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Heterogeneity of Outer Membrane Proteins in *Borrelia burgdorferi*: Comparison of *osp* Operons of Three Isolates of Different Geographic Origins

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Biochemical and immunochemical studies of the outer membrane proteins of *Borrelia burgdorferi* have shown that the OspA and OspB proteins from strains of different geographic origins may differ considerably in their reactivities with monoclonal antibodies and in their apparent molecular weights. To further characterize this variation in Osp proteins between strains, the *osp* operons and deduced translation products from two strains, one from Sweden (ACAI) and one from eastern Russia (Ip90), were studied. Polyacrylamide gel electrophoresis and Western blot (immunoblot) analyses confirmed differences between ACAI, Ip90, and the North American strain B31 in their Osp proteins. The sequences of the *ospA* and *ospB* genes of ACAI and Ip90 were compared with that of the previously studied *osp* operon of B31 (S. Bergström, V. G. Bundoc, and A. G. Barbour, Mol. Microbiol. 3:479–486, 1989). The *osp* genes of ACAI and Ip90, like the corresponding genes of B31, were found on plasmids with apparent sizes of about 50 kb and are cotranscribed as a single unit. Pairwise comparisons of the nucleotide sequences revealed that the *ospA* genes of ACAI and Ip90 were 85 and 86% identical, respectively, to the *ospA* gene of strain B31 and 86% identical to each other. The *ospB* sequences of these two strains were 79% identical to the *ospB* gene of B31 and 81% identical to each other. There was significantly greater similarity between the *ospA* genes of the three different strains than there was between the *ospA* and *ospB* genes within each strain. These studies suggest that the duplication of *osp* genes in *B. burgdorferi* occurred before the geographical dispersion of strains represented by ACAI, Ip90, and B31.

The *ospA* and *ospB* genes encode the major outer membrane proteins of the Lyme borreliosis spirochete *Borrelia burgdorferi*. In *B. burgdorferi* B31, the *osp* genes are situated on a linear plasmid molecule 49 kb in size (5, 9). Earlier studies have shown that the two *osp* genes are organized in one operon, thus giving rise to one single transcript (23). Immunochemical and biochemical studies of the major outer membrane proteins OspA and OspB of *B. burgdorferi* have revealed differences in apparent molecular mass as seen on sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and differences in reactivity with monoclonal antibodies (10, 11, 48). This heterogeneity has been shown to be more prominent among European isolates than among North American isolates (10, 48). Furthermore, in both the European and the North American *B. burgdorferi* isolates, the OspB protein shows more strain variability (4, 10–12).

The isolation and characterization of a plasmid clone carrying the *osp* operon of the *B. burgdorferi* type strain B31 has been described earlier (23, 24). Subsequently, the *ospA* and *ospB* genes were subjected to nucleotide sequence analysis (14). The molecular analysis of the *B. burgdorferi* *osp* operon confirmed the earlier findings that the genes were organized in one operon. The encoded gene products, the OspA and OspB proteins, were 29.3 and 32.7 kDa in size, respectively. Further analysis of the deduced amino acid sequences of the *ospA* and *ospB* genes revealed sequences similar to those of the signal peptides of prokaryotic lipoproteins (14, 49). The lipoprotein nature of the Osp proteins was confirmed by Brandt et al., who used Triton X-114 extraction and immunoprecipitation of [^{3}H]palmitate-labeled proteins with Osp-specific monoclonal antibodies (16).

Persistence and development of chronic disease in a susceptible host may be due to antigenic variation of the proteins on the surface of the pathogenic microorganism. In *Borrelia hermsii*, a closely related *Borrelia* species, this variation is achieved by a genetic switch in which an outer surface protein gene, *vmp*, replaces a former *vmp* gene at a specific site where it can be expressed (8, 18). This antigenic switch is thought to take place through a nonreciprocal recombinational event (6, 7, 34). The finding of clonal polymorphism of the OspB protein (17, 42) and the observation by Craft and colleagues that some patients with Lyme borreliosis developed new antibodies directed against the OspB protein a year or more after the initial infection indicate that antigenic variation of Osp proteins might also occur in *B. burgdorferi* (19).

Recently, various typing systems based on molecular and immunological characteristics have divided *B. burgdorferi* into several different groups. These typing systems differentiate *B. burgdorferi* by protein profiles seen on SDS-PAGE, reactivity with monoclonal antibodies directed against the Osp proteins, 16S rRNA analysis, and genetic analysis with Southern hybridization or polymerase chain reaction (PCR) amplification (1, 28, 35, 36, 47, 48). In an attempt to learn more about the variation of the OspA and OspB proteins and for future evaluation of the gene products, we cloned and sequenced the *ospA* and *ospB* genes from two different *B. burgdorferi* strains. Those two strains, one isolated in Sweden and one isolated in Russia, were placed in two of the different groups (28, 47). The sequences obtained were compared with the previously published *ospA* and *ospB* sequences of *B. burgdorferi* B31 (14) and the *ospA* se-

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quences of strains ZS7 (45) and N40 (20), which all belong to one group.

During recent years, much work has been directed toward developing diagnostic tools for Lyme borreliosis. The most promising and sensitive detection method for the Lyme borreliosis agent is that of detecting single *B. burgdorferi* organisms by PCR amplification (30, 33, 37). The sequence comparison of several *osp* genes obtained from *B. burgdorferi* strains isolated from different geographical locations will be of considerable help in finding, in the *osp* operon, sequences that will be useful for designing primers for specific DNA amplification. Furthermore, these results may also be of importance for molecular epidemiological studies of Lyme borreliosis throughout the world.

