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Purification of *Borrelia burgdorferi* Outer Surface Protein A (OspA) and Analysis of Antibody Binding Domains

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The major outer surface protein, OspA, of *Borrelia burgdorferi* is a lipoprotein which is of particular interest because of its potential as a vaccine candidate. However, serotypic and genetic analyses of OspA from both European and North American strains have demonstrated antigenic and structural heterogeneities. We purified OspA to homogeneity by exploiting its resistance to trypsin digestion. By treating spirochetes with trypsin and then using Triton X-114 extraction and ion-exchange chromatography, we obtained a yield of 2 mg of pure OspA protein per liter of culture. Intrinsic labeling with [^{14}C]palmitic acid confirmed that OspA was lipidated, and partial digestion established lipidation at the amino-terminal end of the molecule. The reactivity of five anti-OspA murine monoclonal antibodies to nine different isolates of *B. burgdorferi* was ascertained by Western blot (immunoblot) analysis. Purified OspA was fragmented by enzymatic or chemical cleavage, and the monoclonal antibodies were able to define four distinct immunogenic domains. Further resolution of the epitope specificity to determine humoral and cellular immune responses to OspA has implications for vaccine development and for the utility of this protein as a reagent in diagnostic testing for Lyme borreliosis.

Infection with *Borrelia burgdorferi* is the most common vector-borne disease in North America and Europe. The major outer surface protein, OspA, of *B. burgdorferi* is a highly basic lipoprotein that has a molecular mass of approximately 31 kDa and that is encoded on a linear plasmid (20). Analysis of isolates of *B. burgdorferi* obtained in North America and Europe has demonstrated that OspA has antigenic variability and that several distinct groups can be serologically defined (34). In murine models of infection, passively transferred anti-OspA monoclonal antibodies (MAbs) have been shown to be protective, and vaccination with a recombinant protein induced protective immunity against a subsequent infection with the homologous strain of *B. burgdorferi* (1b, 4, 31). OspA has been proposed as a vaccine candidate (14, 15, 31); however, protection appears to be serotype specific.

Epitope mapping has been carried out with recombinant fusion proteins or truncated forms of OspA (27–29). Anti-OspA MAbs which bind to specific amino- and carboxy-terminal antigenic determinants have been reported (27). However, only MAbs binding to carboxy-terminal immunogenic domains protect mice against a spirochetal challenge (28). In humans, antibodies occurring during a natural infection also react to domains within the carboxy end of the molecule but not to the same epitopes as those bound by protective murine MAbs (27–29). Structural analysis and epitope mapping of OspA are dependent upon obtaining sufficient quantities for analysis. Full-length OspA is poorly soluble, and the recombinant form is not well expressed in *Escherichia coli* (11). Therefore, it is difficult to obtain OspA in large quantities. Dunn and colleagues (11) were able to express and purify to homogeneity large quantities of a truncated form of OspA in which the first 17 amino acids, including the predicted lipidation site, were deleted. Recently, it was found that this nonlipidated, soluble form of OspA is less antigenic than its lipidated analog (13). Therefore, for vaccination with lipidated OspA to be feasible, it has become imperative to develop the methodology needed to produce large quantities of lipidated OspA.

We report a method for the purification of large amounts of native OspA to homogeneity and describe the mapping of the antigenic specificities of several MAbs to the native protein. Detergent solubilization of *B. burgdorferi* strips the outer surface proteins but yields partially purified preparations containing both OspA and OspB (1b, 6, 9, 10, 26). In contrast to OspB, OspA is resistant to cleavage by trypsin (2, 3). We exploited this characteristic of OspA to remove OspB with trypsin prior to extraction with Triton X-114. The availability of the derived amino acid sequence for OspA from a number of different isolates, combined with peptide mapping and Western blot (immunoblot) analysis, has permitted us to identify the antigenic domains recognized by MAbs and infer the key amino acid residues responsible for specific antibody reactivities.

MATERIALS AND METHODS

Strains of *B. burgdorferi*. Nine strains of *B. burgdorferi* were used in this study, seven European and two North American. The European strains K48, P/Gau, and DK29 (isolated in Czechoslovakia, Germany, and Russia, respectively) were kindly supplied by Russell Johnson, University of Minnesota (35); PKo and TRo (isolated in Germany) were kindly provided by Bettina Wilske and Vera Preac-Mursic, Pettenkhofer Institute, Munich, Germany; and IP3 (isolated in France) and IP90 (isolated in Russia) (19) were generously supplied by Leonard Mayer, Centers for Disease Control and Prevention, Atlanta, Ga. The North American strains included 25015 (16), which was a gift from John Anderson, Connecticut Department of Agriculture, and B31 (ATCC 35210; isolated in Shelter Island, N.Y.) (7).

MAbs. Seven MAbs were used in this study. Five of the

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MAbs, 12, 13, 15, 83, and 336, were produced from hybridomas cloned and subcloned as previously described (27). MAbs H5332 (3) and CIII.78 (28) were supplied by Alan Barbour, University of Texas, and Richard A. Flavell, Yale University, respectively. MAbs 12 and 15 were raised against whole sonicated strain B31, and MAb 336 was produced against whole strain P/Gau. MAbs 13 and 83 were raised against a truncated form of OspA cloned from strain K48 and expressed in *E. coli* by use of the T7 RNA polymerase system (23). All MAbs were typed as immunoglobulin G.

Intrinsic radiolabeling of *B. burgdorferi* B31. Labeling of lipoproteins was performed as described by Brandt et al. (6). [^{14}C]palmitic acid (ICN, Irvine, California) was added to BSKII medium to a final concentration of 0.5 μCi/ml. Organisms were cultured at 34°C in BSKII medium (1a) until a density of 10^8 cells per ml was achieved.

