Binding of DNA to α/β-Type Small, Acid-Soluble Proteins from Spores of Bacillus or Clostridium Species Prevents Formation of Cytosine Dimers, Cytosine-Thymine Dimers, and Bipyrimidine Photoadducts after UV Irradiation

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Small, acid-soluble proteins (SASP) of the α/β-type from spores of Bacillus and Clostridium species bind to DNA; this binding prevents formation of cyclobutane-type thymine dimers upon UV irradiation, but promotes formation of the spore photoprotein, an adduct between adjacent thymine residues. α/β-Type SASP also bound to poly(dG)·poly(dC) and poly(dA-dG)·poly(dC-dT). While UV irradiation of poly(dG)·poly(dC) produced cyclobutane-type cytosine dimers as well as fluorescent bipyrimidine adducts, the yields of both types of photoproduct were greatly reduced upon irradiation of α/β-type SASP-poly(dG)·poly(dC) complexes. UV irradiation of poly(dA-dG)·poly(dC-dT) produced a significant amount of a cyclobutane dimer between cytosine and thymine, as well as a 6-4 bipyrimidine adduct. Again, binding of α/β-type SASP to poly(dA-dG)·poly(dC-dT) greatly reduced formation of these two photoproducts, although formation of the cytosine-thymine analog of the spore photoproduct was not observed. These data provide further evidence for the dramatic change in DNA structure and photoreactivity which takes place on binding of α/β-type SASP and suggest that binding of these proteins to DNA in vivo prevents formation of most deleterious photoproducts upon UV irradiation.

There is abundant evidence that the UV resistance of dormant spores of various Bacillus species is due to the presence in spores of a large amount of small, acid-soluble proteins (SASP) of the α/β-type (9, 12, 16, 19, 20). α/β-Type SASP are present in spores of all members of the Bacillus and Clostridium lines of sporeformers, and their primary sequences have been highly conserved throughout evolution (19). These proteins bind double-stranded DNA in vitro and are associated with the DNA in spores (4, 11, 17). The UV resistance of spores is due to their DNA photochemistry, in that UV irradiation of spores produces no cyclobutane-type thymine dimer (TT), a major lethal photoproduct formed in vegetative cells (2, 20). Instead, a thymyl-thymine adduct termed spore photoprotein (SP) is formed, which is repaired during spore germination by a process which is more error free than is TT repair (20). Spores lacking α/β-type SASP because of mutation are UV sensitive, and TT is formed upon their UV irradiation (15). Recent work has shown that binding of α/β-type SASP to DNA in vitro results in a change to an A-like conformation in the DNA (10), and UV irradiation of α/β-type SASP-DNA complexes in vitro produces SP and not TT (12).

While the findings noted above implicate α/β-type SASP in preventing formation of TT in DNA, many other UV photoproducts can also be formed. These include cyclobutane-type dimers between two adjacent cytosine residues (CC) or adjacent cytosine and thymine residues (CT) as well as adducts between adjacent pyrimidines (22, 25). The latter form most readily between adjacent cytosine and thymine residues and include the various 6-4 photoproducts; there is evidence that 6-4 photoproducts can be extremely mutagenic, possibly more so than pyrimidine dimers (6, 8). In view of the dramatic effect of α/β-type SASP-DNA binding on TT formation, we decided to examine the effect of these proteins on CC, CT, and pyrimidine adduct formation. We report here that binding of α/β-type SASP to DNA in vitro blocks formation of all these photoproducts.

