Small, acid-soluble spore proteins (SASP) of the α/β-type from several Bacillus species were cross-linked into homodimers, heterodimers and homooligomers with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) in the presence of linear plasmid DNA. Significant protein cross-linking was not detected in the absence of DNA. In all four α/β-type SASP examined, the amino donor in the EDC induced amide cross-links was the α-amino group of the protein. However, the carboxylate containing amino acid residues involved in cross-linking varied. In SASP-A and SASP-C of Bacillus megaterium two conserved glutamate residues, which form part of the germination protease recognition sequence, were involved in cross-link formation. In SpS-A and SASP-C of Bacillus subtilis and Bec1 from Bacillus cereus the acidic residues involved in cross-link formation were not in the protease recognition sequence, but at a site closer to the N terminus of the proteins. These data indicate that, although there are likely to be subtle structural differences between different α/β-type SASP, the N-terminal regions of these proteins are involved in protein-protein interactions while in the DNA bound state.

Between 5 and 10% of the total protein in spores of the Bacillus and Clostridium species of bacteria is α/β-type small, acid-soluble spore protein (α/β-type SASP)1 (1, 2). These proteins are encoded by four to seven monocistronic genes in each species, and their amino acid sequences are highly conserved both within and between Bacillus species (1, 2). The α/β-type SASP are nonspecific DNA-binding proteins which are synthesized only within the forespore compartment during sporulation (3, 4). Typically, two major α/β-type SASP accumulate to high levels within the spore, while the minor α/β-type SASP are found at much lower levels. The level of total α/β-type SASP in spores is sufficient to saturate the spore chromosome, and the binding of these proteins to spore DNA is the major determinant of spore resistance to UV radiation and a significant determinant of spore heat resistance (1, 2). Bacillus subtilis spores which lack the two major α/β-type SASP (α and β) are much more sensitive to UV radiation and heat than are wild type spores (5). During the first few minutes of spore germination, α/β-type SASP are quickly degraded by a sequence-specific protease termed germination protease (GPR) (1, 2).

Structural studies of purified α/β-type SASP and α/β-type SASP-DNA complexes have shown that significant changes in these proteins’ structure occur upon binding to DNA, as α/β-type SASP are predominantly unfolded in solution but acquire significant α-helical content upon binding to DNA (6). The α/β-type SASP cover 4–6 base pairs of DNA, and binding of these proteins to DNA is highly cooperative, particularly to DNAs bound with low affinity (7). Electron micrographs of α/β-type SASP-DNA complexes indicate that the protein forms a helical coat along the DNA (8), suggesting that there are extensive interactions between α/β-type SASP when bound to DNA, although these proteins are monomers in solution (9). Consequently, it is possible that interactions between adjacent α/β-type SASP along the DNA backbone may be important for the α/β-type SASP/DNA binding interaction.

To determine which regions of the proteins are involved in interactions between α/β-type SASP bound to DNA, we have performed protein cross-linking studies with the zero-length cross-linking reagent 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC). We have identified EDC-catalyzed protein cross-links in four different α/β-type SASP from Bacillus species, and the identification of these cross-links has yielded new insights into the interaction of α/β-type SASP on DNA.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Growth Conditions**—The Escherichia coli strains used include: JM107 (F’ traD36 proA’ proB’ lacY1 galK1 recA1 thi-1 hsdR17 supE44 relA1 Δ(lac-proAB) mcrA) (Life Technologies, Inc.); JM83 (ara Δ(lac-proAB) rpsL Δ80 lacZM15) (10); BL21(DE3) (T7 RNA polymerase under control of the lac promoter) (11), and BMH 71-18 (F’ proAB lacY1 ΔlacZM15 thi-1 supE Δ(lac-proAB) nutS–Tn10) (CLONTECH Laboratories, Inc.). The Bacillus strains used were Bacillus cereus T (originally obtained from H. O. Halvorson) and Bacillus megaterium QMB1551, ATCC no. 12872 (originally obtained from H. Levinson).

E. coli strains were routinely grown in 2× YT medium (16 g of tryptone, 10 g of yeast extract, 5 g of NaCl per liter) at 37 °C with shaking. For the overexpression of cloned genes encoding α/β-type SASP, the medium was supplemented with 100 μg/ml ampicillin (JMI07) or 200 μg/ml ampicillin and 0.5% glucose (BL21). B. megaterium was sporulated at 30 °C in supplemented nutrient broth, and spores were harvested and cleaned as described previously (12).

