Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 5991–5995, July 1992
Biochemistry

Binding of the *Bacillus subtilis spoIVCA* product to the recombination sites of the element interrupting the σ^K-encoding gene

(sporulation/DNA rearrangement/protein–DNA recognition/DNA bending)

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Communicated by Robert Haselkorn, February 28, 1992 (received for review December 26, 1991)

ABSTRACT **The gene encoding σ^K, a transcription factor controlling mother-cell-specific gene expression at a late stage of sporulation, is interrupted by the *skin* element in *Bacillus subtilis*. The *skin* element is excised from the mother cell chromosome by a DNA rearrangement that depends on the *spoIVCA* gene product. This protein has no other role in sporulation than promoting *skin* excision and exhibits sequence similarity to a family of bacterial site-specific recombinases. An expression library of *B. subtilis* DNA in λgt11 was screened for the presence of a gene encoding a protein able to bind *in vitro* to an oligonucleotide matching the inverted repeat sequences present at the ends of the *skin* element. Several bacteriophages were found to contain the *spoIVCA* gene. A cell extract containing the SpoIVCA protein protected the inverted repeats and their neighboring sequences from DNase I digestion and methylation. SpoIVCA decreased the electrophoretic mobility of a DNA fragment containing its binding sequence and simultaneously bent the DNA. A single molecule of SpoIVCA bound initially to the repeat sequence followed by binding of a second molecule to create a complex straddling the recombination site.**

The late stages of sporulation in *Bacillus* involve the maturation of the spore and its release. The mother-cell compartment of the sporangium produces the coat proteins that surround the spore (1) and then it lyses, completing its terminal differentiation program. Expression of the coat proteins is controlled by a form of RNA polymerase containing the alternate σ factor σ^K (2). The *Bacillus subtilis sigK* gene, encoding σ^K, is interrupted by a 42-kilobase insertion called *skin*, which excises during sporulation from the mother-cell chromosome, joining the *spoIVCB* and *spoIIIC* loci to generate *sigK* (3–5). Transcription of *sigK* is itself restricted to the mother cell (6) and the activity of σ^K is regulated in a temporal manner by processing of its amino-terminal part about 1 hr after it is synthesized (2, 7, 8). Similar DNA rearrangements have been found in other genes from terminally differentiating cells, such as nitrogen fixation genes in heterocysts of *Anabaena* (9, 10) and immunoglobulin genes in B lymphocytes (11).

DNA sequence analysis revealed that *skin* excision takes place within two 5-base-pair (bp) direct repeats and that 21-bp inverted repeats are found just inside these borders (3). Genetic analysis of the recombination reaction demonstrated that the *spoIIID* and *spoIVCA* (*spoIVCcisA*) gene products are required for excision (3–5), the requirement for SpoIVCA being bypassed if an intact *sigK* gene is introduced into the cells (4). The latter result, together with the sequence similarity of the SpoIVCA protein to the Hin Gin Pin family of recombinase proteins (5), strongly suggested that *spoIVCA* encoded the recombinase responsible for *skin* excision. We show here that SpoIVCA binds specifically to the 21-bp repeats flanking the *skin* recombination sites. Detailed analysis of this protein–DNA interaction allows certain comparisons to be made between the SpoIVCA protein and other members of this recombinase family.

MATERIALS AND METHODS

Screening for Site-Specific DNA–Protein Interaction ("Southwestern" Screening). The "*Eco*RI" and "random" expression libraries of *B. subtilis* DNA in λgt11 constructed by Suh *et al.* (12) were screened according to published procedures (13, 14).

Southwestern Blotting. Cell pellets from 1.5-ml induced cultures were resuspended in 80 μl of loading buffer [62.5 mM Tris·HCl, pH 6.8/2% (wt/vol) sodium dodecyl sulfate (SDS)/10% (vol/vol) glycerol/715 mM 2-mercaptoethanol/0.001% (wt/vol) bromophenol blue], heated at 100°C for 5 min, sonicated for 10 sec, and subjected to SDS/polyacrylamide gel electrophoresis (PAGE). The separated proteins were electrophoretically transferred to Amersham Hybond-C extra filters, renatured, and incubated with probes as described (13, 14). Incubation was for 12–16 hr at 4°C with 5×10^6 cpm of probe DNA per ml and washing was carried out three times for 10 min each.