Purification of OspA. *B. burgdorferi*, either unlabeled or [^{14}C]palmitic acid labeled, was harvested and washed as described previously (6). Whole organisms were trypsinized by the protocol of Barbour et al. (2) with some modifications. The pellet was suspended in phosphate-buffered saline (PBS; 10 mM; pH 7.2) containing 0.8% tosyl-L-phenylalanine chloromethyl ketone (TPCK)-treated trypsin (Sigma, St. Louis, Mo.), the latter at 1 μg/10^8 cells. The reaction was carried out at 25°C for 1 h, following which the cells were centrifuged. The pellet was washed in PBS with 100 μg of phenylmethylsulfonyl fluoride per ml. Triton X-114 partitioning of the pellet was carried out as described by Brandt et al. (6). Following trypsin treatment, cells were resuspended in ice-cold 2% (vol/vol) Triton X-114 in PBS at 10^9/ml. The suspension was rotated overnight at 4°C, and the insoluble fraction was removed as a pellet after centrifugation at 10,000 × *g* for 15 min at 4°C. The supernatant (soluble fraction) was incubated at 37°C for 15 min and centrifuged at room temperature at 1,000 × *g* for 15 min to separate the aqueous and detergent phases. The aqueous phase was decanted, and ice-cold PBS was added to the lower, Triton X-114 phase, which was then mixed, warmed to 37°C, and centrifuged at 1,000 × *g* for 15 min. Washing was repeated two more times. Finally, detergent was removed from the preparation with a spin column of Bio-beads SM2 (Bio-Rad, Melville, N.Y.) as described previously (18). Ion-exchange chromatography was performed as described by Dunn et al. (11) with minor modifications. Crude OspA was dissolved in buffer A (1% Triton X-100, 10 mM Tris-HCl [pH 5.0]) and loaded onto SP Sepharose resin (Pharmacia, Piscataway, N.J.) preequilibrated with buffer A at 25°C. After the column was washed with 10 bed volumes of buffer A, bound OspA was eluted with buffer B (1% Triton X-100, 10 mM Tris-HCl [pH 8.0]). OspA fractions were detected by a protein assay using bicinchoninic acid (Pierce, Rockford, Ill.) or as radioactivity when intrinsically labeled material was fractionated. Triton X-100 was removed with a spin column of Bio-beads SM2.

Protein cleavage. Hydroxylamine-HCl, *N*-chlorosuccinimide, and cyanogen bromide cleavage of OspA was done by the methods described by Bornstein (5), Shechter et al. (30), and Gross (17), respectively. Protease cleavage with endoproteinase Asp-N (Boehringer Mannheim, Indianapolis, Ind.) was performed as described by Cleveland et al. (8). Ten micrograms of OspA was used for each reaction. The ratio of enzyme to OspA was approximately 1:10 (wt/wt). Prediction of the various sites of cleavage of OspA from the strains could be accomplished by use of the primary amino acid sequence derived from the full nucleotide sequence of OspA (4, 12).

Detection of the anti-*Borrelia* activities of MAbs. The activities of MAbs against whole OspA and its fragments were determined by Western blot analysis. Proteins and peptides were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) (21) and electroblotted onto Immobilon polyvinylidene difluoride membranes (24). They were detected by amido black staining or by binding with murine MAbs and then by staining with alkaline phosphatase-conjugated goat anti-mouse immunoglobulin G. Specific binding was detected with a 5-bromo-4-chloro-3-indolylphosphate (BCIP)–nitroblue tetrazolium developer system (KPL Inc., Gaithersburg, Md.). The ability of the MAbs to agglutinate various strains of borreliae was assessed with a modified agglutination assay as described by Sadziene et al. (25). In brief, washed spirochetes were suspended at a concentration of 10^9 cells per ml in CMRL 1066 (Sigma) containing 10% bovine serum albumin. Duplicate 100-μl volumes of spirochetes and 100 μl of tissue culture supernatant containing MAbs which had been serially diluted twofold in the modified CMRL 1066 suspension buffer were added to the wells of a polystyrene, round-bottomed, 96-well microtiter plate (Corning Glass, Corning, N.Y.). The spirochete-MAb mixture was coincubated at 22°C for 30 min and examined visually and microscopically. A well was considered positive if a pellet formed at the bottom after gentle shaking. Agglutination was confirmed by dark-field microscopy.

TABLE 1. Antigenic variability of *B. burgdorferi* OspA as determined by Western blot analysis

B. burgdorferi genospecies[a] and strains	Reactivity[b] with the following MAb:					
	15	13	H5332	12	83	336
I						
B31	+	−	+	+	−	+
25015	+	−	+	+	−	+
II						
TRo	−	−	−	−	−	+
K48	+	+	+	−	+	−
DK29	+	+	+	−	+	−
IP90	+	+	−	−	+	−
III						
PKo	−	−	+	−	+	+
P/Gau	−	−	+	−	+	+
IP3	−	−	+	−	+	+

[a] See references 1, 4a, 12, 32, and 34.
[b] +, reactive; −, not reactive.

Amino-terminal protein sequencing. Amino-terminal amino acid sequence analysis was carried out as described by Luft et al. (22). Amido black-stained bands were excised from polyvinylidene difluoride blots and sequenced by Edman degradation by use of an Applied Biosystems model 475A sequencer with a model 120A phenylthiohydantoin analyzer and a model 900A control and data analyzer.