MATERIALS AND METHODS

Bacterial strains and media. The *B. burgdorferi* strains used in this study were the North American reference strain B31 (ATCC 35210) isolated from an *Ixodes dammini* tick; the Swedish isolate ACAI isolated from a patient with acrodermatitis chronica atrophicans (2) who came from a geographical region in which *Ixodes ricinus* is the tick vector; and the Ip90 strain, which was isolated from an *Ixodes persulcatus* tick from the Khabarovsk territory in Russia and was kindly provided by E. I. Korenberg and V. N. Kryuchechnikov of the Gamaleya Institute, Moscow, Russia (28). The *B. burgdorferi* strains were cultivated in BSK II medium as previously described (3). The *Escherichia coli* strains DH5α (Bethesda Research Laboratories, Gaithersburg, Md.) and Y1090 (25) were used for propagation of recombinant plasmids and for growth of λgt11 phage gene library, respectively.

Extraction of *B. burgdorferi* proteins, SDS-PAGE, and Western blotting (immunoblotting). The cells were grown in 200 ml of BSK II medium at 34°C and harvested in mid-log phase (approximately 2×10^7 to 4×10^7 cells per ml) by centrifugation at 8,000 × *g* for 20 min. The cells were washed twice in phosphate-buffered saline (PBS)–5 mM $MgCl_2$, and the pellets were suspended in 2 ml of PBS. To prepare soluble proteins, the cells were sonicated four times for 30 s each time in an ice bath by using Branson Sonifier cell disruptor B15 at setting 3. After centrifugation at 10,000 × *g* for 30 min, the supernatant was collected, and the amount of protein was determined by using the Bio-Rad protein assay (Bio-Rad, Munich, Germany).

SDS-PAGE was performed essentially as described before (12) using either 12.5% or 15% acrylamide running gels and 4% acrylamide stacking gels. In each lane, 10 to 15 μg of protein was added. The gels were either fixed and stained by Coomassie brilliant blue (Sigma Chemical, St. Louis, Mo.) or processed for immunoblotting. Molecular weight standards were obtained from Pharmacia (Uppsala, Sweden) and included proteins ranging in size from 14.4 to 94 kDa. The proteins were transferred to Immobilon filters (Millipore Corp., Bedford, Mass.) by electroblotting at 0.8 mA/cm^2 for 45 min. The nonspecific binding on the filters was blocked by incubation with 5% (wt/vol) nonfat milk powder in PBS overnight. The filters were then incubated with antibodies (1:20 or 1:25 dilution of hybridoma supernatant in 2.5% milk powder in PBS), washed 3 times for 5 min each time in PBS–0.05% Tween 20, and then incubated with an appropriate peroxidase-labeled monoclonal antibody (1:500 dilution in 2.5% milk–PBS) for 1 h. Bound antibodies were then visualized by adding 5-bromo-4-chloro-3-indolylphosphate as peroxidase substrate. All incubations were performed during continuous shaking. The monoclonal antibodies used were the anti-OspA antibodies H5332 (13) and H3TS (10) and the anti-OspB antibody H6831 (12).

DNA techniques and primer extension analysis of mRNA. Restriction enzymes, T4 DNA ligase (BioLabs, Finnzymes, OY, Espoo, Finland), reverse transcriptase (Life Sciences, St. Petersburg, Fla.), Sequenase (U.S. Biochemical, Cleveland, Ohio), and the T7 sequencing kit (Pharmacia) were used as recommended by the manufacturers. The isolation of *B. burgdorferi* DNA was performed as described previously (5, 9, 24). Transcriptional analysis was performed on total mRNA isolated from *B. burgdorferi* B31 by using a method previously described (32, 43). The isolated and purified mRNA was subjected to primer extension in an in vitro reaction with avian myeloblastosis virus reverse transcriptase (Life Sciences) as described previously with minor modifications (15, 18, 46). The primer extension reaction with RNA was carried out with [α-^{32}P]dATP. The primer used in the primer extension reaction was the reverse complement of nucleotides 128 to 160 shown in Fig. 3 (Table 1). Only full-length extension product was synthesized, and the size of the transcript was compared with that of a regular Sanger dideoxy DNA sequence on plasmid pTRH44 (23) obtained when the same oligonucleotide primer was used. In the DNA sequencing reactions, α-^{35}S-dATP was used.

Construction and screening of λgt11 and pUC18 plasmid *B. burgdorferi* gene libraries. *B. burgdorferi* strains were cultured in 400 ml of modified BSK II medium (3) at 34°C and harvested at late mid-log phase. The DNA was extracted and purified as previously described (5, 9, 24). The ACAI DNA was partially digested by *Sau*3AI, and fragments 4 to 8 kb in size were isolated from a 0.7% agarose gel. The fragments were then ligated into *Bam*HI-cut λgt11 arms and packaged into phage heads as described by the vendor (SDS-Promega, Falkenberg, Sweden). The λ phages were propagated in *E. coli* Y1090, and screening of the λ library by DNA hybridization was done according to standard methods (25). The oligonucleotides used for screening (J1, J2, and J3) were synthesized from the previously published *B. burgdorferi* B31 *osp* operon nucleotide sequence (14). All oligonucleotides used for screening of phage and plasmid libraries and the primers for nucleotide sequencing are shown in Table 1. The oligonucleotides were end labeled with [γ-^{32}P]dATP as previously described (31) and purified on a Sephadex G-50 (Pharmacia) spin column. The hybridization was performed at 37°C, i.e., medium stringency, as some differences in the nucleotide sequences of the different strains were expected. Phage DNA was extracted as previously described (29). Purified λ DNA was further subcloned into the *Eco*RI site of pUC18 according to standard cloning methods (31).

