody (no. 115 035 071; Dianova) and chloronaphthol (peroxidase substrate) were used. For two-dimensional SDS/PAGE (24), isoelectric focusing (Pharmacia ampholytes: 1.45% pH 3.5–10; 0.1% pH 2.5–4.0; 0.2% pH 4–6; 0.2% pH 9–11) and SDS/PAGE under reducing conditions were combined.

Radioactive Ligand Binding. Immobilon-P membranes were blocked (2 hr; PBS/2% BSA) and incubated for 1.5 hr with ^{125}I-labeled plasminogen (iodinated by the chloramine T method) at a concentration of 1.65 nM (1×10^5 cpm/ml) in PBS/2% BSA. The membranes were washed (five times in PBS/2% BSA) and analyzed by autoradiography.

Scatchard Analysis. Five hundred nanograms of recombinant OspA per well was coated onto microtiter plates (Maxisorb F16, Nunc) overnight. After being washed with PBS (0.05% Tween 20, vol/vol) the plates were blocked with PBS/2% BSA for 2 hr. Coated wells were then incubated with increased concentrations (78 pmol/ml–10 nmol/ml) of a mixture of 12.8% ^{125}I-labeled plasminogen and 87.2% nonlabeled plasminogen in PBS/2% BSA for 1.5 hr at room temperature. After washing (four times in PBS/2% BSA) the bound radioactivity was solubilized by adding 1 M NaOH and then determined. Binding to 5 μg of BSA per ml instead of OspA was taken as control (unspecific binding) and subtracted. For estimation of the K_d the data were transformed according to Scatchard (25); the slope of the resultant curve represents $-1/K_d$.

RESULTS AND DISCUSSION

To test whether *B. burgdorferi* binds and or activates plasminogen viable spirochetes were incubated in human serum and subsequently analyzed for cell-surface-bound plasminogen with monoclonal or polyclonal antibodies to plasmin(ogen) (26). Only bacteria preincubated with serum (Fig. 1 *A–D*), but not with PBS (Fig. 1 *E–H*), were stained with the antiplasmin(ogen) Igg. The assumption that plasminogen specifically adheres to the surface of *B. burgdorferi* organisms was further substantiated by the finding that spirochetes also bind purified fluorochrome-labeled plasminogen (Fig. 1*I*). The fact that this binding is inhibitable in the presence of the lysine analogue tranexamic acid (Fig. 1*J*) indicates that plasminogen interacts with the surface of *B. burgdorferi* via its lysine-binding sites (27).

To determine whether binding of plasminogen to the spirochete facilitates its activation by human plasminogen activators, *B. burgdorferi* organisms were incubated with plasminogen and either uPA or tPA (Table 1). The proteolytic activity of plasmin was determined with the specific chromogenic substrate S-2251. Activation of plasminogen by both uPA and tPA (Table 1) was shown to be considerably enhanced (4- to 5-fold) in the presence of spirochetes. No or only marginal plasmin activity was seen with tranexamic

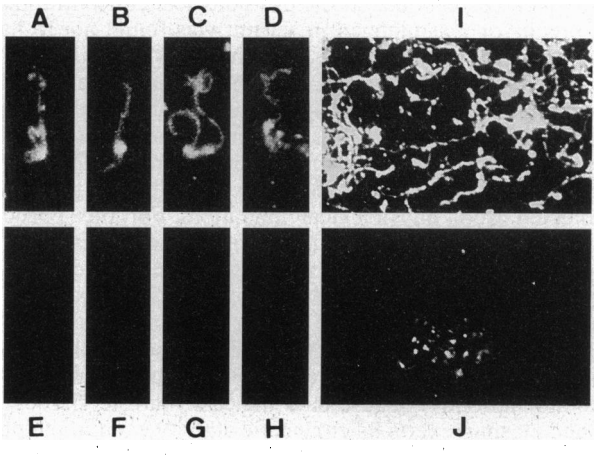

FIG. 1. Binding of serum-derived or purified plasminogen to intact *B. burgdorferi* (strain ZS7) organisms. Fixed spirochetes were preincubated in human serum/PBS, 1:2 (*A–D*) or in PBS (*E–H*) for 1 hr at room temperature. After being washed, bacteria were stained with goat anti-human plasminogen IgG followed by a fluorescein isothiocyanate-labeled swine anti-goat IgG. Alternatively, *B. burgdorferi* organisms were incubated with purified fluorochrome-labeled plasminogen (20 μg/ml) in the absence (*I*) or presence (*J*) of 50 mM tranexamic acid.