**Polynucleotide Chain Reaction Amplification and Site-directed Mutagenesis**—Oligonucleotides were designed to polymerase chain reaction amplify a 512-base pair fragment containing the gene encoding Bec1 (13) from B. cereus genomic DNA; the amplified fragment contained the gene’s ribosome binding site and transcription terminator (13). The upstream primer, BCE1–1 (5’-AAAAAGATCCCTATATTCTCATATTGGTACC; complementary to nucleotides 118–140) (13) and downstream primer, BCE1–2 (5’-AAAAAGATCCCTTTTAAAGTAGCTTTTCTTGG; complementary to nucleotides 592–613) (13), each contained BamHI restriction sites and 5′-flanking sequences (underlined residues) for cloning purposes. The BamHI-digested polymerase chain

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‡ The abbreviations used are: SASP, small, acid-soluble spore proteins; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HPLC, high performance liquid chromatography; MALDI-TOF, matrix-assisted laser desorption/ionization-time of flight; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethy]glycine; PAGE, polyacrylamide gel electrophoresis; GPR, germination protease.

8 S. C. Mohr and P. Setlow, unpublished results.

9 B. Setlow and P. Setlow, unpublished results.
reaction product was agarose gel-purified and ligated into BamHI-digested plasmid pET3 (11), generating plasmid pPS2532 in which Bce1 digestion confirmed that the gene encoding Bce1 was under the control of the T7 promoter. Plasmid pPS2532 was used to transform E. coli strain DE3(BL21) to ampicillin resistance.

The E10K mutant form of Bce1 was generated with the TransfeR™ site-directed mutagenesis kit from CLONTECH according to manufacturer's instructions. Phosphorylated primers complementary, except for designed mismatches (underlined bases), to the unique Bce1 and Bce1E10K were overexpressed in lactopyranoside inducible promoter as described previously (14, 15).

Dialysis in Spectrapor 3 tubing against 1 liter of 10 mM sodium phosphate (pH 7.5) at 24 °C for 20 min prior to addition of EDC to use cross-linking reagents with short- or zero-length linker.

The proteins chosen for this study were SASP-A and SASP-C from B. megaterium, SspC from B. subtilis, and Bce1 from B. cereus (Fig. 1). Most of the variability between these proteins occurs near the N termini which vary both in length and amino acid sequence (Fig. 1) (1). Therefore several protein cross-linking reagents which might be useful in future studies were selected for this study. The a/β-type SASP lack the N-terminal methionine residue which is the only residue that is removed post-translationally (Fig. 1) (1). SASP-A and SASP-C do not contain cysteine residues (1) and therefore several protein cross-linking reagents which rely on thiol chemistry could not be used for this study. The a/β-type SASP only in the presence of DNA (see below) and therefore decided to use protein cross-linking to trap interacting amino acid residues for subsequent biochemical analysis. Protein cross-linking with EDC usually involves the formation of a disulfide bond between cysteine residues. Therefore, in contrast to cross-linkers that react with amine groups, EDC appeared to interact with one another only when bound to DNA. We were interested in regions of close contact between a/β-type SASP and therefore decided to use cross-linking reagents with short- or zero-length linker arms. EDC, a water-soluble carbodiimide, gave efficient cross-linking of a/β-type SASP only in the presence of DNA (see below). Consequently, we chose this reagent for further work. Protein cross-linking with EDC usually involves the formation of an amide bond between either an N-terminal α-amino or lysine ε-amino group and the carbonyl side chain of aspartate/glutamate residues. Therefore, in contrast to cross-linkers that contain flexible linker arms several anssgroms in length, EDC induced cross-links should occur only between residues that are in very close proximity to one another.

The proteins chosen for this study were SASP-A and SASP-C from B. megaterium, SspC from B. subtilis, and Bce1 from B. cereus (Fig. 1). Most of the variability between these proteins occurs near the N termini which vary both in length and amino acid sequence (Fig. 1) (1). All a/β-type SASP lack the N-terminal methionine residue which is the only residue that is removed post-translationally (Fig. 1) (1). SASP-A and SASP-C are major a/β-type SASP in spores, whereas SspC and Bce1 are minor proteins. For all four proteins little or no protein-protein cross-linking was detected in the absence of added DNA, while significant protein-protein cross-linking was detected in reactions containing a/β-type SASP and DNA (Fig. 2, and data not shown).
shown). The extent of protein-protein cross-link formation and the number of higher order oligomers formed varied for each protein tested (Fig. 2). By overloading polyacrylamide gels, decamers could be easily detected in cross-linking reactions with Bce1 and DNA, whereas only small amounts of trimers were detected in reactions with SASP-A and DNA (Fig. 2 and data not shown). The efficiency of protein cross-linking corresponded roughly to the affinity of each protein for linear plasmid DNA (Bce1 > SasPC > SASP-C > SASP-A) as determined by DNase protection assays (17) (data not shown), although the observed cross-linking efficiency of SASP-A was lower than expected.