SpoIVCA Overproducing Plasmids. The 1724-bp *Nsi* I–*Bst*BI *spoIVCA*-containing fragment (5) was converted to an *Hin*dIII–*Xba* I fragment by insertion into a polylinker and was then inserted downstream of the *spac* promoter (16) in pDG148 (17) to create pSK11. A deletion of a 106-bp *Ssp* I–*Stu* I fragment internal to *spoIVCA* produced pDG1106, which expressed a truncated form of SpoIVCA.

Extract Preparation. *Escherichia coli* strain TG1 carrying the indicated *spac* promoter plasmid was grown in 2× YT medium (18) containing ampicillin. When the culture reached an OD_{600} of 0.5, isopropyl β-D-thiogalactoside was added to 1 mM and incubation was continued for 3 hr. All further steps were carried out at 4°C. The cells were collected by centrifugation, washed with B buffer (10 mM Tris·HCl, pH 8.0/50 mM KCl/0.1 mM EDTA/1 mM dithiothreitol/5% glycerol), and resuspended in B buffer to 0.5 g/ml. The cells were broken by passage through a French pressure cell at 6000 psi (1 psi = 6.89 kPa). The lysate was diluted 5-fold with B buffer containing 250 mM KCl and mixed gently to avoid shearing the DNA. The lysate was centrifuged at 16,000 × *g* for 30 min. The supernatant was collected and ammonium sulfate was added to 35% saturation. The precipitated protein was collected by centrifugation and ammonium sulfate was added to the supernatant to a total of 50% saturation. The lysate was again centrifuged and the two ammonium sulfate pellets were re-

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Abbreviation: DMS, dimethyl sulfate.

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suspended in B buffer (20–30 mg/ml). These two fractions were dialyzed against B buffer and stored at −80°C. The 35–50% ammonium sulfate precipitated protein exhibited the majority of the SpoIVCA activity and was used for further experiments.

DNA Probes. Plasmids used to prepare probes for Southwestern screening were made by cloning complementary sets of synthetic oligonucleotides. Two sets of oligonucleotides—5′-TTGTAATTACAAAAGGGGGGTGCATACACCC + 5′-GGGTGTATGCACCCCCCTTTTGTAATTACAA and 5′-TTGTAATTACAAAACCCCCCTGCATACACCC + 5′-GGGTGTATGCAGGGGGGTTTTGTAATTACAA—were synthesized, annealed, and cloned into the *Eco*RV site of pBluescript KS+ (Stratagene). The sequence generated by the former pair of oligonucleotides is referred to as the 31-mer sequence. Large amounts of the 43-bp *Eco*RI–*Hin*dIII restriction fragments from these plasmids were gel purified and blunt-ended using the Klenow fragment of DNA polymerase I in the presence of [α-^{32}P]dATP. The reaction was terminated by heating at 65°C for 5 min. The resulting fragments were ligated overnight at 15°C, producing probes of 200–250-bp average length, which were separated from unincorporated nucleotides by passage over a 1-ml Bio-Gel P-30 column (Bio-Rad).

Probes for mobility-shift assays and DNase protection ("footprinting") experiments were labeled on one end, gel purified, and subjected to chemical sequencing reactions as described (19). The recombination site at the *spoIIIC* locus was inserted between the *Bam*HI and *Eco*RV sites of pBluescript KS+ as a 219-bp *Sau*3A–*Rsa* I fragment to produce plasmid pSK75. This plasmid was later found to also contain a 65-bp *Sau*3A fragment from just downstream of *spoIIIC*. The presence of this extra fragment in some probes had no effect on the results. The recombination site from the *spoIVC* region was inserted between the *Sma* I and *Acc* I sites of pBluescript KS+ as a 231-bp *Hpa* I–*Bst*BI fragment to produce pSK79.