RESULTS

Antigenic variability of OspA. We tested nine strains of *B. burgdorferi* against six different murine anti-OspA MAbs by Western blot analysis (Table 1). Strains K48 and DK29 expressed an OspA band at 31 kDa and no OspB. Western blots with monospecific antibodies against OspB confirmed that both strains were completely deficient in OspB (data not shown). Western blots of replicate gels probed with the different anti-OspA MAbs showed that K48 and DK29 had similar patterns of reactivity, as did strains IP3, P/Gau, and PKo. OspA of TRo was immunologically distinct from the

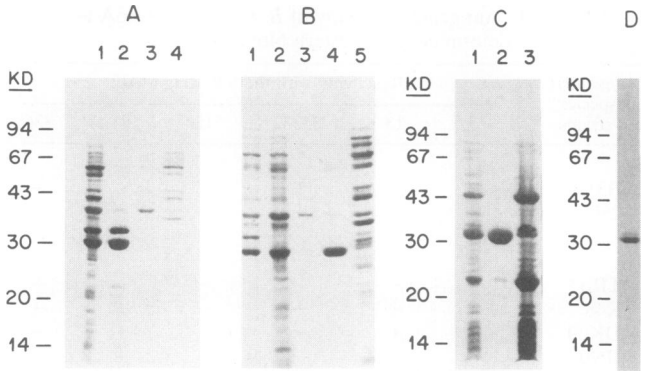

FIG. 1. Purification of OspA from strains B31 and K48. (A) Triton X-114 partitioning of strain B31 borreliae without trypsin treatment. Lanes: 1, whole cells; 2, Triton X-114-soluble fraction, detergent phase; 3, Triton X-114-insoluble fraction; 4, Triton X-114-soluble fraction, aqueous phase. (B) Triton X-114 partitioning of B31 borreliae after trypsin treatment. Lanes: 1, whole cells; 2, trypsin-treated cells; 3, Triton X-114-insoluble fraction; 4, Triton X-114 phase of soluble fraction; 5, aqueous phase of soluble fraction. (C) Triton X-114 partitioning of strain K48 (trypsin treatment was omitted). Lanes: 1, whole cells; 2, Triton X-114 phase of soluble fraction; 3, Triton X-114-insoluble fraction. (D) OspA of B31 purified by SP Sepharose ion-exchange chromatography.

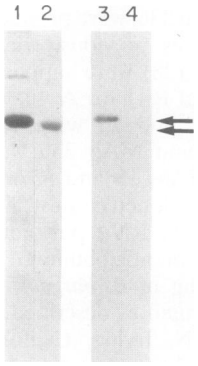

FIG. 2. SDS-PAGE analysis of purified nonreacted endoproteinase Asp-N digests (8) of purified OspA of strain B31 labeled with [^{14}C]palmitic acid. Lanes: 1, nonreacted OspA of B31; 2, OspA of B31 partially digested with endoproteinase Asp-N; 3 and 4, autoradiograms of lanes 1 and 2, respectively.

others, being recognized by MAb 336 only. MAb 12 recognized only the two North American strains, B31 and 25015. When the strains were arranged into genospecies, it was noted that all the MAbs, except for MAb 12, cross-reacted with multiple genospecies.

The MAbs were also evaluated in an agglutination assay. MAbs 13 and 15 failed to agglutinate any of the spirochetes listed in Table 1. In contrast, MAbs 12, 83, and 336 agglutinated the spirochetes in a manner that directly corresponded to their Western blot reactivity.

Purification of lipoprotein OspA from strains B31 and K48. Previous studies reported that although OspA is a lysine-rich protein, it is resistant to trypsin. By using this property, we were able to purify OspA from an outer surface membrane preparation. The results obtained at each step of the purification of OspA are shown in Fig. 1. In the absence of trypsin treatment, OspA and OspB were the major components of the soluble fraction remaining after Triton X-114 partitioning of strain B31 (Fig. 1A, lane 2). In contrast, when Triton X-114 extraction was added after trypsin treatment, the OspB band did not appear (Fig. 1B, lane 4). Because strain K48 lacks OspB, trypsin treatment was omitted, and OspA was again the major component of the soluble fraction remaining after Triton X-114 partitioning (Fig. 1C, lane 2). Further purification of B31 OspA on an SP Sepharose column (Fig. 1D) resulted in a single band in SDS-PAGE. The yield following the removal of detergent for both strains was approximately 2 mg/liter of culture.

Lipidation site of OspA of strain B31. [^{14}C]palmitic acid-labeled OspA of B31 was purified as described above and partially digested with endoproteinase Asp-N (Fig. 2). Following digestion, a new band with a lower molecular weight was apparent in SDS-PAGE (arrows) and found by direct amino-terminal sequencing to begin at Asp-25. This band had no trace of radioactivity in autoradiography (Fig. 2, lanes 3 and 4). OspA and OspB contain a signal sequence (L-X-Y-C) similar to the consensus sequence described for lipoproteins of *E. coli*, and it has been predicted that the lipidation site of OspA and OspB should be the amino-terminal cysteine (6). Our result supports this prediction.

Peptide mapping of OspA of strains B31 and K48. Using hydroxylamine-HCl, we fragmented purified OspA of B31 labeled with [^{14}C]palmitic acid into two peptides, designated HA1 and HA2 (Fig. 3, lane 2). The HA1 band migrated at 27 kDa and retained its radioactivity, indicating that this peptide includes the lipidation site at the N terminus of the molecule (Fig. 3, lane 7). From the predicted cleavage point, HA1 should correspond to residues 1 to 251 of B31 OspA. HA2 had a molecular mass of 21.6 kDa in SDS-PAGE, with amino-terminal sequence analysis indicating that it begins at Gly-72, i.e., residues 72 to 273 of B31 OspA (Fig. 3, lane 2). In contrast, hydroxylamine-HCl cleaved OspA of K48 into three peptides, designated HA1, HA2, and HA3 (Fig. 4A, lane 2), with apparent molecular masses of 22, 16, and 12 kDa, respectively. Amino-terminal sequencing showed HA1 to start at Gly-72 and HA3 to start at Gly-142. HA2 was found to have a blocked amino terminus, as was observed for the full-length OspA

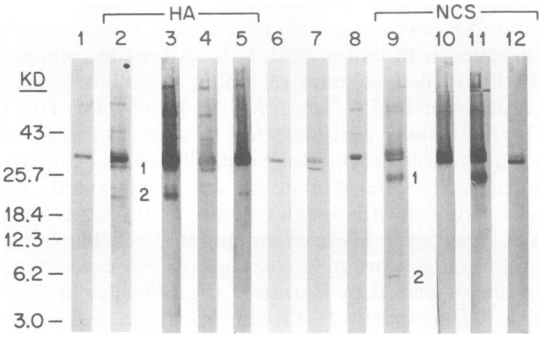

FIG. 3. Chemical cleavage of purified OspA of B31 with hydroxylamine-HCl (HA) and *N*-chlorosuccinimide (NCS). Lanes: 1 and 8, noncleaved OspA of B31; 2 and 5, replica blots of HA-cleaved OspA of B31; 9 and 12, replica blots of *N*-chlorosuccinimide-cleaved OspA of B31. The blots were stained with amido black (lanes 1, 2, 8, and 9) or reacted with MAb 12 (lanes 3 and 10), MAb 15 (lanes 4 and 11), or MAb 336 (lanes 5 and 12). Lanes 6 and 7 are autoradiograms of OspA of B31 labeled with [^{14}C]palmitic acid (lane 6, noncleaved; lane 7, hydroxylamine-HCl cleaved). Specific binding was detected as described in the legend to Fig. 1. Numbers beside gels indicate peptides.