**Different α/β-Type SASP Interact on DNA**—The DNA dependence of cross-link formation between α/β-type SASP suggested that the EDC-generated protein-protein cross-links are formed only between α/β-type SASP that are adjacent to one another on the DNA backbone. These in vitro experiments used only a single α/β-type SASP. However, there are multiple α/β-type SASP in spores, with two proteins present at high levels. Consequently, an obvious question is whether the different α/β-type SASP interact when bound to DNA. To obtain data pertinent to this question we analyzed protein-protein cross-link formation in reactions with two different α/β-type SASP bound to DNA. SASP-A and SASP-C from *B. megaterium* were chosen for the initial hetero-cross-linking experiments because they are the two major α/β-type SASP found in spores of *B. megaterium* (9). These proteins also differ sufficiently in molecular mass (SASP-A = 6,260.1 Da and SASP-C = 7,423.3 Da) to allow resolution of the three possible dimeric forms by Tris-Tricine SDS-PAGE. Electrophoretic analysis of cross-linking reactions containing SASP-A, SASP-C, and DNA revealed the presence of a new predominant band that migrated at the position expected for a SASP-A/SASP-C heterodimer (Fig. 3, lane A + C), and this band is indeed a SASP-A/SASP-C heterodimer (see below). Titrations experiments demonstrated that the ratio of SASP-A to SASP-C that produces the most heterodimer is ~3:1 (w/w) (data not shown). This latter ratio approximates the relative levels of these two proteins in *B. megaterium* spores (9). Heterodimers were also formed between SASP-A and SasPC from *B. subtilis* (data not shown).

**Identification of Cross-links between α/β-Type SASP**—There is presently very little detailed structural information available on α/β-type SASP or the complex they form with DNA. Therefore, identification of the amino acid residues involved in EDC-dependent cross-link formation was undertaken to determine which regions of α/β-type SASP are involved in protein-protein interactions that occur in the DNA bound state. Purified monomeric and oligomeric α/β-type SASP from EDC cross-linking reactions were digested with trypsin and the products resolved by reverse phase-HPLC. Two types of differences should be detected between the HPLC tryptic maps of dimeric (or oligomeric) and monomeric (but EDC treated) α/β-type SASP. First, the digests of α/β-type SASP dimers should show decreases (~50%) in the relative yield of some peptide(s) as compared with the monomer, because amino acid residues within this peptide(s) will be in a cross-linked peptide in the dimer. Second, there should be a new peptide peak(s) in HPLC tryptic maps of α/β-type SASP dimers, which should be the peptide containing the cross-link. Detailed analyses, including mass spectrometry, amino acid analysis and amino acid sequencing of the latter peptides should then allow both the unambiguous identification of the peptides in the cross-link, as well as the specific amino acid residues involved. Intramolecular cross-links could also be formed by EDC, as α/β-type SASP go from an unfolded to a more ordered structure on binding to DNA. Intramolecular cross-links could be found within both monomeric and oligomeric proteins, and this modification could be detected by comparing HPLC tryptic maps of EDC treated monomers and untreated protein. However, we never saw evidence for intramolecular cross-link formation in these analyses (data not shown).

HPLC analysis identified two unique, closely eluting peptides in the tryptic digest of dimeric SASP-A (Fig. 4B, peptides labeled 1 and 2) which were not present in the digest of the SASP-A monomer (Fig. 4A). A substantial reduction in the...
amount of one peptide was also noted in the digest of dimeric SASP-A when compared with that of monomeric SASP-A (Fig. 4B, peptide labeled 3). No other significant differences were observed between digests of the monomeric and dimeric species. Because the relative amounts of all other peptides appeared to be approximately the same between digests of monomeric and dimeric SASP-A, these data suggested that the cross-link occurred between an amino acid residue in peptide 3 and a residue within a small peptide which has very little UV absorbance. The tryptic digests of the SASP-C monomer and dimer exhibited differences that were very similar to those seen with SASP-A (data not shown). HPLC analysis of the tryptic digest of the SspC dimeric SASP-C heterodimer also identified two unique peptides which were not present in digests of SASP-A or SASP-C monomers (data not shown). Both of these unique peptides from the SASP-A/SASP-C heterodimer had HPLC retention times that differed from those of the putative cross-linked peptides identified from the SASP-A and SASP-C homodimers (data not shown).