A plasmid containing a tandem repeat of a SpoIVCA-binding site was created by insertion of a *Xba* I–*Alu* I fragment of pSK79 into *Xba* I–*Sma* I-digested pMTL22 (20). The *Stu* I–*Sca* I and *Nae* I–*Sca* I fragments of this plasmid containing the SpoIVCA binding site were then ligated to create pDG1103. After various restriction enzyme digestions this plasmid was 3′ end-labeled and used without further purification.

Mobility-Shift Gel Electrophoresis. Binding reaction mixtures contained 20 mM Hepes (pH 7.8), 1 mM EDTA, 150 mM NaCl, 7 mM 2-mercaptoethanol, 10 mM glycerol, 25 μg of sonicated calf thymus DNA per ml, and ≈0.3 nM probe DNA in H_2O. Protein was added to the reaction mixtures last (15%, vol/vol) and incubation was carried out at room temperature for 10 min prior to loading onto a gel. Electrophoresis was on 5% acrylamide/0.17% bisacrylamide gels in 0.5× TBE (45 mM Tris-borate/1 mM EDTA) at 8 V/cm for 3–6 hr.

Footprinting Procedures. Twenty-microliter DNA-binding reaction mixtures were prepared and incubated as for mobility-shift gels except (*i*) no competitor DNA was added during the binding reaction, (*ii*) probe DNA was at ≈3.5 nM, and (*iii*) all protein solutions were 10 mg of total protein per ml [extract protein as indicated plus purified bovine serum albumin (Boehringer Mannheim)] in 0.5× B buffer. For DNase I footprinting, 5 μl of a DNase I (Boehringer Mannheim, grade 2) solution at 1 ng/μl (in 10 mM Tris·HCl, pH 7.5/10 mM KCl/10 mM $CaCl_2$/10 mM $MgCl_2$) was added, and incubation was continued at room temperature for 1 min. The reaction was terminated with 7.5 μl of DNase stop solution (2 M ammonium acetate/250 mM EDTA/200 μg of calf thymus DNA per ml) and 75 μl of ethanol. The samples were precipitated, washed with 70% ethanol, and dried. For methylation protection, 1 μg of calf thymus DNA was added immediately prior to addition of 1 μl of dimethyl sulfate (DMS). Incubation was continued for 4 min at room temperature. The reaction was stopped with 10 μl of DMS stop solution (19) and 200 μl of ethanol. The samples were precipitated, treated with piperidine, and prepared for electrophoresis as described for chemical sequencing reactions (19). Reactions involving DNase I treatment prior to loading onto nondenaturing gels were constituted as stated above for these gels. DNase I (100 or 400 pg) was added immediately before loading onto the gel. Radioactive bands were detected by autoradiography and the DNA was recovered as described (19).

RESULTS

Cloning of *spoIVCA*. To identify the protein that binds to the inverted repeats near the borders of the *skin* element, we used a double-stranded DNA probe containing the 31-mer sequence (see *Materials and Methods*) to screen the proteins produced in the plaques of a λgt11 expression library of *B. subtilis* DNA (12, 21). We identified four plaques containing proteins that bound the 31-mer sequence but not a similar sequence in which an internal string of 6 G·C base pairs had been inverted. Restriction analysis of these phages indicated that they contained the *spoIVCA* gene, and this was confirmed by hybridization analysis with a *spoIVCA* internal fragment (not shown).

***spoIVCA* Encodes a 55-kDa DNA-Binding Protein.** We achieved significant expression of *spoIVCA* in *E. coli* through two mechanisms: induction of the *lac* promoter in the λgt11 clones and induction of transcription from the *spac* promoter (16) inserted upstream of *spoIVCA* on a plasmid. The proteins produced in these cells were separated by SDS/PAGE, electrophoretically transferred to a membrane, allowed to renaturate, and screened for their DNA-binding activity with the 31-mer DNA probe. Cells in which *spoIVCA* had been transcribed contained a 55-kDa protein that bound specifically to this DNA probe (Fig. 1, and not shown). This protein did not bind to the control probe mentioned above or to probes containing unrelated sequences (not shown). Induced cells that contained only the parent plasmid or phage did not produce this DNA-binding activity. In all of these strains we observed, in addition, a 69-kDa protein that exhibited a high capacity for DNA binding and no sequence specificity (Fig. 1).