MATERIALS AND METHODS

Nucleic acids and proteins. Poly(dG)·poly(dC) was purchased from the Sigma Chemical Co., and poly(dA-dG)·poly(dC-dT) was from Pharmacia. Poly(dG)·poly(dC) was labeled with [5-3H]dCTP in 200 μl of 50 mM Tris-HCl (pH 8.0)–100 mM HEPES (N-2-hydroxyethylpiperezine-N′-2-ethanesulfonic acid)–50 mM KCl–5 mM MgCl2–1 mM dithiothreitol with 5 μg of polymer and 10 μM [5-3H]dCTP (50 μCi). Polymerization was initiated by addition of the large fragment of Escherichia coli DNA polymerase (10 U), and the mixture was incubated at 37°C. Generally, 15 to 60% of the labeled triphosphate was incorporated into acid-insoluble material in 1 to 3 h, at which time unlabeled polymer was added (25 μg), the solution was made 0.3 M in NaCl, DNA was precipitated with 2 volumes of ethanol, and the pellet was rinsed with 70% ethanol, air dried, and dissolved in 100 to 200 μl of water prior to use. Poly(dA-dG)·poly(dC-dT) was labeled and isolated in a manner similar to that described above, but using either [5-3H]dCTP or [methyl-3H]dCTP. All four deoxynucleoside triphosphates were present in this labeling procedure, with the unlabeled nucleotides at 100 μM and the labeled one at 1 to 10 μM.

The SASP used in this work were all purified as previously described. These included the γ-type SASP, SASP-B, from Bacillus megaterium spores (18) as well as the α/β-type SASP, SASP-A from B. megaterium (18), SASP-I from Bacillus cereus (26), SpS-C from Bacillus subtilis (11), and

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SASP-α and -β from Cloridium bifurcations (1). The mutant SspC derivatives, SspC'Tyr, SspC'His, and SspC'Ala, were also purified as previously described (24). Previous work has shown that the DNA binding characteristics of SspC'Tyr are identical to those of SspC'His (24); therefore, SspC'Tyr and SspC'His were used interchangeably in this work and are referred to as SspC. In contrast, SspC'Ala has lost all DNA-binding activity both in vivo and in vitro, while SspC'His binds only to poly(dG)·poly(dC) (24).

Formation and irradiation of protein-DNA complexes and photoproduct analysis. Complexes between SASP and DNA were routinely formed in 10 mM Tris-acetate (pH 7.0) and 1 mM EDTA, buffer conditions which promote maximum SASP-DNA binding (17). Polynucleotides were present at 100 μg/ml with SASP added to a 5/1 (wt/wt) ratio with the DNA unless otherwise noted; this amount of α/β-type SASP saturates the DNA (11, 17). Complex formation was initiated by SASP addition and proceeded for 2 h at 37°C (11, 17). Aliquots (70 μl) of these incubation mixtures (1 × 10^6 to 2 × 10^6 cpm) were irradiated as described previously with 10 kJ of UV light per m² primarily at 254 nm unless otherwise noted (12). The irradiated sample was rinsed into a hydrolysis vial with 0.1 M NH₄HCO₃ and lyophilized, and the DNA was hydrolyzed with either 400 μl of formic acid for 90 min at 175°C or 400 μl of trifluoroacetic acid for 2 h at 175°C (12, 14). Trifluoroacetic acid was used exclusively for analysis of 6-4 photoproducts because hydrolysis with this acid does not result in breakdown of 6-4 photoproducts, in contrast to hydrolysis with formic acid (14). Polymers labeled with [5-3H]dCTP were boiled for 20 min before lyophilization and hydrolysis. This procedure deaminates C residues with a saturated 5/6 double bond (i.e., in CC or CT) to U residues, thereby preventing loss of the 5'-H label during acid hydrolysis (21). Hydrolyzed samples (5 × 10^5 to 2 × 10^5 cpm) were run on descending paper chromatography on Whatman no. 1 paper with butanol-acetic acid-water (80:12:30, vol/vol) to separate UV photoproducts as described previously (12, 16). Rₜ values of various compounds were: uracil-uracil dimers (UU), a breakdown product of CC, 0.10; uracil-thymine dimers (UT), a breakdown product of CT, 0.21; 6-4′-[pyrimidin-2'-one]-thymine [thy(6-4)pyo], 0.29; cytosine (C), 0.34; uracil (U), 0.47; and thymine (T), 0.62. These Rₜ values are similar to those found previously (12, 14, 22, 25). After the solvent had traveled 45 to 48 cm from the origin, the paper was dried and cut into 1-cm strips, photoproducts were eluted, and their radioactivities were counted in a toluene-based scintillation fluid (12, 16).