Table 1. Additions to enzyme reaction

Spirochete	Additions	A_{405} change rate/min $\times 10^{-3}$
Spirochetes	uPA, plasminogen, S-2251	20.500
Spirochetes	uPA, plasminogen, S-2251, tranexamic acid	1.030
None	uPA, plasminogen, S-2251	4.350
Spirochetes	plasminogen, S-2251	0.001
Spirochetes	tPA, plasminogen, S-2251	4.310
Spirochetes	tPA, plasminogen, S-2251, tranexamic acid	1.000
None	tPA, plasminogen, S-2251	0.750
Spirochetes	plasminogen, S-2251	0.002
$\text{Spirochetes}_{\text{plasmin}}$	S-2251	1.030
$\text{Spirochetes}_{\text{plasmin}}$	Tranexamic acid, S-2251	0.075
$\text{Spirochetes}_{\text{plasmin}}$	S-2251, aprotinin	0.041

Effect of *B. burgdorferi* on activation of plasminogen by uPA and tPA (spirochetes data) and binding of plasmin to *B. burgdorferi* ($\text{spirochetes}_{\text{plasmin}}$ data). uPA- and tPA-mediated plasminogen activation is accelerated in the presence of intact *B. burgorferi* organisms. No or only marginal plasmin activity is seen with tranexamic acid. Plasmin activity was determined by using chromogenic substrate S-2251. Data are presented as absorbance change rate per min $\times 10^{-3}$. For $\text{spirochetes}_{\text{plasmin}}$, whole spirochetes ($4 \times 10^7$) were incubated with plasmin (2×10^{-2} units/ml) in the presence or absence of 50 mM tranexamic acid for 1.5 hr at 37°C. Afterward spirochetes were washed, and enzyme activity of cell-bound plasmin was determined with S-2251. Absorbance at 405 nm was monitored for 4 hr, and data were prepared as absorbance change rate per min $\times 10^{-3}$. Spirochetes bind plasmin, and the binding is inhibited by tranexamic acid. Cell-surface-bound enzyme activity is inhibited by the low-molecular-mass plasmin inhibitor aprotinin (1000 units/ml).

acid, indicating that previous binding of plasmin(ogen) to the spirochete is a prerequisite for optimal cleavage by plasminogen activators. Furthermore, the fact that no plasmin was formed in the absence of plasminogen activators (Table 1 spirochete data) suggests that spirochetes do not express endogenous plasminogen activators.

B. burgdorferi was shown to also bind active plasmin (Table 1 $\text{spirochete}_{\text{plasmin}}$ data). The cell-surface-bound plasmin is enzymatically active and inhibitable by the low-molecular-weight inhibitor aprotinin, which was shown before to inactivate plasmin even after its binding to high-molecular-weight structures (28). No spirochete-associated plasmin activity was seen when *B. burgdorferi* organisms and plasmin were preincubated in the presence of tranexamic acid (Table 1 $\text{spirochete}_{\text{plasmin}}$ data), again indicating that, as for plasminogen, the lysine-binding site of plasmin is essential for its interaction with spirochetal cell-surface structures. In separate experiments (data not shown) plasmin at 0.15 mg/ml, which corresponds to the physiological concentration of its precursor plasminogen in serum, was found not to have any apparent effect on the integrity of *B. burgdorferi*, as judged by the motility and growth of spirochetes in BSK medium, as well as by their unaltered cell-surface phenotype.