Characterization of SpoIVCA DNA Binding. A partially purified protein fraction from *E. coli* cells induced to produce SpoIVCA was used to perform DNase I (22) and DMS (23) methylation protection experiments. Fifty-four-base-pair regions surrounding the *skin* recombination sites, including the 21-bp conserved sequences, were protected from DNase I digestion by SpoIVCA (Figs. 2 and 3). The protection was almost complete throughout this region; only two digestion sites were unprotected near the actual site of recombination. Methylation of many G residues within these binding regions was diminished in the presence of SpoIVCA, whereas the methylation of several others was enhanced (Figs. 2 and 3).

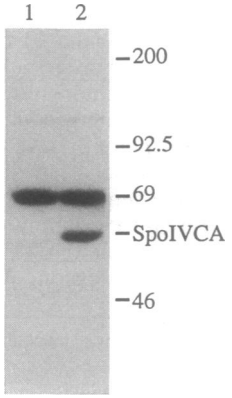

FIG. 1. Southwestern blotting of SpoIVCA. Isopropyl β-D-thiogalactoside-induced *E. coli* cells containing pDG148 (*spac* promoter, lane 1) or pSK11 (*spac-spoIVCA*, lane 2) were broken and loaded onto an SDS/12% polyacrylamide gel. The separated proteins were transferred to a filter, renatured, and detected with a DNA probe containing the 31-mer sequence. The positions of molecular mass standard proteins are indicated in kDa.

Particularly striking was the effect on the string of six or seven G residues found in both sites, three of which were protected in both cases, whereas others were enhanced. Interestingly, G residues on the opposite side of the recombination sites, in regions not conserved between the two sites, were also protected. The ability of SpoIVCA to protect a DNA fragment containing the 31-mer sequence was tested. Only a 27-bp region surrounding the conserved 21-bp sequence was protected (Figs. 2 and 3), corresponding to one-half of the region protected in the natural SpoIVCA-binding sites. No protection was observed in the presence of extract prepared from an induced strain containing only the parental plasmid pDG148 (not shown).

The protein fraction exhibiting SpoIVCA DNA-binding activity was able to produce a shift in the electrophoretic mobility of a DNA fragment containing the *skin* repeat sequence (Fig. 4). Several protein–DNA complexes were observed that were not produced by an identical fraction from induced cells carrying only the parental plasmid. The least retarded band (complex A in Fig. 4) appeared at low protein concentrations, increased to a plateau with increasing protein concentration, and disappeared at the highest protein concentrations (not shown). Complex B, the predominant retarded band, appeared at slightly higher protein concentrations than did complex A and increased proportionally with protein concentration (not shown). A faint band (complex C) was even more retarded than complex B. A single complex with relative mobility similar to that of complex A was observed when a DNA fragment containing the 31-mer sequence was used (not shown). The requirement for SpoIVCA binding to produce complexes A, B, and C was demonstrated by competition with specific and nonspecific DNA (Fig. 4). Addition of competitor plasmid DNA containing the *skin* recombination region led to the disappearance of these complexes, whereas a plasmid identical except for the absence of the *skin* sequences did not. Plasmid DNA that contained only the 31-mer sequence was an equally effective competitor, whereas plasmid containing the mutant 31-mer sequence was not (not shown). We carried out experiments designed to determine if complex A or B might represent a complex containing two DNA fragments held together through interaction with SpoIVCA. When two probes of different sizes were mixed with the SpoIVCA extract, a pattern of bands was produced that equaled the sum of the patterns generated by the two probes individually (not shown). No new intermediate band that would be expected to result from a complex simultaneously containing the two probes was observed.

