Binding of DNA In Vitro by a Small, Acid-Soluble Spore Protein from *Bacillus subtilis* and the Effect of This Binding on DNA Topology

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The DNA within spores of *Bacillus subtilis* is complexed with a large amount of α/β -type small, acid-soluble spore protein (SASP). Measurement of the interaction of a purified α/β -type SASP with DNA in vitro by a filter binding assay showed that the binding saturated at one molecule of SASP per approximately 5 bp. SASP-DNA binding did not require a divalent cation, was optimal at pH 6.7, and was unaffected by salt up to 400 mM. Binding of SASP to relaxed plasmid DNA in the presence of topoisomerase I resulted in the introduction of 18 (for plasmid pUC19) or 36 (for plasmid pUB110) negative supertwists, a superhelical density similar to that found in several plasmids isolated from spores. The SASP-dependent introduction of negative supertwists did not require a divalent cation, was unaffected by salt, and also gave a value of one molecule of SASP per approximately 5 bp at saturation. There was at least one slow step in the binding of SASP to DNA as seen in both the filter binding and supercoiling assays.

There are three lines of evidence which indicate that the state of DNA within dormant spores of various *Bacillus* species is significantly different from that in growing cells. (i) Spores are much more resistant to UV irradiation than are cells (15); (ii) UV irradiation of spores generates different DNA photoproducts than irradiation of cells (5, 15); and (iii) plasmid DNAs from spores have ~50% more negative supertwists than plasmid DNAs from cells (11). It has been suggested, based on this and other evidence, that DNA in spores is in a different conformation than DNA in cells (17). While a detailed explanation for the different properties of spore and cell DNA in vivo is not available, much of the difference appears due to the presence in spores of α/β -type small, acid-soluble spore proteins (SASP) which compose 30 to 50% of total spore core protein (16). The α/β -type SASP are coded for by a family of genes termed *ssp* (which has at least seven members in *B. subtilis*) which direct synthesis of proteins of almost identical primary sequence (16). The α/β -type SASP are associated with spore DNA in vivo (6), and removal of the majority of this protein by deletion of appropriate *ssp* genes results in UV-sensitive spores (9), whose DNA photochemistry and plasmid superhelicity are more similar to those of growing cells (11, 13). Strikingly, all α/β -type SASP tested appear to be interchangeable in their ability to confer spore-type properties on spore DNA in vivo (10). While the exact mechanism by which α/β -type SASP alter spore DNA structure and/or conformation (and thus its photochemistry and topology) is not clear, the amino acid sequences of α/β -type SASP are highly conserved both within and across species (2, 16). This is a further indication of the key role played by these major spore proteins in spore DNA structure. However, α/β -type SASP share no obvious sequence homology with any other proteins in available databases (16).

Given the striking effects these proteins have on spore DNA properties in vivo, it would be of interest to understand

the structural basis for these effects. As a first step in this process, we have begun studies on the interaction between purified α/β -type SASP and DNA. In this communication, we report the characterization of SASP-DNA binding and the effect of this binding on plasmid topology.

MATERIALS AND METHODS

Sources of plasmid DNAs. Plasmids pUC19, pBR325, pUB110, and pDG148 were isolated and purified through two CsCl gradients as previously described (3, 10, 18). Purified unlabeled pUC19 or pBR325 was cut with *Eco*RI (pUC19 and pBR325) or *Eco*RI plus *Hinc*II (pBR325) and labeled at 3' termini with [α -³²P]dATP and reverse transcriptase. Final specific activities of the labeled plasmid were ~5 × 10⁵ cpm/μg. However, for filter binding assays, labeled plasmid was routinely used with a 10- to 60-fold excess of unlabeled cut plasmid.