A plasmid gene library of *B. burgdorferi* Ip90 DNA was constructed by partial *Hin*dIII digestion of total DNA, in which 1 U of enzyme was incubated with 100 ng of DNA at 37°C for 15 min. The reaction was terminated by a phenol-chloroform (1:1) extraction. After ethanol precipitation, the fragments were ligated with 30 ng of *Hin*dIII-digested pUC18 plasmid DNA at 16°C for 4 h. The plasmids were transformed into competent *E. coli* DH5α cells. In order to obtain a full-length *osp* operon clone, an additional plasmid gene library was constructed by complete *Eco*RI digestion of total Ip90 DNA and cloning into the *Eco*RI site of the plasmid pUC18.

The *B. burgdorferi* Ip90 plasmid gene library, obtained by partial *Hin*dIII digestion, was screened with a 259-bp *osp* fragment. This *ospA* fragment was acquired by PCR amplification of a DNA segment located at the end of the *ospA*

TABLE 1. Oligonucleotides used for screening of phage and plasmid gene libraries, nucleotide sequencing of *osp* operons, and primer extension analysis

Oligomer	Strain of origin	Position[a]	Sequence 5′ to 3′
J1	B31	788–760	TTTGAGTCGTATTGTTGTACTGTAATTGT
K1	B31	529–556	TATGTTCTTGAAGGAACTCTAACTGCTG
J2	B31	337–312	GTGTGGTTTGACCTAGATCGT
J3	B31	1237–1217	AGGTTACTGTGTTTAAATCAG
P1	pUC19	334–317	ACGCCAGGGTTTTCCCAG
P2	pUC19	172–189	GTGTGGAATTGTGAGCGG
I1	Ip90	278–256	GTTTTTTCACCTTCAAGTGTTCC
B1	B31	−67–−48	TTATTATCATTTTATTTTTTTT
B2	B31	75–54	GGCTAATATTAGACCTATTCCC
B3	B31	1084–1066	ATTTTTTTCTTTGCTTAC
B4	B31	1066–1084	GTAAGCAAAGAAAAAAAT
B5	B31	1425–1407	TAGAGTTTCTACTGCTTTT
B6	B31	1407–1425	TACAAAAGCAGTAGAAACTC
B7	B31	1603–1622	TTAACAATTAGTGCTGACAG
B8	B31	1642–1661	GTGTTCTTAACAGATGGTAC
B9	B31	1661–1642	GTACCATCTGTTAAGAACAC
B10	B31	160–128	CAAGAACTTTCATTTCACCAGGCAAATCTACTG
A1	ACAI	140–120	GGCAAATCTACTGAAGCGCTG
A2	ACAI	202–223	GCAACAGTAGACAAGATTGAGCT
A3	ACAI	452–472	GAGAAAATGGAACCAAACTTG
A4	ACAI	525–502	TTTTAAAACTTCTTTAGCTTTTCC
A5	ACAI	669–689	AAAAACTGGCGCATGGGATTC
A6	ACAI	840–821	AAGTTCATCAAGTGTTTTAA
A7	ACAI	968–992	TATAAACTCAGACAATACACC
A8	ACAI	1176–1156	ACCTTCAAGCTTGCCAGATCC
A9	ACAI	1291–1313	CAAGGGTCAGTAATAAAAGAATC
A10	ACAI	1802–1779	CCTACAAAGGTATTAGCCGA

[a] All positions are derived from the *B. burgdorferi* B31 *ospA* and *ospB* sequences in Fig. 3 except for the pUC19 sequences, which are derived from the EMBL data base sequence of pUC19 plasmid.

gene, with the J1 and K1 oligonucleotides used as primers (Table 1). This fragment corresponds to a fragment between nucleotide positions 529 and 788 of the *B. burgdorferi* B31 *ospA* gene. The conditions for the PCR amplification were as follows: 16.6 mM $(NH_4)_2SO_4$; 67 mM Tris HCl (pH 8.8 at 25°C); 6.7 mM $MgCl_2$; 10 mM 2-mercaptoethanol; 200 μM each dATP, dGTP, dCTP, and dTTP; 170 μg of bovine serum albumin per ml; 5 pmol of each primer; about 10 pg of total Ip90 DNA; and 1 U of BioLabs *Taq* polymerase. Reactions were performed in a volume of 50 μl overlaid with 40 μl of mineral oil. During the first five cycles, denaturation was done at 94°C for 2 min, annealing was at 45°C for 1 min, and elongation was at 72°C for 1 min. In the next 30 cycles, the denaturation time was shortened to 30 s and the annealing temperature was raised to 55°C; otherwise, the conditions were the same. The PCR fragment was labeled with [α-^{32}P]dATP by using an oligo-labeling kit (Amersham, Buckinghamshire, United Kingdom) and used to screen the *Hin*dIII library for *osp* gene-containing clones. The screening was performed as previously described (31), and the hybridization was performed at high stringency (60°C). The Ip90 gene library constructed by *Eco*RI-digested total DNA was screened with an oligonucleotide, I1, specific for the *ospA* gene of *B. burgdorferi* Ip90.