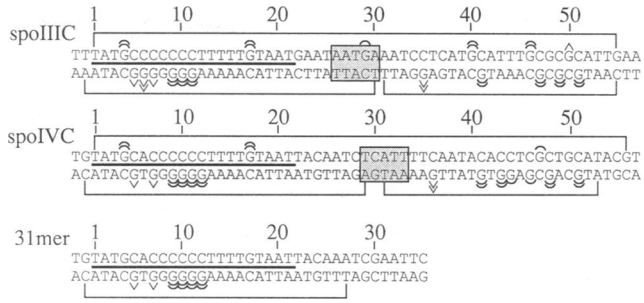

FIG. 3. Summary of DNA protection by SpoIVCA. Data are from Fig. 2 (where the run of GC pairs close to the recombination sites can be used to locate each sequence) and experiments not shown. Brackets indicate the extent of DNase I protection. Semicircles and carats indicate methylation protections and enhancements, respectively; double symbols indicate stronger effects. The bars between the strands indicate the inverted 21-bp repeats.

To further characterize the complexes observed in nondenaturing gels, we treated the binding reaction mixtures with DNase I just prior to loading onto the gel. The bands containing the various complexes were isolated from the gels and the DNA was recovered and run on a sequencing gel (Fig. 5). The DNA in complex A was protected from DNase I digestion in a 27-bp region equivalent to that previously observed with the 31-mer sequence. In complex B, the DNA was protected over a larger region, as observed in earlier experiments (see Fig. 2). Protection from DNase I digestion in complex C was nearly identical to that observed in complex B with the exception of a cutting site by DNase I at position 42. The difference in mobility between complexes B and C may be due to a conformational change resulting in a

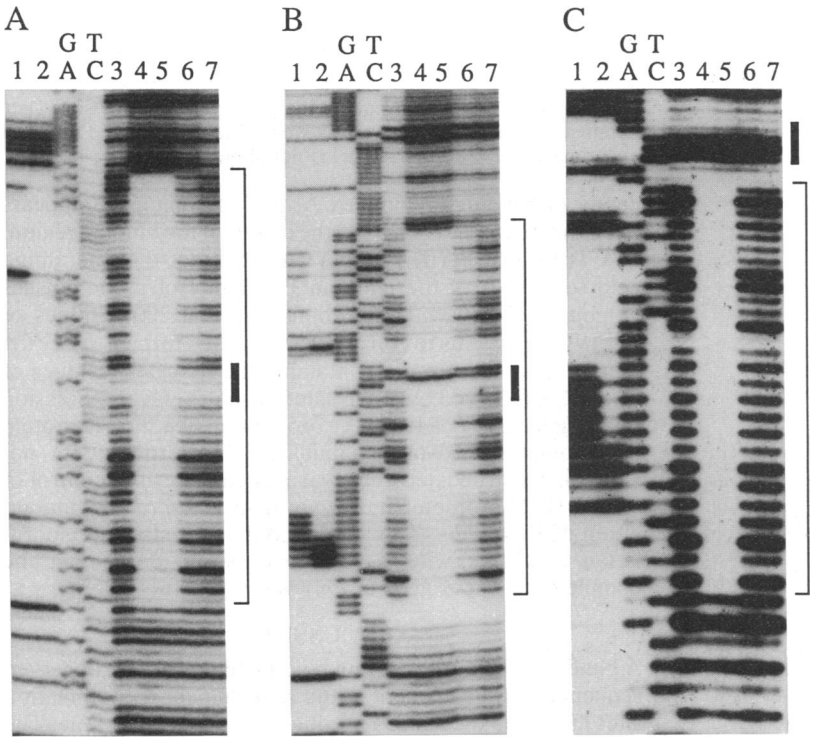

FIG. 2. DNase I and methylation protection by SpoIVCA. (*A*) Protection of the *spoIVC* recombination site. (*B*) Protection of the *spoIIIC* recombination site. (*C*) Protection of the 31-mer sequence. In all panels lanes 1 and 2 contain DMS-treated DNA in the presence of 0 μg and 375 μg of SpoIVCA-containing extract per ml, respectively. GA and TC indicate chemical sequence reactions. DNase I reaction mixtures in lanes 3–7 contained 0, 375, 75, 25, and 5 μg of SpoIVCA-containing extract per ml, respectively. Brackets indicate the extent of the region protected from DNase I and the heavy bar indicates the position of (or "position corresponding to" in *C*) the 5-bp site of recombination.