In one experiment, larger amounts of irradiated poly(dA-dG)·poly(dC-dT) (3 × 10^6 cpm) or poly(dG)·poly(dC) (2 × 10^6 cpm) were applied in a 10-cm strip to the chromatography paper. After separation as described above, the location of radioactive compounds was determined by counting one edge of the resolved sample. The sample on the remaining paper was eluted with water and then analyzed again by paper chromatography with or without reirradiation as described above to cleave cyclobutane dimers (3).

For eventual fluorescence spectral analysis of bipyrime dine photoproducts (25), we added samples (10 μg) of poly(dG)·poly(dC) or poly(dA-dG)·poly(dC-dT) to 400 μl of 10 mM Tris-acetate (pH 7.0) without SspC'Ala (5 μg). After incubation for 2 h at 37°C, these samples were UV irradiated in 25-μl aliquots with 25 kJ/m². The fluorescence spectra of the samples with or without irradiation, as well as a buffer control, were obtained on a Perkin-Elmer MPF-3 spectrofluorometer with excitation and emission slit widths of 6 nm and excitation at 317 nm (25). All spectra reported have been corrected for the small amount of fluorescence in the unirradiated polymer control. This latter value was essentially identical to that of buffer alone and was not increased by SspC'Ala addition. Note that SspC'Ala has neither tryosine nor tryptophan (22).

DNAse protection assays. Complexes for DNase protection analyses were formed in 25 μl of 10 mM Tris-acetate (pH 7.0) with 1.5 μg of labeled poly(dA-dG)·poly(dC-dT) (3 × 10^5 to 1 × 10^6 cpm) and various amounts of SASP. After incubation for 2 h at 37°C, MgCl₂ and DNase were added, and DNase-resistant DNA was quantitated by determining acid-precipitable radioactivity as described previously (17).

RESULTS

Previous work has shown that α/β-type SASP binding to an essentially random-sequence DNA (plasmid pUC19) blocks UV-induced TT formation and promotes SP formation (12). While analysis of the effect of α/β-type SASP on CC, CT, and bipyrimidine photoduct formation in such a biologically relevant DNA would be likely, the low levels of these UV photoproducTs in particular CC) generated in random-sequence DNAs make their analysis difficult. Consequently, we chose to use synthetic deoxynucleotide polymers (i) to maximize the yields of CC, CT, and bipyrimidine photoducts and (ii) to facilitate radioactive labeling of DNAs with deoxycytidine, thus permitting ready analysis of cytosine-containing photoducts. We chose two synthetic DNAs for study, poly(dG)·poly(dC) and poly(dA-dG)·poly(dC-dT). UV irradiation of the polymers should generate high levels of CC or CT, as well as significant levels of bipyrimidine photoducts. Note that we did not use poly(dA)·poly(dT) in this work, because α/β-type SASP do not bind to this polymer (18).

Effect of SASP on photoproduct formation in poly(dG)·poly(dC). UV irradiation of poly(dG)·poly(dC) at 254 nm produces a significant amount of CC (most likely the cis, syn-isomer [14], which is detected on chromatograms as UU (21) (Fig. 1; note change of scale on vertical axis). The identification of this material as UU was made because (i) its Rₜ was similar to that reported previously for UU (14, 21), and (ii) its isolation and reirradiation converted it into a compound which comigrated with uracil on paper chromatography (data not shown). In contrast, irradiation of an SspC-poly(dG)·poly(dC) complex gave no CC, as essentially no UU was detected on chromatograms (Fig. 1). Similarly, irradiation of all other α/β-type SASP-poly(dG)·poly(dC) complexes gave little or no UU (Table 1). The only exception was SspC'Ala, which does not bind to poly(dG)·poly(dC) (17, 24). Note that SspC'His prevented UV formation in poly(dG)·poly(dC), as this mutant protein binds to this synthetic polymer (24) (Table 1). However, SspC'His does not bind to other DNAs including plasmids and poly(dG-dC)·poly(dG-dC) (24), as well as poly(dA-dG)·poly(dC-dT) (see below).