Pulsed-field agarose gel electrophoresis. The electrophoretic separation of plasmid and chromosomal *B. burgdorferi* DNA was carried out by pulsed-field electrophoresis using a Gene Navigator machine (Pharmacia). About 0.5 μg of total *B. burgdorferi* DNA was separated on 1.2% agarose (15 by 15 cm) using the following running parameters: 300 V; stepping with switch times of 0.3, 0.5, and 0.7 s, each lasting for 1 h, followed by switch times of 2.0 and 4.0 s, each lasting for 5 h. The agarose gel was stained with ethidium bromide (0.2%), and the DNA was blotted onto a nylon filter (Hybond-N; Amersham, Stockholm, Sweden) and hybridized with an *osp*-specific oligonucleotide probe.

Nucleotide sequence analysis. Deletion libraries in one direction were constructed for the full-length clone of ACAI and the shorter of the Ip90 clones as described earlier (22). Plasmid minipreparations were performed either by the boiling method (31) or by a CsCl method previously described (40). Nucleotide sequencing was performed by using the dideoxy chain termination method described by Sanger et al. (39), with the use of Pharmacia T7 sequencing kit (Pharmacia). From the nucleotide sequences obtained, internal primers were synthesized to enable sequencing of the other strand plus the beginning of the Ip90 *osp* operon. The complete sequences for both strands were determined. The sequences obtained from the DNA deletion sequencing method were assembled by using GENEUS (21) software for VAX computers (Digital Equipment Corp.). Additional nucleotide sequence analyses were performed with the University of Wisconsin GCG Sequence Analysis software for the VAX computer and PC-Gene (Genofit) for personal computers.

RESULTS

Biochemical and immunochemical characterizations of the OspA and OspB proteins of *B. burgdorferi* ACAI and Ip90. Whole-cell protein extracts prepared from *B. burgdorferi* B31, ACAI, and Ip90 separated on a 12.5% SDS–PAGE gel are shown in Fig. 1. These three *B. burgdorferi* isolates were obtained from different geographical locations: North America, Sweden, and the eastern part of Russia. We chose these

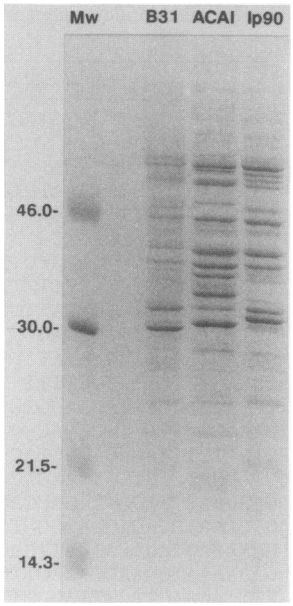


FIG. 1. Coomassie blue-stained 12.5% SDS-PAGE gel of whole-cell lysates of *B. burgdorferi*. Whole-cell extracts were from *B. burgdorferi* B31, ACAI, and Ip90. The sizes of the molecular weight (Mw) standards (in thousands) are shown to the left.

three different isolates because of the long geographical distances between them and their probable different tick origins. Another reason for choosing these strains was that they were representatives of three different *B. burgdorferi* groups (28, 47). The three strains showed different apparent molecular masses for both of the major outer surface proteins, OspA and OspB. This difference has also been seen earlier, when strains B31 and ACAI were compared (11). The molecular masses of the OspA and OspB proteins as determined by SDS-PAGE were as follows: for B31, 31 and 34 kDa; for ACAI, 32 and 36 kDa; and for Ip90, 33.5 and 34 kDa, respectively. The Osp proteins from the three different isolates were further characterized by Western blot analysis using different monoclonal antibodies directed against the OspA and OspB proteins of *B. burgdorferi* B31. As can be seen in Table 2, there was an immunochemical variability of the OspA and OspB proteins in these three strains.

Cloning of the *osp* operons from *B. burgdorferi* isolates ACAI and Ip90. The *osp* operon from the Swedish *B. burgdorferi* strain ACAI was isolated from a λgt11 library. The isolation of the ACAI *osp* operon was performed by screening with a mixture of three oligonucleotides (J1, J2, and J3 [Table 1]) which were synthesized from the nucleotide sequence of the previously published *B. burgdorferi* B31 *osp* operon (14). One positive λ clone containing the ACAI *ospA* and *ospB* genes was isolated and characterized by restriction endonuclease mapping (data not shown). The isolated *osp* operon was subcloned into pUC18, and the genetic organization of the genes was confirmed with the same three oligonucleotides, J1, J2, and J3. The *osp* operon of strain Ip90 was cloned from a pUC18 plasmid library by using *Hin*dIII partially digested total Ip90 *B. burgdorferi* DNA. One positive clone containing the entire Ip90 *osp* operon except for the first 175 bp of *ospA* was obtained. An oligonucleotide, I1, constructed from the start of this *Hin*dIII clone was used to pick up a clone containing the whole Ip90 *osp* operon from a pUC18 plasmid library of completely *Eco*RI-digested Ip90 DNA.

TABLE 2. Reactivity of *B. burgdorferi* B31, ACAI, and Ip90 in Western blots with monoclonal antibodies H5332, H3TS, and H6831

Strain	Reactivity with monoclonal antibody[a]:		
	H5332	H3TS	H6831
B31	+	+	+
ACAI	+	−	−
Ip90	−	−	−

[a] Visualized as a band on Immobilon filter with peroxidase substrate; −, no band on Immobilon filter.