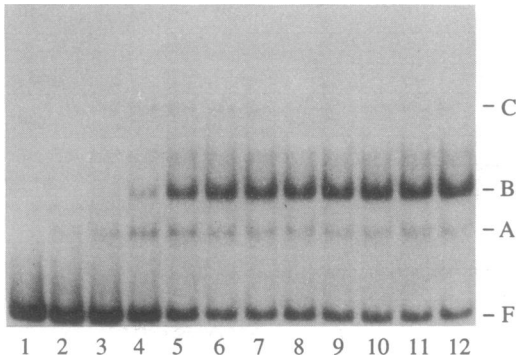

FIG. 4. DNA electrophoretic mobility shift produced by SpoIVCA. The 255-bp *Xba* I–*Xho* I fragment of pSK79 was incubated with 0 μg (lane 1) or 45 μg (lanes 2–12) of SpoIVCA-containing extract per ml prior to loading onto a polyacrylamide gel. Lane 7 contained no competitor DNA, whereas lanes 2–6 and 8–12 contained 12.5, 5, 1.9, 0.63, and 0.25 nM of pSK79 and pBluescript KS+, respectively. The top of the gel is not shown.

slight alteration in protection or may result from the binding of an additional protein present in the preparation.

SpoIVCA Bends DNA. We determined whether SpoIVCA bends the DNA around its binding site by using circularly permuted DNA fragments (24). Although the unbound DNA fragments did not exhibit any significant differences in electrophoretic mobility, binding of SpoIVCA near the center of the fragments resulted in significantly reduced mobility in comparison to fragments on which it bound near an end (Fig. 6). Interestingly, the patterns of altered mobilities for complexes A and B were slightly different. Plotting the relative mobility of the fragments against the positions of their ends produces a symmetrical curve in the case of complex A (Fig. 6*B*), indicating the presence of a simple DNA bend (24). Plotting of the data for complex B does not produce a simple curve (the slopes of the left and right sides of the curve shown in Fig. 6*B* are not equal and a symmetrical curve does not fit the data points), which suggests that these complexes may contain multiple bends. The large size of the binding site and a lack of restriction sites prevented a detailed determination of the center of the DNA bend. However, a comparison of the fastest migrating fragments in complexes A (Fig. 6*A*, lane 6) and B (Fig. 6*A*, lane 7) indicates that, with respect to the center of bending in complex A, that of complex B appears to be shifted toward or across the center of the binding region.

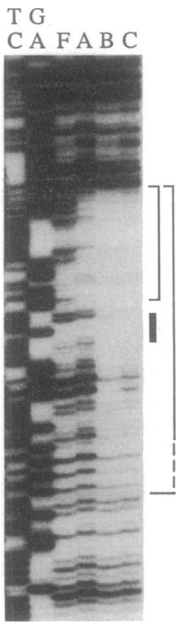

FIG. 5. DNase I protection in isolated SpoIVCA–DNA complexes. SpoIVCA–DNA complexes were treated with DNase I prior to electrophoresis on a nondenaturing polyacrylamide gel. The free DNA (lane F) and that from complexes A, B, and C (Fig. 4) were isolated and loaded onto a sequence gel. The DNA was a fragment of pSK79 3′ end-labeled at the *Xho* I site. The smaller, inside bracket marks the extent of protection in complex A. The larger bracket marks protection in complexes B and C; the dashed portion indicates weak protection. The bar marks the 5-bp recombination site.