While no photoproducT other than UU was detected in acid hydrolysates of poly(dG)·poly(dC) (pH 7.0), UV irradiation of this polymer should generate some bipyrimidine adducts (5, 25). These compounds can readily be detected in irradiated DNA by their fluorescence emission in the 400-nm range, since unirradiated nucleic acid generally exhibits no such fluorescence. Indeed, while unirradiated poly(dG)·poly(dC) had no detectable fluorescence between 350 and 450 nm, the irradiated polymer did and its spectrum was similar to that expected for bipyrimidine adducts between
without paper chromatography poly[\(^3\)H]dC) irradiated with (dG) cytosines (Fig. 2) (5, 25). A sample of poly(dG) poly(dA-dG) poly(dC-dT) was also hydrolyzed without irradiation and analyzed similarly. Symbols: C, unirradiated; Δ, irradiated plus SspC\(^{3yr}\); ◯, irradiated without SspC\(^{3yr}\). Note the change in the scale on the vertical axis.

cytosines (Fig. 2) (5, 25). As found with CC formation, binding of SspC greatly reduced UV-induced formation of these bicytosine adducts (Fig. 2).

Effect of SASP on photoproducts from poly(dA-dG) poly(dC-dT). The major photoproduct detected on chromatograms of formic acid hydrolysates of UV-irradiated poly(dA-dG) poly(dC-dT) labeled with [\(^5\)H]cytosine was CT (again, most likely the cis, syn-isomer [14]), which was detected on chromatograms as UT (Fig. 3; note change of scale on vertical axis). Use of trifluoroacetic acid for hydrolysis gave not only UT, but also a compound migrating with an \(R_f\) of 0.29 (Fig. 4; note change of scale on vertical axis). This is most likely thy(6-4)pyo as this compound has been reported to have this \(R_f\) and is destroyed by formic acid hydrolysis (14). Other evidence that this new compound is thy(6-4)pyo is given below. As found with poly(dG) poly(dC), binding of SspC to poly(dA-dG) poly(dC-dT) suppressed UV-induced formation of all photoproducts (Fig. 3 and 4). The absence of any new photoproduct from UV-irradiated SspC-poly(dA-dG) poly(dC-dT) is noteworthy, as it was possible that \(α/β\)-type SASP binding to this polymer could have promoted formation of an adduct between adjacent T and C residues analogous to SP (12). Tests of other \(α/β\)-type SASP for their effects on poly(dA-

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**FIG. 1.** Chromatogram of UV photoproducts from poly(dG) poly(\(^3\)H)dC) irradiated with or without SspC\(^{3yr}\). Aliquots of poly(dG) poly(\(^3\)H)dC) (6 μg) with or without SspC\(^{3yr}\) (30 μg) were incubated, irradiated, hydrolyzed with formic acid, and analyzed by paper chromatography as described in Materials and Methods. One sample of poly(dG) poly(\(^3\)H)dC) (\(10^3\) cpm) was also hydrolyzed without irradiation and analyzed similarly. Symbols: C, unirradiated; Δ, irradiated plus SspC\(^{3yr}\); ◯, irradiated without SspC\(^{3yr}\). Note the change in the scale on the vertical axis.

**FIG. 2.** Fluorescence spectra of poly(dG) poly(dC) UV irradiated with or without SspC\(^{3yr}\). Aliquots of poly(dG) poly(dC) with or without SspC\(^{3yr}\) were incubated and irradiated as described in Materials and Methods, and the fluorescence spectra were recorded. Note that spectra have been corrected for fluorescence in comparable unirradiated samples; these latter values were \(<10\%\) of peak values obtained from samples irradiated without SspC\(^{3yr}\).

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**TABLE 1.** Photoproducts produced in UV-irradiated poly(dG) poly(dC) and poly(dA-dG) poly(dC-dT) complexed with various SASP