Determination of the transcriptional start of the *osp* operon. Prior to sequence analysis of the *ospA* and *ospB* genes of *B. burgdorferi* ACAI and Ip90, we further characterized the sequences 5′ to the start of the *ospA* gene of strain B31. Previous analysis of the B31 *osp* operon had revealed two possible σ^{70}-type promoters for the *osp* genes in this region (14). In our comparisons of the different sequences and their 5′ and 3′ flanking regions, it was important, therefore, to identify the transcriptional start site for the *osp* operon. Accordingly, primer extension analysis was carried out on mRNA from *B. burgdorferi* B31 by using antisense primer B10 (Table 1, Fig. 2). The in vitro transcription analysis identified the transcriptional start site as the G at position +1 (Fig. 3). This transcriptional start site was situated 36 bp upstream of the AUG translational start codon for *ospA*. This study indicated that the likely promoter was the TTGTTA (−35 box) at positions −35 to −30 and TATAAT (−10 box) at positions −13 to −8 and not the other candidate promoter sequences just downstream of these two locations.

Nucleotide sequence analysis of the *osp* operons from *B. burgdorferi* B31, ACAI, and Ip90. The *osp* operons of strains ACAI and Ip90 were sequenced. The obtained nucleotide sequences are aligned in Fig. 3 and compared with the previously published nucleotide sequences of the *ospA* and *ospB* genes of strain B31 (14) and the *ospA* sequences of strains ZS7 (45) and N40 (20). The nucleotide sequence comparisons revealed that the *ospA* genes from strains ACAI and Ip90 have a sequence identity of 85 and 86%, respectively, with the *ospA* gene of strain B31. Compared with each other, the *ospA* genes of ACAI and Ip90 showed an 86% identity. In contrast, the two previously published *ospA* sequences from strains ZS7 and N40 were almost identical (>99%) to the *ospA* sequences of strain B31. The *ospB* sequences of strains ACAI and Ip90 were 79% identical to the *ospB* gene of strain B31 and 81% identical to each other. Approximately half of the differences in the various sequences were located in the wobble position of the codon triplets. Thus, the *ospA* and *ospB* structural genes of strains B31, ACAI, and Ip90 all showed a similar sequence variability from each other. Furthermore, a high degree of sequence similarity between the *ospA* and *ospB* genes of the three different strains was shown. However, when the sequences upstream and downstream of the structural genes were compared, the ACAI and Ip90 strains showed a much higher sequence similarity to each other than to the B31 sequence of these regions (Fig. 3). The *osp* operons in these two strains are also preceded by a control region consisting of a σ^{70} promoter and a Shine-Dalgarno ribosome-binding site, as shown in Fig. 3 (38). In addition, in both ACAI and Ip90, we found an extra 6 bp between the promoters and the AUG

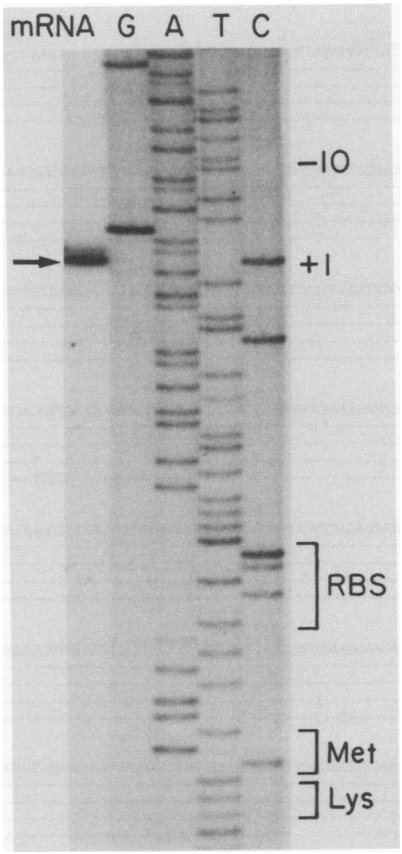


FIG. 2. Determination of transcriptional start of the *B. burgdorferi* B31 *osp* operon. The figure shows the primer extension of the *osp* mRNA together with the corresponding sequence ladder of the *osp* DNA. The −10 region, transcriptional start base (+1), ribosome-binding site (RBS), and first two amino acid codons (Met and Lys) are indicated. The arrow points to the full-length extension product.

start codons. All strains, including B31, had a similar nucleotide sequence in the promoter region. It is therefore likely that the transcriptional start point is located at position +1 in all three strains.