Source of α/β -type SASP. The SASP we chose for analysis of its DNA-binding properties was termed SspC and is the product of the *B. subtilis* *sspC* gene (4). The 3.2-kb *Eco*RI fragment containing the *sspC* coding sequence (4) was isolated, the ends were filled with DNA polymerase I Klenow fragment, *Hind*III linkers were added, and the DNA was digested with *Hind*III. This resulted in the *sspC* coding region on a 0.6-kb fragment, with its ribosome-binding site only 21 nucleotides from the *Hind*III site (Fig. 1A). This fragment was ligated with *Hind*III-cut pDG148 to place the translation initiating codon of the *sspC* gene 56 nucleotides from the inducible *spac* promoter (18). The ligation mix was used to transform *Escherichia coli* JM107 to ampicillin resistance, and colonies with plasmid carrying an insert were detected by colony hybridization with the 0.6-kb *Hind*III fragment as a probe (3). Plasmid was isolated from colonies carrying the insert, and the orientation of the insert in the plasmid was determined by *Eco*RV digestion. One such strain, PS708, was used for SspC production.

Strain PS708 was grown at 37°C in a 10 liters of 2× YT medium (3) containing ampicillin (50 μg/ml). At an optical density at 600 nm of 0.5, isopropyl-β-D-thiogalactospyranoside (IPTG) was added to 1 mM final concentration to induce

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FIG. 1. (A) Map of the DNA fragment containing the *sspC* gene; (B) polyacrylamide gel of purified SspC. (A) The restriction map of the *sspC* gene was taken from reference 4. The solid box denotes the ribosome-binding site, the shaded box shows the coding sequence, and the horizontal arrow gives the direction of translation. The restriction enzyme sites are denoted as follows: H3, *Hind*III; RV, *Eco*RV. (B) Aliquots of 5 μ g (lane b) or 50 μ g (lane c) of purified SspC as well as 20 μ g of starting acid extract from strain PS708 (lane a) were electrophoresed on an acid polyacrylamide gel and stained as described in Materials and Methods. The direction of migration on the gel is from left to right; the arrow notes the position of SspC.

the *spac* promoter, and growth continued for an additional 3 h. Cells were harvested by centrifugation (15 min; $10,000 \times g$) and frozen and lyophilized; the yield was ~ 8 g (dry weight) of cells. The dry cells were broken in 100-mg aliquots in a dental amalgamator (Wig-L-Bug) with glass beads (100 mg) as the abrasive material (3); 2 min of shaking sufficed to disrupt the cells. The dry powder was extracted with 600 ml of 3% acetic acid–30 mM HCl at 4°C for 30 min and centrifuged (15 min, $10,000 \times g$), and the pellet was reextracted with 400 ml of acetic acid–HCl. The supernatant fluids were pooled and dialyzed at 4°C in Spectrapor no. 3 tubing against six changes of 6 liters of 1% acetic acid, and the dialyzed material was lyophilized. The dry protein was dissolved in 80 ml of 8 M urea–10 mM Tris-maleate (pH 5.5) at room temperature and applied to a column (5 by 20 cm) of carboxymethyl cellulose-52 in 10 mM Tris-maleate (pH 5.5) (buffer A) at room temperature. After the column was washed with buffer A, protein was eluted with a linear salt gradient from 0 to 0.3 M NaCl in buffer A with 800 ml of each solution. Protein was detected by the Lowry reaction (8); SspC eluted in the middle of the gradient. Peak fractions were pooled and dialyzed at 4°C in Spectrapor no. 3 tubing against buffer A, and protein was concentrated by layering dry Sephadex G-200 over the dialysis bag, followed by dialysis overnight at 4°C against buffer A. The final yield of protein was ~ 60 mg at a concentration of 5 mg/ml as determined by amino acid analysis. The purified protein gave only a single band on polyacrylamide gel electrophoresis at low pH (3), even when large amounts of protein were applied to the gel (Fig. 1B), and $>90\%$ bound to a DNA-cellulose column at pH 7.0 (data not shown).

Filter binding assay. Filter binding assays of SspC-DNA interaction were routinely done in 50 μ l of binding buffer (25 mM Tris-maleate [pH 6.7], 50 mM potassium acetate [KOAc]) with 5×10^3 to 1×10^4 cpm of 32 P-labeled pUC19, which corresponded to 100 ng to 5 μ g of DNA. All solutions were at 4°C, and binding reactions were begun by SspC

addition. After 10 to 15 s at 4°C, samples were passed through a 2.5-cm HAWP membrane (0.45- μ m pore size; Millipore) and rinsed with 40 drops of room temperature binding buffer, the filters were dried, and their radioactivities were counted in a toluene-based scintillation fluid. Routinely, the percentage of total counts in a reaction which bound to the filter in the absence of SspC was $<1\%$.