The results described so far show that *B. burgdorferi* organisms can bind both human plasminogen and plasmin. We next determined whether the cell-surface-bound plasmin is protected from physiological inhibition. For this purpose, spirochetes were incubated in human serum, which contains high amounts of plasmin inhibitors—in particular, α_2-antiplasmin, the main inhibitor of plasmin (29)—in the presence of exogenous tPA. As shown in Fig. 2*A* considerable plasmin activity was generated under these conditions; the enzyme was inhibited by the low-molecular-weight plasmin-inhibitor aprotinin. These data indicate that the spirochete-bound plasmin exerts its enzymatic activity even in the presence of abundant α_2-antiplasmin. This assumption is further substantiated by experiments in which the enzymatic activity of equivalent amounts of spirochete-bound or free plasmin was tested after incubation with increased concentrations of α_2-antiplasmin (Fig. 2*B*). At a concentration of 1 mg of α_2-antiplasmin per ml, >70% of the plasmin activity was seen in the presence of bacteria, but only 4% or less was seen in the absence of bacteria. These data strongly suggest that spirochetes protect cell-surface-attached plasmin from binding to α_2-antiplasmin. This conclusion is corroborated by the finding that complex formation between α_2-antiplasmin and plasmin only occurred when plasmin was added in solution but was not spirochete-associated. This result was shown by incubating spirochetes with an excess of ^{125}I-labeled plasminogen and adding uPA in concentrations allowing processing of both bound and free zymogen into ^{125}I-labeled plasmin. Subsequently, α_2-antiplasmin was added, and after centrifugation spirochete-attached and soluble compounds were separated by SDS/PAGE and analyzed by autoradiography (Fig. 2*B Inset*). Complexes between plasmin/α_2-antiplasmin of molecular mass ≈160 kDa were only detected in the soluble fraction (Fig. 2*B*, lane b), but were not detected in the spirochete-associated fraction, which mainly contains noncomplexed plasmin (≈90 kDa; Fig. 2*B*, lane a).

So far the presented data indicate that *B. burgdorferi* (*i*) binds serum-derived plasminogen via its lysine-binding sites, (*ii*) facilitates processing of cell-surface-associated plasminogen by host-derived plasminogen activators, and (*iii*) protects the active enzyme against inhibition by serum-derived plasmin inhibitors. To analyze the proteolytic activity of spirochete-associated plasmin for physiological substrates, *B. burgdorferi* organisms were pretreated with plasmin and subsequently incubated with the radiolabeled high-molecular-mass glycoprotein fibronectin. SDS/PAGE and autoradiography of the incubation mixture reveal that spirochete-bound plasmin can completely degrade ^{125}I-labeled fibronectin to low-molecular-mass fragments (Fig. 3, lane a). No or only partial degradation of fibronectin was seen when spirochetes were preincubated (*i*) in the absence of plasmin (Fig. 3, lane b), (*ii*) with plasmin in the presence of tranexamic acid (Fig. 3, lane c), or (*iii*) with plasmin in the presence of aprotinin (Fig. 3, lane d).

Although plasmin(ogen) receptors have been isolated from bacteria in the past (6), corresponding structures from spirochetes have not been identified so far. To elucidate the putative plasminogen-binding structure(s) of *B. burgdorferi* bacterial lysates from strains corresponding to the genotypes *B. burgdorferi sensu strictu* and *B. burgdorferi garinii* (30) were subjected to SDS/PAGE. After their transfer to Immobilon-P membranes (ligand-blotting), the separated proteins (Fig. 4*A*, silver stain) were incubated with ^{125}I-labeled plasminogen. For all *B. burgdorferi* strains tested, the autoradiograms (developed after 42 hr) revealed one major binding