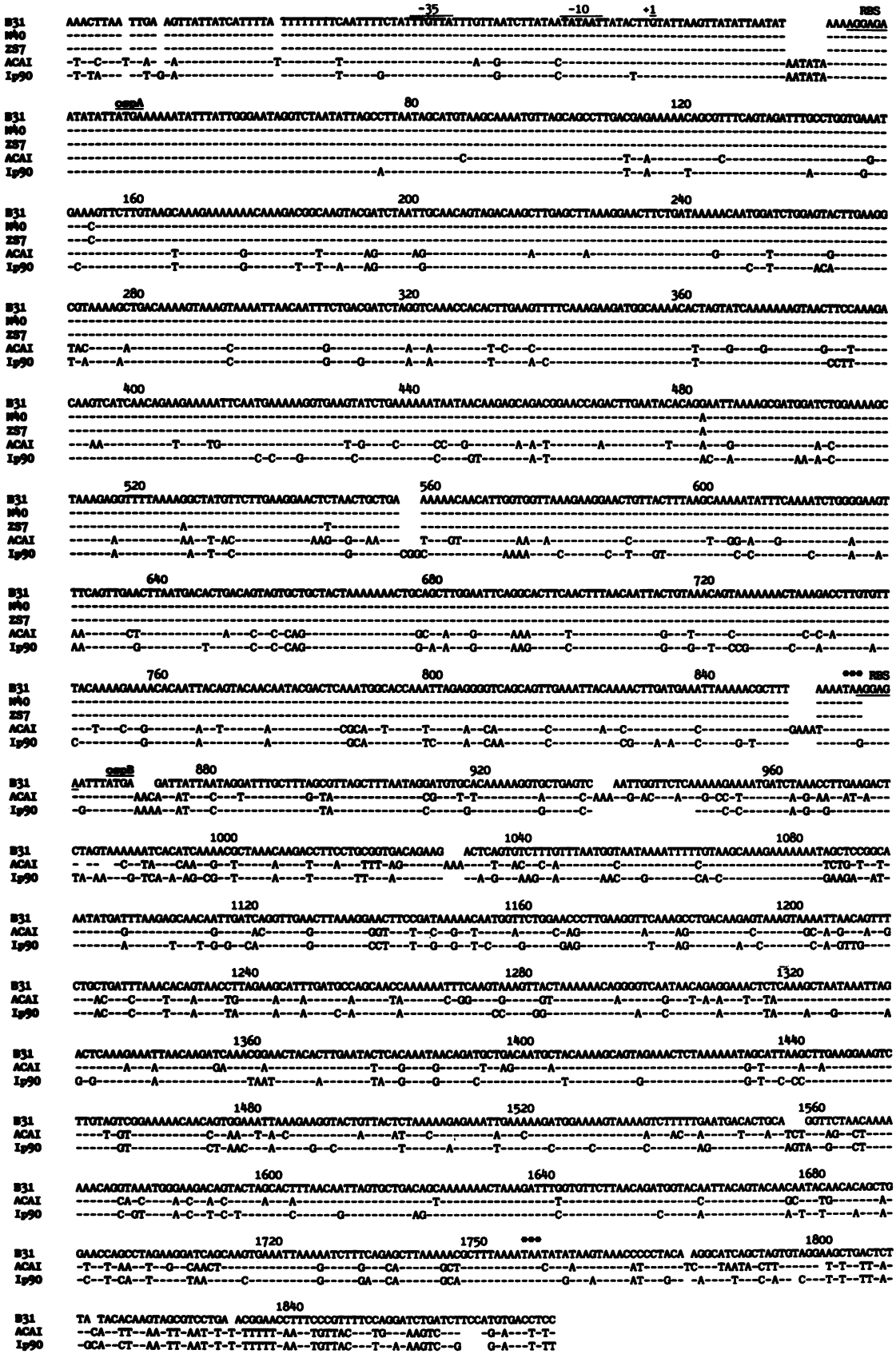
Further analysis of the *osp* genes of strains ACAI and Ip90 revealed that the *ospA* and *ospB* genes were organized in one operon and were separated by just a few base pairs. In order to elucidate where in the genome the *osp* operons of strains ACAI and Ip90 were located, total *B. burgdorferi* DNA was separated by hexagonal pulsed-field gel electrophoresis followed by blotting onto a nylon filter and hybridization with a mixed oligonucleotide probe made up of oligonucleotides J1, B6, B7, A4, and I1 (Fig. 4). The plasmid profiles of the three strains were found to be different both in apparent molecular sizes and numbers of linear plasmids (Fig. 4A), indicating heterogeneity between the different *B. burgdorferi* isolates. The oligonucleotide probe hybridized in all three strains to a plasmid molecule about 50 kb in size (Fig. 4B). In strains ACAI and Ip90, the plasmids were slightly larger than the linear plasmid of 49 kb in *B. burgdorferi* B31 known to harbor the *osp* operon (5, 9).

Sequence analysis of the translated OspA and OspB proteins of strains ACAI and Ip90. The translated products of the *ospA* genes of ACAI and Ip90 were compared with the deduced translation products of strains B31, ZS7, and N40. The comparison of the different OspA proteins in an optimal alignment is shown in Fig. 5A. The deduced OspA protein for ACAI was 273 amino acids long with a theoretical molecular weight of 29,629, and the protein for Ip90 was 274 amino acids long with a theoretical molecular weight of 29,673. The three different *ospB* gene products are compared in Fig. 5B. The deduced OspB proteins had molecular weights of 32,432 encoded by 299 amino acids for ACAI and 32,105 encoded by 294 amino acids for Ip90. From the amino acid sequence comparison of the OspA and OspB proteins, it was evident that the N termini of the OspA proteins were very conserved between the different strains, while the middle and C-terminal parts of the proteins showed a higher degree of variation. In the OspB protein, the same overall variability in the sequence was seen. The amino acid sequence comparisons revealed an identity of 77 and 79% when the OspA protein in B31 was compared with the OspA proteins in ACAI and Ip90, respectively. When compared with each other, the OspA proteins of ACAI and Ip90 showed an 81% identity. The OspA proteins of N40 and ZS7 were 99% identical to the B31 OspA. The OspB protein in B31 was 66 and 67% identical to the OspB proteins in ACAI and Ip90, respectively, while the OspB proteins in ACAI and Ip90 showed a 68% identity to each other. From the deduced amino acid sequences of the OspA and OspB proteins of *B. burgdorferi* B31, the sequence similarity with prokaryotic lipoproteins was shown (14). The deduced OspA and OspB proteins of *B. burgdorferi* ACAI and Ip90 also contained the typical consensus tetrapeptide (LXYC) in their signal peptides. The putative signal peptidase II recognition sites of the OspA and OspB proteins are indicated in Fig. 5.