Analysis of effects of SspC on plasmid supercoiling. Purified supercoiled plasmid was relaxed with calf topoisomerase I (Bethesda Research Laboratories) and then repurified by phenol extraction and ethanol precipitation. The relaxed plasmid (1 to 14 μ g) was incubated for ~ 18 h at 4°C in 20 μ l of 25 mM Tris-maleate (pH 7.5)–50 mM KOAc–10 mM magnesium acetate–1 mM dithiothreitol–50 μ g of bovine serum albumin per ml with various amounts of SspC. This incubation time was found to be sufficient for the SspC-DNA interaction to reach equilibrium. Topoisomerase (~ 2 U/ μ g of DNA) was then added, samples were incubated for an additional 2 h at 37°C, and DNA was purified by phenol extraction and ethanol precipitation. The purified DNA was dissolved at ~ 0.1 μ g/ μ l in 10 mM Tris hydrochloride (pH 8.0)–1 mM EDTA, and ~ 0.5 μ g of DNA was run on agarose gel electrophoresis in the presence of chloroquine (2 or 4 μ g/ml) as previously described (11). The average number of superhelical turns (τ_{av}) in this treated plasmid was determined by electrophoresis with a set of standards prepared by incubating plasmid DNA with topoisomerase in the presence of different amounts of ethidium bromide as described previously (11).

RESULTS

Choice of α/β -type SASP. The α/β -type SASP we chose for study of SASP-DNA binding was SspC, the product of the *sspC* gene of *B. subtilis* (4). We chose SspC because we obtained high yields of this protein from an *E. coli* expression system, yields which were 3- to 20-fold higher than those for other SASP produced with the same vector (data not shown). SspC was by far the most predominant protein in acid extracts of IPTG-induced strain PS708 (Fig. 1B, lane a). The SspC obtained was $>98\%$ pure as judged by acrylamide gel electrophoresis at low pH (Fig. 1B, lanes b and c), and its amino acid composition was identical to that predicted from the sequence of the gene (data not shown). While SspC is only a minor SASP in *B. subtilis* spores (B. Setlow and P. Setlow, unpublished data), previous work has shown that all α/β -type SASP, including minor ones such as SspC, are interchangeable in so far as their effects on spore DNA properties are concerned (10; F. Tovar-Rojas and P. Setlow, unpublished data).

Analysis of SspC-DNA interaction by filter binding. Measurement of SspC-DNA interaction by filter binding showed that SspC caused retention of DNA on a mixed cellulose acetate-cellulose nitrate filter (Fig. 2). With constant DNA present, DNA retention on the filter increased as the SspC level was increased up to values of 80 to 90% of input DNA retained. With DNA concentrations of 20 and 100 μ g/ml, maximum DNA retention was achieved at SspC/DNA weight ratios of $\sim 3/1$. However, with 2 μ g of DNA per ml, maximum binding required a higher SspC/DNA ratio, suggesting that the binding of SspC to DNA is not very tight (Fig. 2, and see legend). Strikingly, the binding curves at the two lower DNA concentrations were sigmoid (Fig. 2), suggesting that SspC binding to DNA is cooperative and/or that binding of multiple SspC molecules is needed to achieve DNA retention on the filter (see below).

FIG. 2. Filter binding assay of SspC-DNA interaction at various DNA concentrations. The filter binding assay was done with 50- μ l reaction mixtures as described in Materials and Methods with 100 ng (○), 1 μ g (●), or 5 μ g (□) of 32 P-labeled pUC19 per assay. The SspC/DNA ratios given are the weight ratios. Maximum binding in this experiment represents binding of 85% of the input DNA.