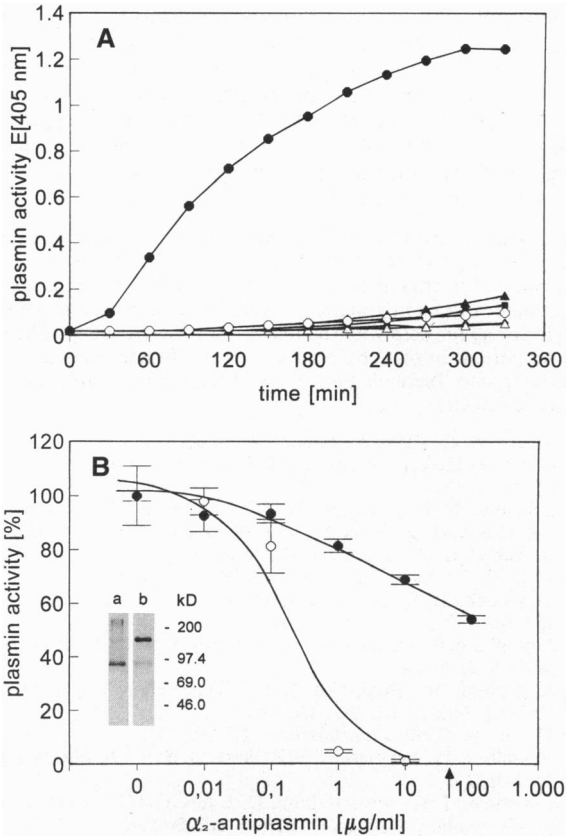

FIG. 2. (*A*) Effect of *B. burgdorferi* on activation of serum-derived plasminogen by tPA. *B. burgdorferi* organisms (7.5 μg) were mixed with human serum (1:50 in PBS). Plasminogen was converted into plasmin by tPA addition (70 ng/ml). Plasmin activity was determined by using the chromogenic substrate S-2251 (0.4 mg/ml). Enhanced plasmin activity was seen when serum and tPA were incubated in the presence (●) but was not seen in the absence (○) of spirochetes. tPA-mediated plasminogen activation in the presence of spirochetes was inhibited by 200 mM tranexamic acid (▲). When serum and spirochetes were incubated in the absence of tPA (■), only weak plasmin activity was seen. Serum alone (△) showed minimal plasmin activity. (*B*) Effect of α_2-antiplasmin on the enzymatic activity of spirochete-bound plasmin. Enzyme activity of cell-surface bound plasmin (●) was much less susceptible to inhibition by α_2-antiplasmin as compared with free plasmin (○). Graded concentrations of α_2-antiplasmin were added either to plasmin-loaded spirochetes or to an equivalent amount of free plasmin in the presence of chromogenic substrate S-2251. Residual enzyme activity was determined after 1 hr. Enzyme activity is presented as percentage of that obtained without α_2-antiplasmin. (*Inset*) Effect of spirochetes on complex formation of plasmin with α_2-antiplasmin. Spirochetes were incubated with ^{125}I-labeled plasmin and α_2-antiplasmin, as described. Sedimented bacteria (lane a) and supernatant (lane b) were analyzed by SDS/PAGE and autoradiography. Complex formation between plasmin is seen only with free plasmin (in supernatants) and is not seen with spirochete-associated enzyme. The molecular mass of plasmin is ≈90 kDa (kD) and that of the plasmin/α_2-antiplasmin complex is ≈160 kDa.

structure(s) in the molecular mass range of ≈31 kDa (Fig. 4*C*). Upon longer exposure (100 hr), two–five additional bands with molecular masses of ≈21 kDa, ≈34 kDa, ≈55 kDa, and ≈80 kDa were seen (Fig. 4*D*). Concomitant analyses of the gel with an anti-OspA immune serum (Fig. 4*B*) or mAbs (data not shown) (32) suggest that the major plasminogen-binding structure of spirochetes is OspA or a distinct molecule with a similar molecular mass. Further analyses revealed that the major plasmin(ogen)-binding structure comigrates with OspA in two-dimensional immunoblots (Fig. 4 *E* and *F*) and that various purified preparations of recombinant OspA, including the lipidated (not shown) and unlipidated forms, can bind ^{125}I-labeled plasminogen (Fig. 5, lane e). Binding of ^{125}I-labeled plasminogen to native OspA (Fig. 5, lane c) and to recombinant OspA (Fig. 5, lane e) was