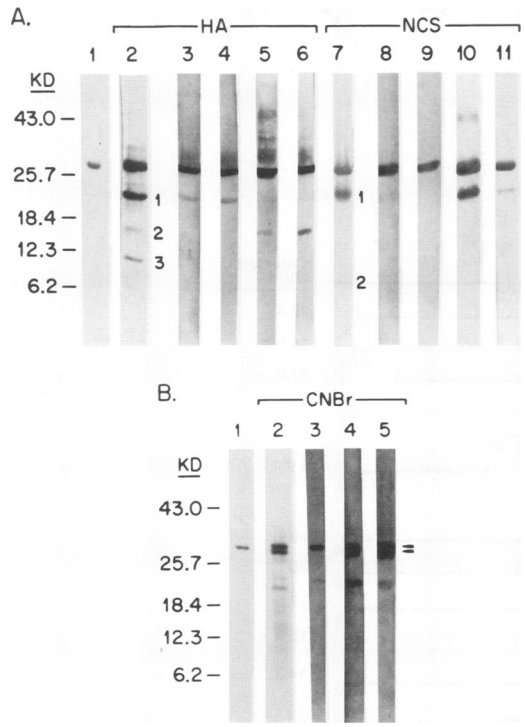

FIG. 4. Chemical cleavage of purified OspA of K48. (A) Lanes: 1, noncleaved OspA of K48; 2 and 6, replica blots of hydroxylamine-HCl (HA)-cleaved OspA of K48; 7 and 11, replica blots of *N*-chlorosuccinimide (NCS)-cleaved OspA of K48. The blots were stained with amido black (lanes 1, 2, and 7) or immunoblotted with MAb 83 (lanes 3 and 8), MAb H5332 (lanes 4 and 9), MAb 13 (lanes 5 and 10), or MAb 15 (lanes 6 and 11). The secondary antibody was as above. Numbers beside gels indicate peptides. (B) Lanes: 1, noncleaved OspA of K48; 2, blot of cyanogen bromide (CNBr)-cleaved OspA of K48. The blots were stained with amido black (lanes 1 and 2) or incubated with MAb 13 (lane 3), MAb 15 (lane 4), or MAb H5332 (lane 5). Upper arrow, uncleaved OspA; lower arrow, NCS-cleaved OspA.

protein. HA1, HA2, and HA3 of K48 OspA are predicted to be residues 72 to 274, 1 to 141, and 142 to 274, respectively.

N-Chlorosuccinimide cleaves the carboxy terminus at tryptophan (W), which is at residue 216 of B31 OspA or residue 217 of K48 OspA. As shown in Fig. 3, lane 9, *N*-chlorosuccinimide cleaved B31 OspA into two fragments, NCS1, of 23 kDa and representing residues 1 to 216 of the protein, and NCS2, of 6.2 kDa and representing residues 217 to 273. Similarly, K48 OspA was divided into two fragments, NCS1, representing residues 1 to 217, and NCS2, representing residues 218 to 274 (Fig. 4A, lane 7).

Cleavage of OspA by cyanogen bromide occurs at the carboxy-terminal methionine, residue 39. The major fragment, CNBr1, has a molecular mass of 25.7 kDa and represents residues 39 to 274, as determined by amino-terminal amino acid sequence analysis (Fig. 4B, lane 2). CNBr2 (about 4 kDa) could not be visualized by amido black staining; instead, lightly stained bands of about 20 kDa were observed. These bands reacted with anti-OspA MAbs and were likely to be degradation products resulting from cleavage by formic acid.

Determination of antibody-binding domains for anti-OspA MAbs. The cleavage products of OspA of strains B31 and K48 were analyzed by Western blotting to assess their ability to bind to six different MAbs (Fig. 3 and 4). MAb 12, specific for B31 OspA (Table 1), bound to both HA1 and HA2 of B31 OspA. However, cleavage of B31 OspA by *N*-chlorosuccinimide at residue Trp-216 created two fragments which did not react with MAb 12 (Fig. 3, lane 10), suggesting that the relevant domain is near or is structurally dependent upon the integrity of this residue. MAb 13 bound only to K48 OspA (Table 1) and to peptides containing the amino terminus of the molecule (e.g., HA2, Fig. 4A, lane 5, and NCS1, lane 10). It did not bind to CNBr1 (Fig. 4B, lane 3) (residues 39 to 274). Thus, the domain recognized by MAb 13 is in the amino-terminal end of K48 OspA, near Met-38.

MAb 15 reacted with OspA of both strains B31 and K48 (Table 1) and with peptides containing the N terminus of OspA, such as HA1 of B31 OspA (Fig. 3, lane 4) and NCS1 of B31 OspA and K48 OspA (Fig. 3, lane 11, and Fig. 4A, lane 11, respectively) but not with peptides HA2 of B31 OspA and HA1 of K48 OspA (Fig. 3, lane 4, and Fig. 4A, lane 6, respectively). Both of the former peptides include residues from position 72 to the C terminus of the molecules. MAb 15 bound to CNBr1 of K48 OspA (Fig. 4B, lane 4), indicating the domain for this antibody to be residues 39 to 72, specifically, near Gly-72.