One potential problem with the filter binding assay for measurement of SspC-DNA interaction is whether retention of DNA on the filter requires association with one or with many SspC molecules. However, it appears that much (possibly saturating) SspC must be bound to the DNA to obtain retention of DNA on the filter, because with labeled DNAs of either 2.7 kb (pUC19, Fig. 2) or 0.5 or 4.8 kb (*Eco*RI- or *Eco*RI- plus *Hinc*II-cut pBR322, data not shown) at 20 μ g/ml, the binding saturated at a weight ratio of SspC/DNA of 3/1. If weak binding of only a few SspC molecules was needed for retention of DNA on the filter, then the smaller fragments would have been expected to require higher SspC levels to achieve equivalent filter binding. That the 3/1 weight ratio of SspC/DNA needed for maximum filter binding does reflect a requirement for binding of multiple (and possibly saturating) SspC molecules to cause maximum DNA filter binding is also consistent with the 3/1 weight ratio of SspC/DNA needed for maximum effects of SspC on plasmid DNA supercoiling (see below).

The filter binding assay was easily used to measure the effect on SspC-DNA interaction of pH, ionic strength, and divalent cations. Binding of SspC to DNA was found to be optimal at pH 6.7 but was only slightly decreased at pH 6.1 and 7.5 (Table 1). However, binding decreased dramatically at pH 8.5. SspC-DNA binding was not appreciably decreased by KOAc concentrations up to 400 mM and was essentially identical with EDTA or Mg^{2+} present (Table 1). However, use of higher concentrations (10 mM) of Mg^{2+} decreased binding somewhat (Table 1).

Surprisingly, when binding was initiated by the addition of subsaturating amounts of SspC, the DNA retained on the filter decreased significantly over time, with the rate of loss slower at 4°C than at 25°C, although at 25°C an equilibrium

TABLE 1. Effect of various parameters on SspC-DNA interactions as measured by filter binding^a

pH	[KOAc] (mM)	[EDTA] (mM)	DNA bound to filter (% of maximum)
Expt 1			
6.1	50	1	70
6.7	50	1	100 ^b
7.5	50	1	67
8.5	50	1	3
Expt 2			
6.7	50	1	100 ^b
6.7	100	1	100
6.7	200	1	99
6.7	400	1	97
Expt 3			
6.7	50	1	100 ^b
6.7	50	0 (5 mM $MgCl_2$)	100
6.7	50	0 (10 mM $MgCl_2$)	78

^a SspC-DNA interaction was measured by the filter binding assay as described in Materials and Methods, with changes as noted. Each reaction had 100 ng of DNA and 1 μ g of SspC.

^b For each experiment, these values were set at 100% and represent 60 to 70% of binding achieved at saturating levels of SspC.

FIG. 3. Change in filter-bound DNA as a function of time. The filter binding assay was done as described in Materials and Methods with 1 μ g of pUC19 per assay. Binding reactions were initiated by the addition of 2 μ g (□, ■) or 5 μ g (○, ●) of SspC, and reaction mixtures were incubated at 4°C (●, ■) or 25°C (○, □). Aliquots were removed at appropriate times and assayed for filter binding. At zero time, the samples with the 2/1 SspC/DNA weight ratios gave 56% (25°C) and 69% (4°C) of the binding seen with the 5/1 SspC/DNA weight ratios.

FIG. 4. Chloroquine-agarose gel analysis of plasmid incubated with SspC. Samples were incubated overnight at 4°C with 1 µg of either pUC19 or pUB110 with or without SspC followed by topoisomerase I addition and further incubation for 2 h at 37°C. Samples were then treated and analyzed by agarose gel electrophoresis with chloroquine as described in Materials and Methods. The direction of migration on the gels is toward the bottom of the figure. For pUC19: lanes 1 and 5, no SspC plus topoisomerase; lanes 2 and 6, 12 µg of SspC without topoisomerase; lanes 3 and 7, 5 µg of SspC plus topoisomerase; lanes 4 and 8, 12 µg of SspC plus topoisomerase; lanes 1 to 4, gel run with 2 µg of chloroquine per ml; lanes 5 to 8, gel run with 4 µg of chloroquine per ml. For pUB110: lanes 1 and 3, no SspC plus topoisomerase; lanes 2 and 4, 12 µg of SspC plus topoisomerase; lanes 1 and 2, gel run with 2 µg of chloroquine per ml; lanes 3 and 4, gel run with 4 µg of chloroquine per ml. The arrow lettered *a* denotes the migration position of nicked plasmid DNA. The arrows numbered 0, -18, and -38 denote the number of supercoils in plasmid migrating at these positions as determined with reference to standards as described in Materials and Methods.