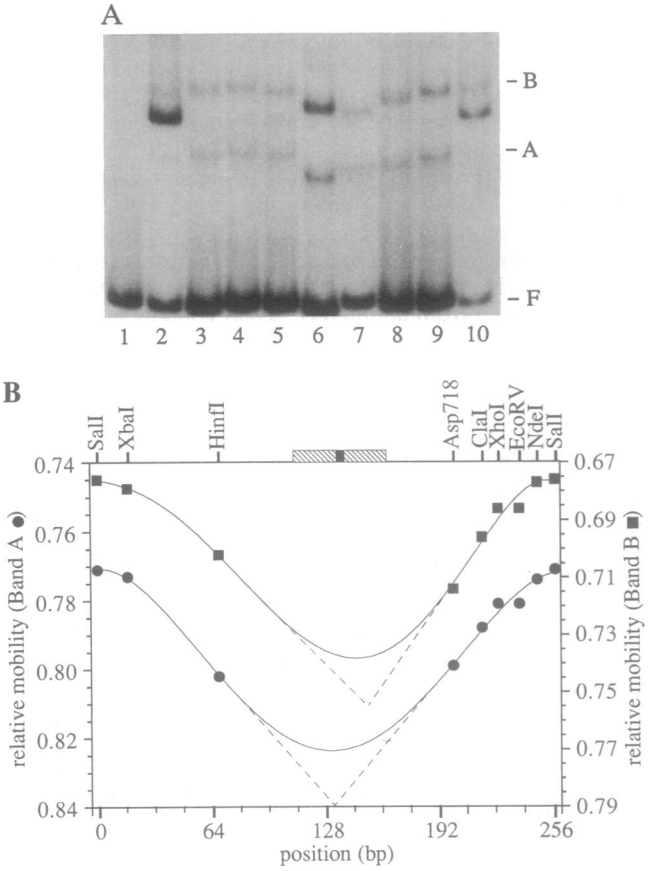

FIG. 6. DNA bending induced by SpoIVCA. (*A*) Circularly permuted fragments containing a *skin* recombination sequence were generated from plasmid pDG1103 by digestion with *Cla* I (lanes 1 and 8), *Eco*RV (lanes 2 and 10, where an additional 308-bp fragment originating from the pMTL22 vector is present), *Nde* I (lane 3), *Sal* I (lane 4), *Xba* I (lane 5), *Asp*718 (lane 7), or *Xho* I (lane 9) and ^{32}P labeling. The 257-bp *Hin*fI fragment of pDG1103 (lane 6) was gel purified prior to labeling. The DNA was mixed with 0 μg (lane 1, which contains only unbound fragment designated F) or 45 μg (lanes 2–10) of SpoIVCA-containing extract per ml prior to loading onto a polyacrylamide gel. (*B*) Relative mobilities of complexes A and B plotted against the position of the end points of the various DNA fragments. On the top of the plot frame are shown the positions of the region protected by SpoIVCA (hatched box) and of the recombination site (solid box). Position 0 is the center of the *Sal* I site. Data points are from *A* (similar results were obtained in three different experiments). The top of the gel is not shown.

A Truncated Form of SpoIVCA. The fact that the amino acid sequences of the Hin Gin Pin family of recombinases exhibit similarity with the amino-terminal 200 residues of SpoIVCA (5) led us to construct a truncated form of *spoIVCA* that expressed a protein containing the first 221 residues of SpoIVCA followed by 7 unrelated residues. Overexpression of this gene in *E. coli* led to the production of a 26-kDa protein that bound the 31-mer-containing DNA probe (data not shown). The truncated gene was placed under the control of the *spoIVCA* promoter at the *amy* locus in a *spoIVCA* mutant strain of *B. subtilis*. This strain exhibited a stage IV sporulation blockage, whereas a similar strain containing the complete *spoIVCA* gene at *amy* was Spo^+ (not shown).