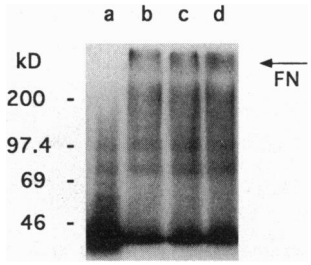

FIG. 3. Degradation of fibronectin by spirochete-associated plasmin. Spirochetes were preloaded with plasmin, as described in the legend to Table 1 and incubated with ^{125}I-labeled fibronectin. The reaction mixture was then separated by SDS/PAGE under reducing conditions and processed for autoradiography. Cell-surface-bound plasmin degrades high-molecular-mass ^{125}I-labeled fibronectin (FN) into small fragments (lane a); ^{125}I-labeled fibronectin is not degraded by untreated spirochetes (lane b) or by spirochetes preincubated with plasmin in the presence of tranexamic acid (lane c). Degradation of ^{125}I-labeled fibronectin by plasmin-loaded spirochetes is prevented in the presence of aprotinin (1000 units/ml) (lane d). The arrow indicates ^{125}I-labeled fibronectin. kD, kDa.

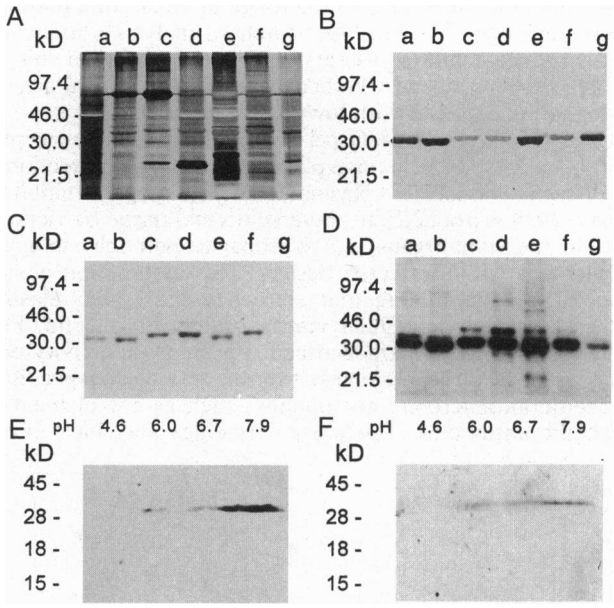

FIG. 4. Identification of plasminogen-binding structures of *B. burgdorferi*. Solubilized preparations of *B. burgdorferi* isolates ZS7 (lane a), B31 (lane b), NE58 (lane c), NE11H (lane d), GeHo (lane e), ZQ1 (lane f), and Z37 (lane g) were analyzed (≈15 μg per lane) by SDS/PAGE under reducing conditions, and proteins were visualized by silver staining (*A*). Alternatively, the separated proteins were electroblotted onto immobilon-P membranes and analyzed by immuno- (*B*) and ligand- (*C*) blotting. OspA of the genotype *B. burgdorferi sensu strictu* (lanes a, b, e, and g) and genotype *B. burgdorferi garinii* (lanes c, d, and f) (30) in the expected molecular mass range of ≈31 kDa (kD) was visualized by immunoblotting with an immune serum against purified recombinant OspA of *B. burgdorferi* strain ZS7 (*B*). Immobilon-P membranes were incubated with ^{125}I-labeled plasminogen. Upon short-term exposure (42 hr) one major binding protein was seen in the range of ≈31 kDa (*C*). Upon prolonged exposure of the blot (100 hr) additional weaker bands of ≈21, ≈34, ≈55, ≈60, and ≈80 kDa were seen (*D*). Cell lysates of spirochete (strain ZS7) were also separated by two-dimensional gel electrophoresis (31) and analyzed by immunoblotting using a mixture of the anti-OspA mAb LA 31.1 and LA 2.2 (32) (*E*) or by ligand-blotting using ^{125}I-labeled plasminogen (*F*) as described above.