point was reached (Fig. 3). However, at saturating SspC levels, binding remained relatively constant (Fig. 3). The decrease in apparent binding at low SspC levels was not due to DNA degradation or exonuclease removal of labeled 3' termini, as isolation of DNA after 3 h of incubation and analysis by agarose gel electrophoresis revealed no degradation (data not shown). The reason for the slow decrease in filter binding with time at subsaturating SspC/DNA ratios is not clear. However, a slow interaction between SspC and DNA was also seen in analysis of the topological effects of binding of SspC to DNA (see below).

Effect of SspC binding on apparent DNA supercoiling. The binding of SspC to DNA *in vitro* is not surprising given the association between α/β -type SASP and DNA *in vivo* (6). Similarly, the effects of α/β -type SASP on plasmid DNA supercoiling in spores (11) suggested that SspC might have profound effects on plasmid DNA supercoiling *in vitro*. Consequently, we mixed SspC with relaxed plasmid DNA, added topoisomerase I to relax any supertwists generated by SspC-DNA interaction, and then determined the number of supertwists present in the plasmid DNA after deproteinizing the complex. Striking, maximum SspC binding resulted in introduction of a large number of negative supertwists into the two plasmids tested, pUC19 (Fig. 4, lanes 4 and 8) and pUB110 (Fig. 4, lanes 2 and 4). While approximately twice as many negative supertwists were introduced into pUB110 as into pUC19, the latter plasmid is only 60% the size of pUB110. Consequently, the superhelical density induced in both plasmids by SspC binding was similar and was only slightly below that seen with plasmids isolated from spores (11). With pUC19, the same final superhelical density ($\tau_{av} = -18$) was also achieved when SspC was added to extremely supercoiled plasmid ($\tau_{av} = -54$) and then incubated with topoisomerase I (data not shown). However, without topoisomerase I addition, SspC caused no change in the supercoiling of relaxed pUC19 (Fig. 4, lanes 1, 2, 5, and 6) or pUB110 (data not shown).

Titration of the effect of SspC binding on plasmid super-

helicity gave a sigmoid curve, again indicative of cooperative interactions between SspC molecules in DNA binding. These curves reached saturation at an SspC/DNA weight ratio of $\sim 10/1$ (Fig. 5). At values below saturation, an even distribution of plasmid was observed around the τ_{av} (Fig. 4, pUC19, lanes 3 and 7, and data not shown), as was also observed when the effect of HU protein on plasmid supercoiling was examined (12). The high weight ratio of SspC/DNA required to achieve saturation is probably a reflection of the weakness of SspC-DNA interactions under these conditions, because use of higher plasmid DNA concentrations gave maximal change in supercoiling at SspC/DNA weight ratios of $\sim 3/1$ (Table 2). Note that these reactions were performed under somewhat different conditions than the filter binding assay (pH 7.5 instead of 6.7; 10 mM magnesium acetate instead of 1 mM EDTA). These changes were needed for maximal topoisomerase activity but were found to weaken SspC-DNA interaction in the filter binding assay. Mg^{2+} is not essential for calf topoisomerase I activity, but it does exert a stimulating effect. This was especially true for topoisomerase action on pUB110 (data not shown), for reasons that are not clear. Consequently, while Mg^{2+} was not essential for the effect of SspC on plasmid DNA topology (see below), it was routinely added to ensure that the topoisomerase reaction went to completion.