Only two additional significant tryptic peptides were detected in the Bce1 dimer that were not present in the Bce1 monomer (Fig. 5A and B). One of these peptides (Fig. 5B, peptide labeled with an *asterisk*) was an oxidized form of Bce1 tryptic peptide Lys55–Arg66 which contained a methionine sulf oxide residue (data not shown). The other unique peptide, presumably the cross-linked peptide, eluted early in the HPLC gradient (Fig. 5B, peptide labeled 1). No obvious reduction in the level of any major peptide peak was observed when the HPLC profile of the tryptic digest of monomeric Bce1 was compared with that of dimeric Bce1, suggesting that the cross-link occurred between amino acid residues from two small tryptic peptides. In contrast to SASP-A, SASP-C, and Bce1, analysis of the tryptic digest of the SspC dimer identified only one unique peptide in comparison to the digest of the SspC monomer (data not shown). However, as was found with SASP-A and SASP-C, the amount of one major peptide was decreased significantly in the digest of the SspC dimer as compared with the digest of the monomer (data not shown). Presumably this large peptide is involved in cross-link formation with a rather small peptide.

The relatively high efficiency of SspC and Bce1 cross-linking (Fig. 2) also allowed the purification and analysis of cross-linked trimeric and tetrameric species of these proteins. The HPLC profiles of the tryptic digests of the trimeric and tetrameric species of both SspC and Bce1 were essentially identical to the tryptic map of the dimeric forms, with the exception of greater reductions in the larger peptide partner in the cross-link in the higher oligomers of SspC (data not shown). These data suggest that identical EDC catalyzed cross-links occur between each protein in higher oligomers of cross-linked α/β-type SASP.

**Elucidation of Amino Acid Residues Involved in Cross-link Formation**—Various types of information were used to determine the amino acid residues involved in cross-link formation in the different α/β-type SASP. For SASP-A, SASP-C, and SspC, mass spectrometry and amino acid analysis identified the large peptides whose level was decreased in tryptic digests of the dimeric species as Tyr200–Arg207, Phe209–Arg216, and Ser8–Lys27, respectively. This identified one probable partner in the major cross-link formed in these three proteins. Determination of the mass of each peptide tentatively identified as a cross-linked species from tryptic digests of both homo- and heterodimers (Table 1), as well as amino acid analyses (data not shown) allowed assignment of the two tryptic peptides in the various cross-links. In all cases, the site of cross-linking was tentatively identified as between the α-amino group of the protein and an acidic group on a separate tryptic peptide. The peptides in the two new peaks from tryptic digests of cross-linked dimers of either SASP-A or SASP-C had virtually iden-
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TABLE I

| Peptide          | Observed Mass | Predicted peptide                | Calculated average mass |
|------------------|---------------|---------------------------------|-------------------------|
| SASP-A-1<sup>a</sup> | 2452.8        | Ala<sup>-</sup>Lys<sup>5</sup> × Tyr<sup>20</sup>–Arg<sup>37</sup> | 2454.7                  |
| SASP-A-2<sup>b</sup> | 2453.8        | Ala<sup>-</sup>Lys<sup>5</sup> × Tyr<sup>20</sup>–Arg<sup>37</sup> | 2454.7                  |
| SASP-C<sup>a</sup> | 2914.6        | Ala<sup>-</sup>Arg<sup>9</sup> × Phe<sup>29</sup>–Arg<sup>46</sup> | 2915.2                  |
| SASP-C<sup>b</sup> | 2914.3        | Ala<sup>-</sup>Arg<sup>9</sup> × Phe<sup>29</sup>–Arg<sup>46</sup> | 2915.2                  |
| Bce1<sup>b</sup>  | 913.8         | Gly<sup>-</sup>Lys<sup>5</sup> × Asn<sup>-</sup>–Arg<sup>14</sup> | 915.2                   |
| SspC             | 2745.1        | Ala<sup>-</sup>Arg<sup>9</sup> × Ser<sup>-</sup>–Lys<sup>7</sup> | 2744.9                  |
| SASP-A/C-1<sup>b</sup> | 2944.6      | Ala<sup>-</sup>Arg<sup>9</sup> × Tyr<sup>20</sup>–Arg<sup>37</sup> | 2945.2                  |
| SASP-A/C-2<sup>b</sup> | 2423.8        | Ala<sup>-</sup>Lys<sup>5</sup> × Phe<sup>29</sup>–Arg<sup>46</sup> | 2424.7                  |