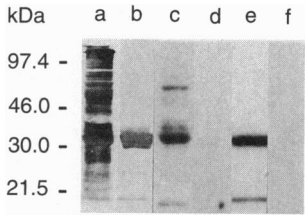

FIG. 5. Plasminogen binds to native and recombinant OspA. Solubilized preparations of *B. burgdorferi sensu strictu* (ZS7) or purified recombinant OspA (≈5 μg) corresponding to the OspA of ZS7 were separated by SDS/PAGE under reducing conditions. Silver stain of whole-cell lysate (lane a) or immunoblot of whole-cell lysate with anti-OspA mAbs as described in the legend to Fig. 4 (lane b). Ligand blot of total cell lysate (lanes c and d) or recombinant OspA (lanes e and f) with ^{125}I-labeled plasminogen in the absence (lanes c and e) or presence (lanes d and f) of 150 mM tranexamic acid.

inhibited by tranexamic acid (Fig. 5, lanes d and f). These results clearly show that OspA of *B. burgdorferi* is a prominent receptor for plasmin(ogen) and that the interaction is mediated by the lysine-binding site of the zymogen. However the data also indicate that *B. burgdorferi* expresses additional receptors for plasmin(ogen).

Radiolabeled plasminogen binds to immobilized recombinant OspA in a dose-dependent manner (Fig. 6). The calculated K_d of 0.26 mM estimated from the nonlinear Scatchard blot (data not shown) is in the range of eukaryotic plasminogen receptors (13). Because Scatchard analysis is applicable only to free solution systems, it is unclear whether this K_d value is accurate and whether there is an additional plasmin(ogen)-binding site with lower affinity on OspA.

Our results show that OspA is a major cell-surface receptor of *B. burgdorferi* for human plasmin(ogen). The protection of cell-surface-associated plasmin from physiological inhibitors may allow spirochetes to traverse normal tissue barriers, to colonize distant organs, and to propagate pathophysiological processes within affected tissues. This assumption is supported by the finding that spirochete-associated plasmin degrades the extracellular matrix protein fibronectin. Furthermore, the cell-surface-attached proteolytic activity may also protect spirochetes against serum-derived nonspecific or specific antimicrobial compounds, such as complement or specific antibodies. The present findings may have broad implications for invasive bacterial infections and support the concept of association of bacterial invasiveness with the generation of plasmin. Together with previous reports on the expression of endogenous plasminogen activators on Gram-positive and Gram-negative bacteria, these data suggest that prokaryotes have evolved at least two independent mechanisms to enhance their pathogenicity by use of the plasminogen-activator system.

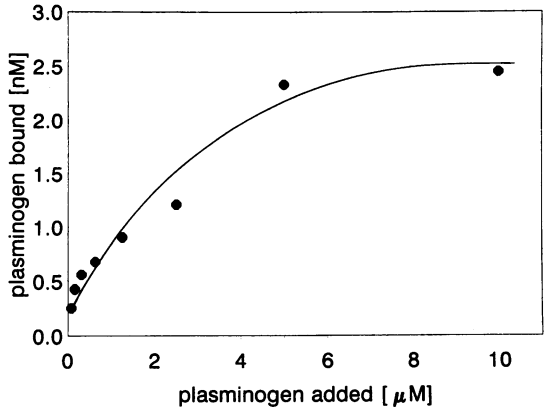

FIG. 6. Concentration-dependent binding of ^{125}I-labeled plasminogen to recombinant OspA (21, 33). Recombinant OspA was immobilized to microtiter plates and incubated with increased concentrations of ^{125}I-labeled plasminogen (abscissa). After being washed, the OspA/plasminogen complex was solubilized with 1M NaOH. Radioactivity of the solubilized material was determined in triplicates; the amount of bound plasminogen was calculated (ordinate).

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