The interaction between SspC and DNA which gave a high negative superhelical density in plasmids had a number of similarities to the SspC-DNA interaction measured by filter binding. Thus, generation of the high negatively supercoiled plasmid was not affected by up to 200 mM KOAc (Fig. 6A and B, compare lanes 2 and 4) (unfortunately, higher concentrations inhibited the topoisomerase I significantly) and was not abolished when EDTA replaced Mg^{2+} (Fig. 6A and B, lanes 3). Indeed, substitution of EDTA for Mg^{2+} resulted in plasmid with two to three more negative supertwists than in plasmid incubated with Mg^{2+} (Fig. 6A and B, compare lanes 3 and 4). The interaction between SspC and plasmid DNA leading to high negative supercoiling also had at least

FIG. 5. Effect of increasing SspC levels on induction of negative supertwists in plasmids. A 1- μ g sample of pUC19 or pUB110 was incubated overnight at 4°C in the standard reaction mixture with various amounts of SspC, followed by topoisomerase I addition and further incubation for 2 h at 37°C. After deproteinization, samples were analyzed by electrophoresis on agarose gels containing chloroquine and the average value of negative supertwists (τ_{av}) was determined as described in Materials and Methods.

one slow step in the process (Fig. 7A and B). Note that in this latter experiment all samples were incubated for 2 h at 37°C to allow topoisomerase action.

DISCUSSION

As indicated previously by analysis of the effects of SASP on DNA melting temperature (14), SspC exhibits no signifi-

TABLE 2. Induced negative supercoiling at various concentrations of DNA and SspC^a

SspC/DNA ratio	DNA (μ g/assay)	τ_{av}
pUB110		
4.25	5	-38
7.5	1	-27
15	1	-38
pUC19		
0.35	14	-2
2.5	10	-14
9.5	1	-16
10	5	-18
18	1	-18

^a DNA and SspC were incubated in 20- μ l volumes as described in Materials and Methods. After incubation overnight at 4°C, topoisomerase I was added and incubation continued for 2 h at 37°C. Samples were deproteinized and analyzed for numbers of negative supertwists as described in Materials and Methods.

cant sequence specificity for DNA, as it binds equally well to pUC19 and pUB110. Analysis of SspC-DNA interaction by measurement of the generation of negative supertwists in relaxed plasmid DNA indicates that saturation of DNA with SspC can be achieved with an approximately 3/1 weight ratio of SspC/DNA. A similar value was obtained with the filter binding assay. This value translates into one molecule of SspC bound per $\sim$ 5 bp of DNA. Analysis of the binding of another nonspecific DNA-binding protein, HU, has generally indicated binding of one HU monomer to 10 to 15 bp (1, 12, 19), although in one report this value was much lower (7); HU protein is $\sim$ 25% larger than α/β -type SASP. It is possible that the SspC/DNA stoichiometry we measured is actually too low, because some of the SspC used may have been damaged in some way. Thus, the value of 5 bp taken as defining the SspC binding site should be considered a minimum value.

Analysis of the α/β -type SASP/DNA weight ratio in spores has given values of 2 to 3 to 1 (14; B. Setlow and P. Setlow, unpublished data). This suggests that the amount of α/β -type SASP in spores is close to sufficient to saturate all spore DNA. However, it is possible that proteins other than α/β -type SASP are bound to spore DNA and are involved in bringing about spore DNA properties. In support of this latter suggestion, removal of $\sim$ 80% of the total α/β -type SASP from spores by deletion of appropriate *sps* genes reduces the increased negative supercoiling of plasmid DNA and formation of spore-type UV photoproducts in spore DNA by only $\sim$ 50% (11, 13). However, at present the identity of such additional proteins, other than minor α/β -type SASP, is unclear (16). It is, of course, possible that the environment in the spore core (pH, water content, etc.), which is quite different from that in the growing cell (15), contributes significantly to spore DNA properties.

As noted above, if the 3/1 weight ratio for SspC/DNA binding at saturation is correct, this indicates binding of one SspC molecule to about 5 bp. However, it appears that SspC may not bind to DNA as a monomer, given the cooperativity seen in both the filter binding and supercoiling assays. Studies of purified SASP in solution have not shown any evidence of formation of dimers or higher oligomers (P. Setlow, unpublished data). However, productive binding to DNA may involve interaction of SspC not only with DNA but also with other SspC molecules. This cooperativity cannot be an all-or-none binding to a single DNA molecule, because plasmids with intermediate levels of supercoiling were observed at subsaturating SspC/DNA ratios. Interestingly, there are at least two reports that the HU protein from *Bacillus* species also exhibits cooperativity in binding to DNA (7, 19), although *E. coli* HU protein may not (1, 12).