MAb 83 bound to K48 OspA (Table 1) and to peptides containing the C-terminal portion of the molecule, such as HA1 (Fig. 4A, lane 3). It did not bind to HA2 of K48 OspA, most likely because the C terminus of HA2 of K48 OspA ends at residue 141. Like MAb 12 binding to B31 OspA, the binding of MAb 83 and MAb CIII.78 to B31 OspA was eliminated by cleavage of OspA at the tryptophan residue (data not shown). Thus, the binding of MAb 12, MAb 83, and MAb CIII.78 to OspA depends on the structural integrity of the Trp-216 residue, which appears to be critical for antigenicity.

Although there was a similar loss of binding activity of MAb 336 with cleavage at Trp-216, this MAb did not bind to HA1 of B31 OspA (Fig. 3, lane 5), suggesting that the domain for this antibody includes the carboxy-terminal end of the molecule, including residues 251 to 273. Low-molecular-mass peptides, such as HA3 (10 kDa) and NCS2 (6 kDa) of K48 OspA, did not bind to this MAb on Western blots. To confirm this observation, we tested the binding of the six MAbs with a recombinant fusion construct, p3A/EC, that contains a TrpE leader protein fused to residues 217 to 273 of B31 OspA (27). Only MAb 336 reacted with this construct (data not shown). Peptides and antigenic domains localized by fragmentation of OspA are summarized in Fig. 5.

Comparison of the sequences of OspA in nine strains of *B. burgdorferi*. To define the molecular basis for our serotype analysis of OspA, we compared the derived amino acid sequences of OspA for nine strains (12) (Fig. 6). At the amino terminus of the protein, the predictions can be more precise, given the relatively small number of amino acid substitutions in this region compared with the carboxy terminus. Domain 1, which is recognized by MAb 13, includes residues Leu-34 to Leu-41. Since MAb 13 binds only to OspA of strains K48, DK29, and IP90, the relevant residue for these three strains must be Gly-37. When Gly-37 is changed to Glu-37, as it is in OspA of strains B31, TRo, P/Gau, and PKo, MAb 13 does not recognize the protein (Table 1). By a similar analysis, it can be shown that Asp-70 is a crucial residue for domain 2, which includes residues 65 to 72 and is recognized by MAb 15. Domain 3 is reactive with MAb H5332, MAb 12, and MAb 83 and includes residues 190 to 220. It is clear that significant heterogeneity exists between MAbs reactive with this domain (Table 1) and that more than one conformational epitope must be contained within the sequence. Domain 4 binds MAb 336 and includes residues 250 to 270. In this region, residue 266 is variable and therefore may be an important determinant. It is apparent that other determinants of the reactivity of MAb 336

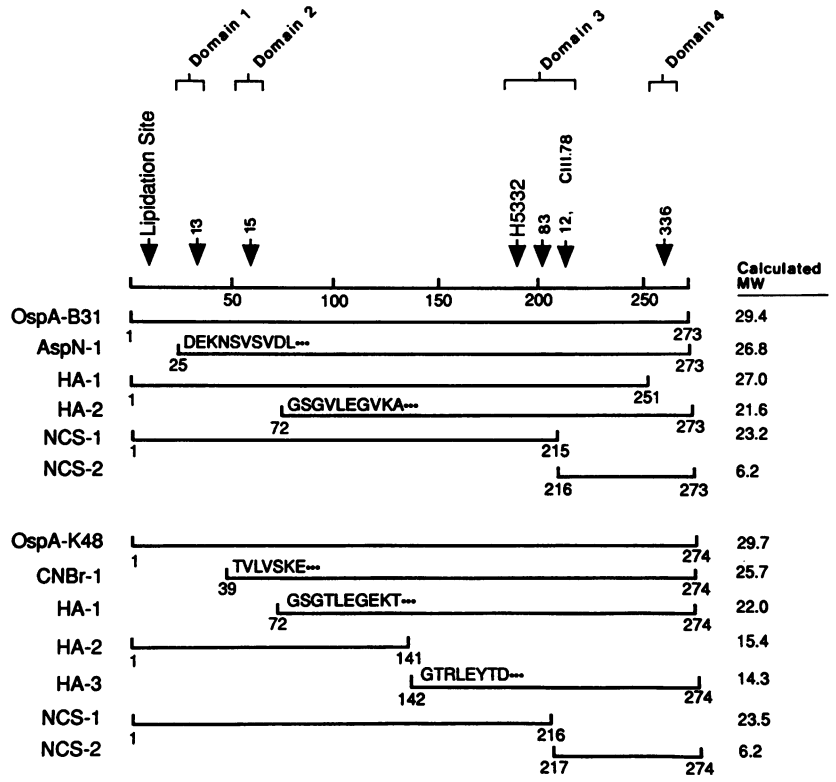

FIG. 5. Maps of OspA of B31 and OspA of K48 indicating the MAb-binding domains (top) and the N- and C-terminal residues of the fragments obtained by chemical or proteolytic cleavage (bottom). The N-terminal sequences obtained are shown to the left of cleavage points. MW, molecular weight, in thousands.

reside in the region comprising amino acids 217 to 250. Furthermore, the structural integrity of Trp-216 is essential for antibody reactivity in the intact protein. Finally, it is important to stress that Fig. 6 indicates the locations of the domains only and does not necessarily encompass the entirety of the domains. Exact epitopes are being analyzed by site-directed mutagenesis of specific residues.

DISCUSSION

In this report, we describe a direct method for the isolation and purification of native OspA. Full-length lipidated OspA is expressed very poorly in *E. coli* compared with truncated nonlipidated constructs. It has been postulated that the protein may be toxic in this prokaryotic expression system (34, 35). In contrast, for *B. burgdorferi* cultured in BSKII medium, OspA and OspB constitute approximately 30% of the total cellular protein (3). Therefore, our ability to easily purify large quantities of native protein may have important implications for strategies used to obtain reagents for the development of an OspA-based vaccine, as well as for diagnostic tests.