DISCUSSION

Genetic, biochemical, and immunological studies have suggested that *B. burgdorferi* can be placed in two to seven different groups (1, 28, 35, 36, 47, 48). In this study, we analyzed and compared the *osp* operons from three *B. burgdorferi* strains: B31, ACAI, and Ip90. Because of the apparent molecular mass differences as seen in SDS-PAGE and their different reactivities with the OspA and OspB monoclonal antibodies used, these three strains fall into three different groups of *B. burgdorferi* strains. The placement of strains B31, ACAI, and Ip90 into three different groups is also similar to recent findings by Wilske et al. (47) and Kryuchechnikov et al. (28). Thus, strains B31, ACAI, and Ip90 can be regarded as representatives of different defined *B. burgdorferi* groups. From the results obtained in this study, we can conclude that the characterized *osp* operons and their translated products from the *B. burgdorferi* isolates tested are different at the molecular level. We have also compared the *ospA* sequences of strains ZS7 and N40 with the *ospA* sequences of strains B31, ACAI, and Ip90. This sequence comparison indicated that strains ZS7 and N40 belong to the same *B. burgdorferi* group as strain B31. Moreover, these data may be of practical benefit for the construction of oligonucleotide primers for PCR DNA diagnosis of Lyme borreliosis and for further studies of *osp* operons from various *B. burgdorferi* isolates. The variability seen between different Osp proteins must also be considered if the Osp proteins are going to be used as a vaccine all over the world, i.e., where all possible classes of Osp proteins will exist.

In a previous study, we showed that the *ospA* and *ospB*


-35
-10
+1
RBS
B31
M40
ZS7
ACAI
Ip90
ompA
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ompB
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1800
1840

FIG. 3. Comparison of nucleotide sequences of *osp* operons from *B. burgdorferi* B31, ACA1, and Ip90. The *ospA* sequences from ZS7 (45) and N40 (20) are also included. The *ospA* and *ospB* sequences of *B. burgdorferi* B31 are shown in the top row (14). Hyphens indicate homology between the *ospA* and *ospB* genes of the different strains, capital letters indicate differences between the *osp* genes, and gaps indicate missing (or inserted) bases. The −10 and −35 regions of the promoter are indicated, and the ribosome-binding sites (RBS) (Shine-Dalgarno sequences) are underlined. Three asterisks are shown above the predicted stop codons.

genes and their translation products exhibit a high degree of sequence identity, indicating a recent evolutionary duplication of an ancestral *osp* gene (14). A similar sequence identity was also seen when the *ospA* and *ospB* genes of ACAI and Ip90 were compared. When all the *ospA* and *ospB* sequences were compared with each other, the similarity between the respective *ospA* and *ospB* genes of the three strains was significantly greater than that between the *ospA* and *ospB* genes within each strain. Thus, it is evident from the results that the ancestral *osp* gene diverged into *ospA* and *ospB* before strains B31, ACAI, and Ip90 separated. It is also likely that the *ospA* gene was first because of its location closer to the promoter.

The *osp* operons of strains ACAI and Ip90 characterized in this study had the same genetic organization as the previously characterized *osp* operon of the reference strain B31. Thus, the *ospA* and *ospB* genes are tandemly arrayed on a linear plasmid about 50 kb in size, are organized in one operon, and are cotranscribed. Note that in Ip90, as in strain B31, the Osp proteins are translated in one reading frame, whereas in strain ACAI, the frame changes between the *ospA* and *ospB* genes. This difference between the *osp* operons could indicate that the region between the two genes in the operon is a region where a shift between different Osp protein genes can take place. It was earlier shown that the OspB protein could undergo changes to different but antigenically related proteins, seen as clonal polymorphism, after in vitro growth on solid medium (17). In that study, it was also found that this change in expression of the OspB protein was not due to any detectable DNA rearrangement within the *B. burgdorferi* genome. However, it is still too early to conclude if there is any true antigenic variation of the OspB proteins in *B. burgdorferi* or if the differences seen are due to expression of different OspB proteins located at different sites in the genome. This could easily be tested by hybridizing with oligonucleotides from different regions of the *ospB* gene and testing to see if multiple copies of *ospB*-like genes exist. Another reason for this polymorphism of the OspB protein could be due to small intragenic changes within the *ospB* gene that result in local amino acid changes, inserts of smaller *osp* related sequences, or introduction of premature stop codons.

The N-terminal regions of the OspA and OspB proteins in all strains analyzed showed characteristics of typical signal peptides, i.e., a basic amino acid terminus, a hydrophobic central core, and a recognition site for signal peptidase cleavage (26, 44). From the sequence analysis, it was also evident that the predicted OspA and OspB proteins of strains ACAI and Ip90 had similarities in the N termini of prokaryotic lipoproteins, as was shown earlier for the OspA and OspB proteins of strain B31 (14, 49). The exact processing of the OspA and OspB proteins in strains ACAI and Ip90 in this study is not known. However, it is probable that it is similar to the processing shown for the Vmp proteins of the related organism *B. hermsii* (18). The Vmp proteins also belong to the group of prokaryotic lipoproteins which are processed by signal peptidase II, because the cleavage is inhibited by the specific signal peptidase II inhibitor globomycin. An unprocessed protein accumulates after globomycin treatment, and the protein is larger than the processed protein corresponding to the addition of the signal peptide to the cysteine residue (18). Further characterization and comparison of the different OspA and OspB proteins with each other showed that the most hydrophobic parts of the proteins were located in the conserved parts of the respective proteins. In contrast, the hydrophilic regions, which are on the surfaces of the proteins, were more variable.