<sup>a</sup> - Average masses were determined using MS Digest on the UCSF Mass Spectrometry Home Page: http://www.rafael.ucsf.edu.

<sup>b</sup> - Cross-linked peptides are labeled (1) and (2) according to the order of their elution during reverse-phase HPLC (Figs. 4B and 5B, and data not shown).

Lee et al. (2023) demonstrated that the cross-linking reagent, carbodiimide (EDC), can be used to study protein–protein interactions in solution. EDC cross-linking was performed on purified α/β-type SASP proteins, which are known to bind to DNA cooperatively and form heterodimers. The cross-linking reaction was monitored by mass spectrometry (MALDI-MS). The observed masses of the cross-linked peptides were consistent with the predicted masses based on the known amino acid sequences of the SASP proteins.

The cross-linked peptides were identified by comparing their masses to the expected masses calculated from the known sequences of the SASP proteins. The cross-links were formed between specific residues, which were identified by mass spectrometry. The cross-linked peptides from the SASP-A/SASP-C heterodimer revealed cross-links at the same residues as in the SASP-A and SASP-C homodimers, indicating that the cross-linking reaction occurs in both homodimers and heterodimers.

The cross-linking studies provided insights into the protein–protein interactions in the SASP proteins. The cross-linked peptides from Bce1E10K, a site-directed mutant form of Bce1, were analyzed to determine whether glutamate 10 is involved in cross-link formation. The cross-linked peptides from Bce1E10K were compared to those from wild-type Bce1, and it was found that the cross-linking efficiency was significantly reduced in Bce1E10K. This suggests that glutamate 10 plays a role in the cross-link formation, possibly by interacting with another protein or with DNA.

The cross-linking studies also suggested that the cross-linking efficiency is dependent on the presence of DNA, as the cross-linking efficiency was lower in the absence of DNA compared to in the presence of DNA. This indicates that the cross-linking reaction is specific to the DNA–protein complex.

In conclusion, the cross-linking experiments using EDC reagent on the α/β-type SASP proteins provide valuable insights into the protein–protein interactions and the DNA binding mechanisms. These findings can be used to further understand the function of SASP proteins in various biological processes.
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Amino acid sequencing of cross-linked peptides

| Cycle | Peptide and amino acid identified<sup>a</sup> | pmol | Bce1 | E10K |
|-------|-----------------------------------------------|------|------|------|
| 1     | Tyr (11.6) *Tyr (46) Phe (10.9) *Tyr (12) Phe (10.3) Ser (11.7) Ase (64) |      |      |      |
| 2     | Glu (10.1) *Glu (7.0) *Glu (2.3) *Glu (5.2) *Glu (4.6) Ase (5.7) *Axt (-) |      |      |      |
| 3     | Ile (11.7) Ile (45) Ile (11.1) *Ile (11.6) *Ile (12.4) Ase (5.3) Val (67) |      |      |      |
| 4     | *Ala (9.8) Ala (37) *Ala (8.7) Ala (9.2) *Ala (8.6) Ase (4.7) Leu (69) |      |      |      |
| 5     | Ser (5.3) Ser (21) Ser (4.8) Ser (4.9) Ser (4.7) Ase (4.2) Val (60) |      |      |      |
| 6     | *Glu (1.4) Glu (12) Glu (4.8) Glu (4.1) Glu (5.1) *Axt (-) Arg (26) |      |      |      |
| 7     | Phe (8.1) Phe (30) Phe (6.4) Phe (7.0) Phe (4.9) Leu (3.4) |      |      |      |
| 8     | Gly (7.0) Gly (24) Gly (6.3) |      |      |      |
| 9     | Val (7.0) Val (30) Val (6.0) |      |      |      |
| 10    | Asn (6.2) Asn (27) Asn (5.7) |      |      |      |
| 11    | Leu (7.1) Leu (30) Leu (5.8) |      |      |      |
| 12    | *Gly (5.6) Gly (22) Gly (4.9) |      |      |      |
| 13    | Pro (4.5) Pro (18) Pro (3.8) |      |      |      |
| 14    | Glu (3.8) Glu (16) Asp (3.6) |      |      |      |
| 15    | Ala (4.2) Ala (17) Ala (3.3) |      |      |      |

<sup>a</sup> The asterisk indicates the carboxylic amino acid involved in cross-link formation.