Detergent solubilization has been used by other investigators to purify outer envelope proteins of various microorganisms. The relative resistance of OspA in comparison with OspB to trypsin digestion was first noted by Barbour et al. (2). Both proteins were sensitive to proteinase K digestion. The relative insensitivity of OspA to trypsin digestion is surprising in view of the fact that OspA has a high lysine content (16% for B31) and may relate to the relative configurations of OspA and OspB in the outer membrane or perhaps the presence of the salt bridges which form. Arguing against the latter hypothesis is the observation that truncated recombinant OspA is also resistant to trypsin, plasmin, and the bacterial protease OmpT (11).

Earlier efforts to purify the surface proteins of *B. burgdorferi* employed low concentrations of SDS (9, 26), *N*-laurylsarcosine (1b, 3), Triton X-100 (26), and Triton X-114 (6, 10). Brandt et al. (6) used Triton X-114 phase partitioning with labeling of cells by [^{3}H]palmitate to demonstrate the presence of Osp proteins in the outer envelope, as well as other lipoproteins apparent by autoradiography. In this protocol, we used trypsin to remove OspB (and perhaps other surface proteins) from strains in which this protein is expressed and ion-exchange chromatography, which has proven useful for the purification to homogeneity of recombinant OspA expressed in the T7 RNA polymerase system (11). Since the cloud point of Triton X-114 is 20°C, this detergent has a significant advantage for separating membrane proteins from many hydrophilic proteins. The protocol that we employed is simple and provides a high yield (2 mg/1,000 ml of culture) of purified OspA. In addition to its usefulness for strains B31 and K48, we have used it successfully for strains DK29 and P/Gau and expect it to be generally applicable for other strains of *B. burgdorferi*.

We previously mapped antibody-binding domains for a series of deletion constructs of OspA expressed as TrpE fusion proteins in *E. coli* (27). That study confirmed that two MAbs with distinct immunoreactivities in Western blot analysis of whole *B. burgdorferi* were indeed directed at distinct epitopes of the molecule. MAb 184.1, whose reactivity is similar but not identical to that of MAb 15 in this report, and MAb 105.5, analogous to MAb 12 in this report, were specific for a region

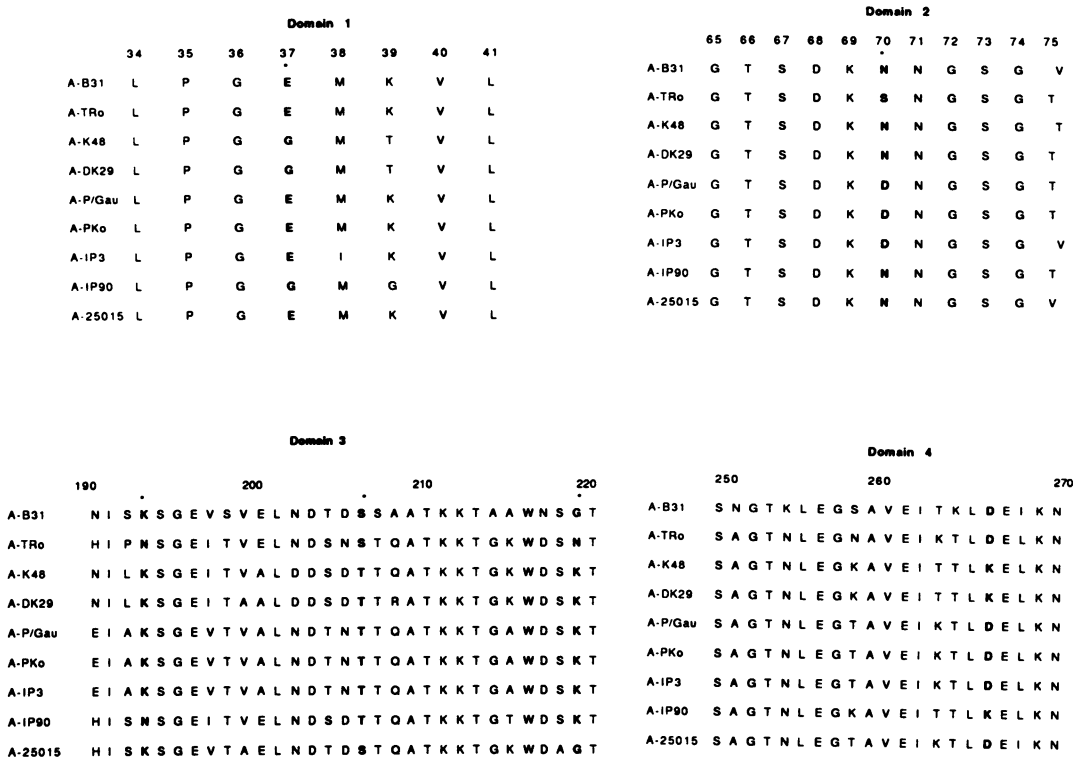

FIG. 6. Comparison of epitopes of OspA in nine strains of *B. burgdorferi*. The numbers at the top indicate positions in the published sequence of OspA of B31. The amino acid sequence was deduced from previously published DNA sequence data: A-B31 (4); A-TRo, A-IP3, A-K48, A-DK29, and A-P/Gau (12); A-PKo (33); A-IP90 (19); and A-25015 (16). Key residues are indicated in boldface type and were determined on the basis of the MAb-binding patterns listed in Table 1.

near residue 61 and centered around residues 214 to 217, respectively. Sera from two patients diagnosed with chronic Lyme borreliosis reacted with distinct epitopes between those recognized by the MAbs. Sears et al. (28) studied the reactivity of human and mouse sera, as well as both protective and nonprotective MAbs, with a series of truncated and overlapping OspA fragments expressed as recombinant glutathione-*S*-transferase fusion proteins. They included MAb CIII.78, a neutralizing antibody which recognizes a determinant between amino acids 133 and 273, an immunodominant domain that also bound to nonprotective antibodies and human sera. The authors noted an inability to finely map segments shorter than 133 to 200 amino acids, a result which they attributed to discontinuous conformational epitopes that survive SDS-PAGE (28). In contrast, we were able to determine in a previous study (27) that MAb 105.5, which has binding properties similar to those of MAb CIII.78, is also able to agglutinate the organism in vitro and has a binding site centered around amino acids 214 to 217. Similar results are reported in the present investigation for lipidated native OspA and also have been obtained for recombinant Osp expressed in the T7 RNA polymerase system and then subjected to chemical and proteolytic cleavage (unpublished results).