Recent studies have shown that both passive and active immunization with the OspA protein can be obtained in mice (20, 41). However, the finding by Johnson and coworkers in a hamster model system that antibodies to a *B. burgdorferi* isolate from one geographical area did not elicit protection against challenge by an isolate from a different geographical location suggests that there is a marked heterogeneity among isolates from diverse geographical areas (27). Thus, the diversity among antigens that is important for formation of protective antibodies has to be studied further. The Osp proteins are candidates for vaccines against Lyme borreliosis, and we believe that the knowledge of the nucleotide sequences and deduced protein sequences of various *osp* genes will be of importance for future studies of antigenic epitopes of the OspA and OspB proteins.

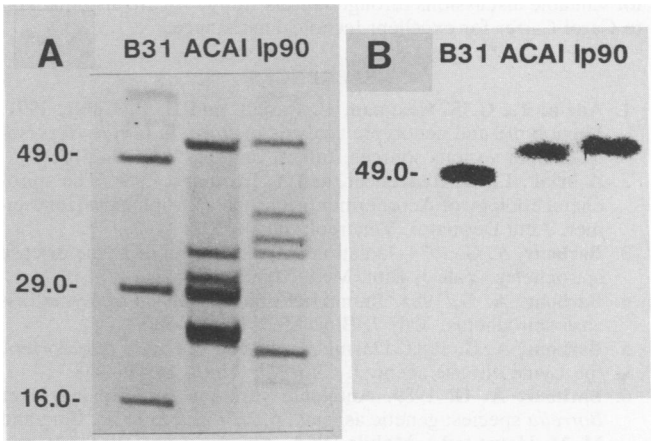

FIG. 4. Analysis of the genetic localization of the *osp* operon by Southern blot analysis of total *B. burgdorferi* DNA. (A) Separation of *B. burgdorferi* DNA from strains B31, ACAI, and Ip90 by hexagonal pulsed-field electrophoresis in 1.2% agarose. DNA was visualized by staining the agarose gel with 0.05% ethidium bromide. The pulse length and time of electrophoresis were set in order to get a maximum separation of linear DNA in the molecular size range of 10 to 100 kb. The previously determined molecular sizes of the three linear plasmids of *B. burgdorferi* B31 are indicated to the left (5). (B) Southern blot analysis of the *osp* operons from *B. burgdorferi* B31, ACAI, and Ip90 with an *osp*-specific mixed-oligonucleotide probe. The size of the 49-kb linear plasmid of *B. burgdorferi* B31 is marked to the left.

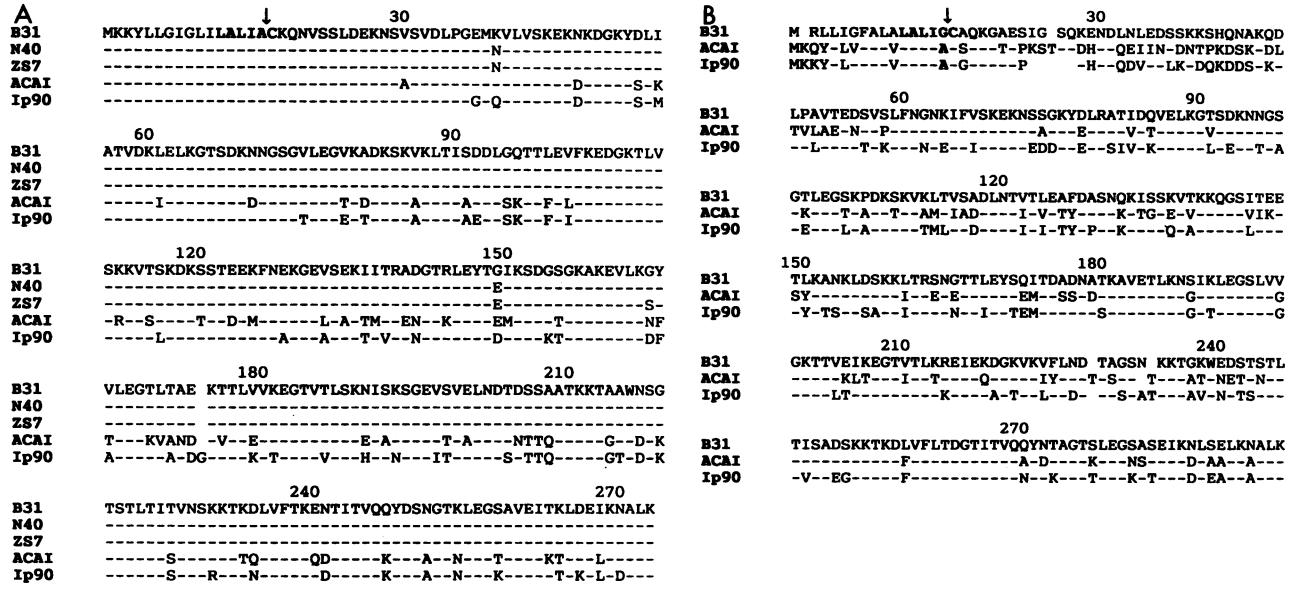

FIG. 5. Comparison of deduced amino acid sequences of the *B. burgdorferi* OspA and OspB proteins. (A) Comparison of the OspA proteins of strains B31, N40, ZS7, ACAI, and Ip90. (B) Deduced amino acid sequences of the OspB proteins of *B. burgdorferi* B31, ACAI, and Ip90. All sequences are compared and aligned with the *B. burgdorferi* B31 sequence (14). Capital letters indicate differences between the strains, hyphens indicate homologies, and gaps indicate missing amino acids. The possible consensus signal peptidase II recognition site is indicated with boldface letters, and the cleavage site is indicated by a vertical arrow.

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