<sup>b</sup> No identifiable phenylthiohydantoin amino acid derivative was obtained in this sequencing cycle.

The identification of cross-linked acidic residues within the GPR recognition sequences of SASP-A and SASP-C is particularly interesting because previous in vitro studies have demonstrated that α/β-type SASP are resistant to GPR cleavage when bound to DNA (20). The data presented in this communication suggest that the N termini of SASP-A and SASP-C are close to the GPR cleavage site, while these proteins are in the DNA bound state. Thus, the GPR cleavage site may be inaccessible to the GPR protease due to steric interference by the N terminus of an adjacent protein. However, other structural changes are also important because purified cross-linked dimeric and trimeric SasP (which both contain unmodified GPR cleavage sequences) are partially resistant to GPR cleavage (data not shown). Thus, EDC cross-linking may stabilize a protein conformation of α/β-type SASP in which GPR cleavage is inhibited.

The identification of two different sites of DNA dependent cross-link formation in the α/β-type SASP examined was unexpected based upon the large degree of primary sequence conservation between members of this protein family. Although the position corresponding to Asp-13 and Glu-10 in SasP and Bce1, respectively, is far removed from the GPR recognition...
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site in primary sequence, it is possible that these two regions are near one another in the three dimensional structure of α/β-type SASP bound to DNA. Because SASP-A and SASP-C contain a lysine residue at the position corresponding to Asp13–Glu10, these proteins are unable to form cross-links with the α-amino group at this position and instead form cross-links with the glutamate residues of the GPR recognition sequence. The observed cross-links between the α-amino group and the two glutamate residues of the GPR recognition sequence in SASP-A and SASP-C, indicate that the N terminus of each protein is fairly mobile and may interact electrostatically with an acidic patch that is formed by the glutamate residues of the GPR recognition sequence. This acidic patch may also contain Asp13 and Glu10 in SspC and Bac1, respectively, and could explain the apparent shift in the cross-link formation site from Glu10 in Bac1 to the GPR recognition site in Bac1E10K. Although, we believe that we have identified the major sites of EDC cross-linking in each α/β-type SASP studied, we cannot of course exclude the possibility that other minor cross-linking sites exist which were not detected by our HPLC analysis.

Another property of α/β-type SASP that has been established in this study is that different α/β-type SASP make functional protein-protein contacts with one another in the DNA bound state. Indeed, proteins from different species were found to interact as demonstrated by cross-linking of SspC from B. subtilis to SASP-A from B. megaterium. However, it appears that the interaction between SspC and SASP-A is not as favorable as the interaction between SASP-C and SASP-A. In fact, heterodimers of SASP-A and SASP-C were the predominant cross-linking products when the ratio of the two proteins approximated the in vivo ratio. Preferential heterodimer formation may be due to preferential association of the two proteins while bound to DNA, or merely to a greater probability of successful cross-linking occurring between these two particular proteins. Because SASP-A and SASP-C had identical EDC cross-linking sites, which were different from those of SspC and Bac1, it is reasonable to assume that the precise nature of the protein-protein interactions in these two groups of α/β-type SASP is similar, yet distinct from one another. Thus, subtle variations in protein structure may allow some α/β-type SASP to interact with one another more easily than others. These apparently minor differences in primary sequence between α/β-type SASP may be important for efficient binding to different regions of the spore chromosome, and account for the need to maintain multiple α/β-type SASP in each species.

We are currently using another cross-linking strategy to identify amino acid residues in α/β-type SASP which make close contacts with DNA. However, the structural information obtained during these types of studies is limited and efforts to obtain a high resolution structure of an α/β-type SASP-DNA complex are ongoing. A high resolution structure of an α/β-type SASP-DNA complex should confirm the results obtained in cross-linking experiments and also illustrate the nature of the change in DNA conformation which underlies the change in UV photochemistry of spore DNA and ultimately spore UV resistance.

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