Fine mapping of the immunogenic domains recognized by several anti-OspA MAbs became possible with the availability of detailed primary amino acid sequence information for Osp proteins of a number of *B. burgdorferi* strains. Overall, considerable evidence exists to suggest that the N-terminal portion is not the immunodominant domain of OspA, possibly by virtue of its lipidation and the putative function of the lipid moiety in anchoring the protein to the outer envelope. In contrast, the C-terminal end is immunodominant and includes domains that account in part for structural heterogeneity (33), may provide epitopes for antibody neutralization (28), and may relate to other activities, such as the induction of T-cell proliferation (29). Our studies provide a structural basis for the observation that some anti-OspA MAbs agglutinate *B. burgdorferi* when added to BSKII medium. We found that agglutination occurred with anti-OspA C terminus MAbs, such as H5332, 12, 83, 336, and CIII.78, but not with anti-OspA N terminus MAbs, such as 13 and 15. This result indicates that these MAbs recognize surface-exposed epitopes. The strain specificity of agglutination corresponded exactly to that apparent on Western blots. Significantly, we found that antibodies which react to antigenic domains located on the carboxy terminus of the protein and agglutinate viable *B. burgdorferi* are not necessarily restricted in their activity to a given genospecies (Table 1). This observation may have important implications for vaccine development. Fikrig et al. (16) demonstrated that mice immunized with recombinant OspA derived from strain 25015, isolated from a tick in Millbrook, N.Y., were not protected from infection with strain N40 but were protected from infection with strain 25015. However, mice immunized with recombinant OspA derived from strain N40 were not protected from infection with 25015 (16). Our results indicate that there are common epitopes in the carboxy end of the protein that are shared by genospecies (33), although their immunoprotective potential needs to be determined.

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REFERENCES

1. **Baranton, G., D. Postic, I. S. Girons, P. Boerlin, J. Piffaretti, M. Assous, and P. A. D. Grimont.** 1992. Delineation of *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* sp. nov., and group VS 461 associated with Lyme borreliosis. Int. J. Syst. Bacteriol. **42:**378–383.

1a. **Barbour, A. G.** 1984. Isolation and cultivation of Lyme disease spirochetes. Yale J. Biol. Med. **57:**521–525.

1b. **Barbour, A. G., S. F. Hayes, R. A. Heiland, M. E. Schrumpf, and S. L. Tessier.** 1986. A borrelia-specific monoclonal antibody binds to a flagellar epitope. Infect. Immun. **52:**549–554.

2. **Barbour, A. G., S. L. Tessier, and S. F. Hayes.** 1984. Variation in a major surface protein of Lyme disease spirochetes. Infect. Immun. **45:**94–100.

3. **Barbour, A. G., S. L. Tessier, and W. J. Todd.** 1983. Lyme disease spirochetes and *Ixodid* tick spirochetes share a common surface antigenic determinant defined by a monoclonal antibody. Infect. Immun. **41:**795–804.

4. **Bergstrom, S., V. G. Bundoc, and A. G. Barbour.** 1989. Molecular analysis of linear plasmid-encoded major surface proteins, OspA and OspB, of the Lyme disease spirochete *Borrelia burgdorferi*. Mol. Microbiol. **3:**479–486.

4a. **Boerlin, P., A.-G. Peter, D. Brete, D. Postic, G. Barranton, and J. C. Piffaretti.** 1992. Population genetic analysis of *Borrelia burgdorferi* isolates by multilocus enzyme electrophoresis. Infect. Immun. **60:**1677–1683.

5. **Bornstein, P.** 1970. Structure of alpha-1 CB 8, a large cyanogen bromide produced fragment from the alpha-1 chain of rat collagen. The nature of a hydroxylamine-sensitive bond and composition of tryptic peptides. Biochemistry **9:**2408–2421.

6. **Brandt, M. E., B. S. Riley, J. D. Radolf, and M. V. Norgard.** 1990. Immunogenic integral membrane proteins of *Borrelia burgdorferi* are lipoproteins. Infect. Immun. **58:**983–991.

7. **Burgdorfer, W., A. G. Barbour, S. F. Hayes, J. L. Benach, E. Grunwaldt, and J. P. Davis.** 1982. Lyme disease—a tick-borne spirochetosis? Science **216:**1317–1319.

8. **Cleveland, D. W., S. G. Fisher, M. W. Kirschner, and U. K. Laemmli.** 1977. Peptide mapping by limited proteolysis in SDS and analysis by gel electrophoresis. J. Biol. Chem. **252:**1102–1106.

9. **Coleman, J. L., and J. L. Benach.** 1987. Isolation of antigenic components from the Lyme disease spirochetes: their role in early diagnosis. J. Infect. Dis. **155:**756–765.

10. **Cunningham, T. M., D. D. Thomas, S. D. Thompson, J. N. Miller, and M. A. Lovett.** 1988. Identification of *Borrelia burgdorferi* surface components by Triton X-114 phase partitioning. Ann. N. Y. Acad. Sci. **539:**376–378.

11. **Dunn, J., B. N. Lade, and A. G. Barbour.** 1990. Outer surface protein A (Osp A) from the Lyme disease spirochete, *Borrelia burgdorferi*: high level expression and purification of a soluble recombinant form of Osp A. Protein Exp. Purif. **1:**159–168.

12. **Dykhuizen, D., D. S. Polin, J. J. Dunn, B. Wilske, V. Preac-Mursic, R. J. Dattwyler, and B. J. Luft.** 1993. *Borrelia burgdorferi* is clonal: implications for taxonomy and vaccine development. Proc. Natl. Acad. Sci. USA **90:**10163–10167.