DISCUSSION

A blind search for the protein binding to the 21-bp conserved sequences found adjacent to the *skin* element recombination sites identified the 55-kDa product of the *spoIVCA* gene. The

SpoIVCA DNA-binding activity was characterized by using partially purified extracts from bacteria in which expression of *spoIVCA* was induced. Binding of SpoIVCA to a DNA fragment containing only the 21-bp conserved sequence afforded protection from DNase I digestion over a 27-bp region. The same amount of protein protected a 54-bp region around both natural recombination sites. This protected region included that observed with the isolated 21-bp conserved sequence and an equivalent region on the opposite side of the recombination site. This result suggests that a single molecule of SpoIVCA binds to the conserved sequence as a half-site, followed by a second molecule binding to a half-site on the opposite side of each recombination site. In support of this idea was the observation of two complexes of SpoIVCA bound to its natural site exhibiting different electrophoretic mobilities. The DNase I footprints of these two complexes indicated that in one case only the conserved half-site was protected, whereas in the more retarded predominant complex both half-sites were protected. The protein concentration dependence of the appearance of these two complexes suggests that this change is due to binding of additional protein rather than to a conformational change. We were not able to determine if the form of SpoIVCA binding to each half-site is a monomer or a dimer but we suspect that it is a monomer for three reasons: (*i*) there is no dyad symmetry within a half-site as is found in the binding sites of many other DNA-binding proteins that bind as dimers, (*ii*) we were unable to detect complexes containing two DNA molecules that would result from binding of dimers in which each subunit was capable of binding a half-site, and (*iii*) other members of this family of recombinases bind a monomer to each half-site (25–28). The half-site outside of *skin* is a weaker binding site but it does contain SpoIVCA-binding determinants, as evidenced by the lack of SpoIVCA binding in the corresponding position when this sequence is removed and by the methylation protection of G residues at homologous positions in the two half-sites. The coding sequence of *sigK* may impose constraints on the sequence of the second half-site precluding a stronger binding sequence.

DNA binding by SpoIVCA is analogous to the situation observed for binding of the related Gin recombinase to the *gix* sites of the phage Mu invertible G region. This protein binds as a monomer to a strong binding site inside the G region and to a second weak binding site flanking the recombination point (25). In this case also removal of the weak binding site results in binding of only one molecule of Gin and no protection in the region corresponding to the normal position of the second site. The binding of the γδ transposon resolvase to normal *res* sites and isolated half-sites follows this same pattern (26). A related but slightly different situation is observed in the case of the Hin recombinase binding to the *hix* recombination sites of the *Salmonella typhimurium* flagellin gene inversion system. In this case the protein always binds as a dimer and removal of half of the *hix* sequence results in protection of the nonspecific sequence that replaces it (27). A 52-amino acid peptide containing the DNA-binding region of Hin does not dimerize and when bound to an isolated *hix* half-site does not protect the neighboring sequence (28).

We used circularly permuted DNA fragments containing a SpoIVCA-binding site to determine whether this protein bends the DNA (24). The differences in electrophoretic mobility of complexes in which the binding site was near the center or an end of the fragment demonstrated that SpoIVCA bends the DNA within the binding site. Binding of SpoIVCA to a single half-site bends the DNA within that half-site, whereas binding of a second molecule seems to introduce a more complex bend or multiple bends. The latter conclusion is drawn from extrapolated curves and remains speculative in the absence of convenient restriction sites near the SpoIVCA-binding site. SpoIVCA shares this ability to bend DNA with the related Gin invertase and γδ resolvase (25, 29).

DNA binding by SpoIVCA is similar to that of other members of this recombinase family, raising a question about the function of the protein domain found in SpoIVCA that is not present in the other family members. Preliminary results suggest that a 26-kDa truncated form of SpoIVCA, containing only slightly more than the recombinase-conserved sequences, retained the ability to bind to a SpoIVCA half-site but was unable to carry out *skin* excision *in vivo* (unpublished data). This would suggest that SpoIVCA resembles Hin (28) and γδ resolvase (15) in the positioning of the DNA-binding domain within the protein. It differs, however, in requiring additional domains to carry out the recombination reaction. Determining whether these added protein sequences are required simply for stability of SpoIVCA or for further interactions with the DNA, with itself, or with other proteins will require further biochemical characterization of the *skin* excision reaction.

We are grateful to Chet Price for his gift of the λgt11 libraries and to Claude Desplan for advice on the Southwestern screening technique. This work was supported by a grant from Centre National de la Recherche Scientifique (URA 1139). D.L.P. was a fellow of the Fogarty Foundation and the Human Frontier Science Program.

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