One obvious question about the SspC-DNA interaction is the tightness of binding. Clearly, initial binding of SspC to DNA cannot be extremely tight, because at low DNA concentrations high SspC/DNA ratios are required to saturate the DNA. Unfortunately, neither of the assays used in this work allow precise calculation of K_D values for an SspC-DNA complex. However, from the supercoiling data at various SspC/DNA ratios, we can calculate that the K_D must be $<5 \times 10^{-4}$ M and $>1 \times 10^{-6}$ M. While this binding looks weak if the higher number is correct, the concentration of α/β -type SASP in spores is extremely high (>2 mM) (14, 16).

One surprising observation in both the filter binding and supercoiling assays was the presence of a slow step in the SspC-DNA interaction. The nature of this slow step is not clear, but slow steps have been observed in binding of other

FIG. 6. Effect of salt and EDTA on SspC-induced plasmid supercoiling. A 1- μ g sample of pUC19 was incubated with 12 μ g of SspC under standard conditions with various changes as noted below, topoisomerase was added, and the samples were incubated for 2 h at 37°C and then processed and run on agarose gel electrophoresis with either 2 μ g (A) or 4 μ g (B) of chloroquine per ml as described in Materials and Methods. The direction of migration on the gels is toward the bottom of the figure. The samples run are as follows: lane 1, relaxed plasmid DNA; lane 2, with SspC and 200 mM KOAc; lane 3, with SspC and EDTA (10 mM) but without magnesium acetate; lane 4, with SspC. The arrows lettered a denote the migration position of nicked plasmid; the arrows numbered 0 and -18 give the number of supercoils in plasmid migrating at these positions as determined with reference to standards as described in Materials and Methods.

FIG. 7. Kinetics of change in plasmid supercoiling upon SspC binding. Plasmid pUC19 (1 μ g) was incubated with 12 μ g of SspC at 4°C (lanes 2 to 5), and at various times samples (20 μ l) were withdrawn, topoisomerase was added, and samples were incubated for 2 h at 37°C and processed for analysis on agarose gel electrophoresis containing 2 μ g (A) or 4 μ g (B) of chloroquine per ml. The samples applied were as follows: lane 1, relaxed plasmid; lane 2, no incubation at 4°C; lane 3, incubated for 1 h at 4°C; lane 4, incubated for 4 h at 4°C; lane 5, incubated for 16 h at 4°C. The arrow lettered a gives the migration position of nicked plasmid DNA; the numbered arrows give the number of supertwists in plasmid migrating at these positions.

nonspecific DNA-binding proteins such as HU to DNA (12). A more fundamental question is the exact nature of the SspC-DNA interaction and the resulting effect on DNA structure. The dramatic change in plasmid supercoiling upon SspC binding suggests there must be concomitant drastic changes in DNA structure. This could be due either to DNA winding around the SspC, as with DNA binding to HU protein (1, 12), or to induction of a conformational change in DNA, possibly from the B form to the A form (11, 15). As noted previously, conversion by SspC binding of a covalently closed DNA from the B form to the A form in the presence of topoisomerase I would result in the introduction of a large number of negative supertwists when the plasmid is deproteinized (11). While it is not clear that DNA in spores is in the A form, this is consistent with its UV photochemistry (15, 17). Furthermore, preliminary circular dichroism spectral analyses of the binding of α/β -type SASP to DNA have indicated that these proteins cause DNA to change to an A-like conformation *in vitro* (S. Mohr and P. Setlow, unpublished data). It is of course possible that α/β -type SASP affect DNA structure by both the wrapping of DNA around the SASP and induction of a B-to-A conformational change. In this regard, note that binding of protein HU, which does not induce a B-to-A conformational change in DNA (1), only induces ~40% the number of negative supertwists per unit of DNA as does SspC, while binding of other basic proteins generally has no effect on plasmid topology (1, 12). Clearly, it will be of great interest to determine the details of the interaction between DNA and α/β -type SASP, as the latter may represent a new class of DNA-binding proteins.

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