13. **Erdile, L. F., M.-A. Brandt, D. J. Warakomski, G. J. Westrack, A. Sadziene, A. G. Barbour, and J. P. Mays.** 1993. Role of attached lipid in immunogenicity of *Borrelia burgdorferi* OspA. Infect. Immun. **61:**81–90.

14. **Fikrig, E., S. W. Barthold, F. S. Kantor, and R. A. Flavell.** 1990. Protection of mice against the Lyme disease agent by immunizing with recombinant Osp A. Science **250:**553–556.

15. **Fikrig, E., S. W. Barthold, F. S. Kantor, and R. A. Flavell.** 1992. Long-term protection of mice from Lyme disease by vaccination with OspA. Infect. Immun. **60:**773–777.

16. **Fikrig, E., S. W. Barthold, D. H. Persing, X. Sen, F. S. Kantor, and R. A. Flavell.** 1992. *Borrelia burgdorferi* strain 25015: characterization of Osp A and vaccination against infection. J. Immunol. **148:**2256–2260.

17. **Gross, E.** 1967. The cyanogen bromide reaction. Methods Enzymol. **11:**238–255.

18. **Holloway, P. W.** 1973. A simple procedure for removal of Triton X-100 from protein samples. Anal. Biochem. **53:**304–308.

19. **Jonsson, M., L. Noppa, A. G. Barbour, and S. Bergström.** 1992. Heterogeneity of outer membrane proteins in *Borrelia burgdorferi*: comparison of *osp* operons of three isolates of different geographic origins. Infect. Immun. **60:**1845–1853.

20. **Kalish, R. A., J. M. Leong, and A. C. Steere.** 1991. Delay in the immune response to outer surface proteins (Osp) A and B of *B. burgdorferi*: correlation with arthritis and treatment failure in susceptible patients with Lyme disease. Arthritis Rheum. **34(9):** S66. (Abstract.)

21. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.

22. **Luft, B. J., W. Jiang, P. Munoz, R. J. Dattwyler, and P. D. Gorevic.** 1989. Biochemical and immunological characterization of the surface proteins of *Borrelia burgdorferi*. Infect. Immun. **57:**3637–3645.

23. **McGrath, B. C., J. J. Dunn, L. L. France, W. Jiang, D. Dykhuisen, D. Polin, G. Gorgone, and B. Luft.** 1993. Biochemical and biophysical characteristics of the major outer surface protein from North American and European isolates of *Borrelia burgdorferi*, p. 365–370. *In* H. S. Ginsberg, F. Brown, R. M. Chanock, and R. Lerner (ed.), Vaccines 1993. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

24. **Ploskal, M. G., M. R. Kavorian, C. Vecoli, and D. A. Hicks.** 1986. Immobilon PVDF transfer membrane: a new membrane substrate for Western blotting of proteins. BioTechniques **4:**272–283.

25. **Sadziene, A., P. A. Thompson, and A. G. Barbour.** 1993. In vitro inhibition of *Borrelia burgdorferi* growth by antibodies. J. Infect. Dis. **167:**165–172.

26. **Sambri, V., and R. Cevenini.** 1991. Comparative ability of various detergents to extract proteins of *Borrelia burgdorferi* and *Borrelia hermsii*. Microbiologica **14:**307–314.

27. **Schubach, W. H., S. Mudri, R. J. Dattwyler, and B. J. Luft.** 1991. Mapping antibody binding domains of the major outer surface membrane protein (OspA) of *Borrelia burgdorferi*. Infect. Immun. **59:**1911–1915.

28. **Sears, J. E., E. Fikrig, T. Y. Nakagawa, K. Deponte, N. Marcantonio, F. S. Kantor, and R. A. Flavell.** 1991. Molecular mapping of Osp A mediated immunity against *Borrelia burgdorferi*, the agent of Lyme disease. J. Immunol. **147:**1995–2000.

29. **Shanafel, M. M., J. Anzola, C. Soderburg, H. Yssel, C. W. Turck, and G. Peltz.** 1992. Epitopes of the Osp A of *B. burgdorferi* recognized by antibodies and T cells of patients with Lyme disease. J. Immunol. **148:**218–224.

30. **Shechter, Y., A. Patchornik, and Y. Burstein.** 1976. Selective chemical cleavage of tryptophanyl peptide bonds by oxidative chlorination with N-chlorosuccinimide. Biochemistry **15:**5071–5075.

31. **Simon, M. M., U. E. Schaible, M. D. Kramer, C. Eckerskorn, C. Museteanu, H. K. Muller-Hermelink, and R. Wallich.** 1991. Recombinant OspA from *Borrelia burgdorferi* induces antibodies protective against spirochete infection in mice. J. Infect. Dis. **164:**123–132.

32. **Wallich, R., C. Helmis, V. Schable, Y. Lobet, S. E. Notere, M. D. Kramer, and M. M. Simon.** 1992. Evaluation of the genetic divergence among *Borrelia burgdorferi* isolates by use of OspA, F1a, Hsp60, and Hsp70 gene probes. Infect. Immun. **60:**4856–4866.

33. **Wilske, B., B. J. Luft, W. H. Schubach, G. Zumstein, S. Jauris, V. Preac-Mursic, and M. D. Kramer.** 1992. Molecular analysis of the outer surface protein A (Osp A) of *Borrelia burgdorferi* for conserved and variable antibody binding domains. Med. Microbiol. Immunol. **181:**191–207.

34. **Wilske, B., V. Preac-Mursic, R. Fuchs, and E. Soutscheck.** 1991. Immunodominant proteins of *Borrelia burgdorferi*, the etiological agent of Lyme borreliosis. World J. Microbiol. Biotechnol. **7:**130–136.

35. **Wilske, B., V. Preac-Mursic, G. Schierz, R. Kuhbeck, and A. G. Barbour.** 1988. Antigenic variability of *Borrelia burgdorferi*. Ann. N. Y. Acad. Sci. **539:**126–143.