<table>
<thead>
<tr>
<th>SASP added</th>
<th>Photoproduct produced (% of maximum amount with):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Poly(dG)-poly((^3)H)dC) (UU)</td>
</tr>
<tr>
<td>None</td>
<td>100(^c)</td>
</tr>
<tr>
<td>(α/β)-type SASP</td>
<td></td>
</tr>
<tr>
<td>B. subtilis SspC(^{3yr})</td>
<td>6</td>
</tr>
<tr>
<td>B. subtilis SspC(^{3yr})</td>
<td>91</td>
</tr>
<tr>
<td>B. subtilis SspC(^{3yr})</td>
<td>11</td>
</tr>
<tr>
<td>B. megaterium SASP-A</td>
<td>14</td>
</tr>
<tr>
<td>C. bifermentans SASP-(α)</td>
<td>8</td>
</tr>
<tr>
<td>C. bifermentans SASP-(β)</td>
<td>12</td>
</tr>
<tr>
<td>(γ)-type SASP (B. megaterium SASP-B)</td>
<td>97</td>
</tr>
</tbody>
</table>

\(^a\) Complexes between nucleic acid and SASP were formed and irradiated, and photoproducts were analyzed as described in Materials and Methods. The values for poly(dG)-poly(\(^3\)H)dC) and poly(dA-dG)-poly(\(^3\)H)dC-dT) were obtained after hydrolysis with formic acid. Values for poly(dA-dG)-poly(dC-\(^3\)H)dT) were obtained after hydrolysis with trifluoroacetic acid. All values have been corrected for the amount of radioactivity comigrating at photoproduct positions in hydrolysates of unirradiated polymers. The estimated range of error for all values is \(±10\%\) of the reported value.

\(^b\) This value was set at 100\%; UU composed 2.2\% of total cytosine.

\(^c\) This value was set at 100\%; UT composed 2.8\% of total cytosine.

\(^d\) This value was set at 100\%; UT composed 3.8\% of total thymine.

\(^e\) This value was set at 100\%; thy(6-4)pyo composed 6.2\% of total thymine.

\(^f\) ND, not determined.
FIG. 3. Chromatogram of formic acid-derived UV photoproducts from poly(dA-dG)·poly[3H]dC-dT) irradiated with or without SspC<sup>CTYr</sup>. Aliquots of poly(dA-dG)·poly[3H]dC-dT (6 μg) with or without SspC<sup>CTYr</sup> (30 μg) were incubated, irradiated, and hydrolyzed with formic acid, and photoproducts were analyzed as described in Materials and Methods. One sample of polymer (~10<sup>7</sup> cpm) was also analyzed without irradiation. Symbols: ○, unirradiated; Δ, irradiated with SspC<sup>CTYr</sup>; □, irradiated without SspC<sup>CTYr</sup>. Note the change in the scale on the vertical axis.

FIG. 4. Chromatogram of trifluoroacetic acid-derived UV photoproducts from poly(dA-dG)·poly[3H]dC-dT) irradiated with or without SspC<sup>CTYr</sup>. Aliquots of poly(dA-dG)·poly[3H]dC-dT (6 μg) with or without SspC<sup>CTYr</sup> (30 μg) were incubated, irradiated, and hydrolyzed with trifluoroacetic acid, and photoproducts were analyzed as described in Materials and Methods. Symbols: ○, irradiated without SspC<sup>CTYr</sup>; □, irradiated with SspC<sup>CTYr</sup>. Thy(6-4)pyo is abbreviated 6-4. The identity of the material at the origin is not known, but it was not always seen. Note that there is significant C→U conversion in this hydrolysis and that the scale on the vertical axis changes.

dG)·poly[3H]dC-dT) UV photochemistry revealed that all wild-type αβ-type SASP reduced UT formation (Table 1), although often less than SspC or SASP-β from C. bifermentans. However, several of these proteins, i.e., B. cereus SASP-I and B. megaterium SASP-A, bound more weakly to poly(dA-dG)·poly(dC-dT) than did SspC (see below). SspC<sup>CTYr</sup> and SspC<sup>Gln</sup>, which provide no DNase protection to this polymer (see below) and presumably do not bind to it, also had no effect on UT formation. As was found with SspC, the other SASP also did not promote formation of any new photoproduct from poly(dA-dG)·poly[3H]dC-dT (data not shown).

While the absence of a new photoproduct in UV-irradiated SspC-poly(dA-dG)·poly(dC-dT) suggested that a cytosine-thymine adduct analogous to SP might not form, it was possible that the 5-3H in the C moiety of such a product might be acid labile or that such a photoproduct comigrated with C. To address these possibilities, and to more readily detect pyrimidine adducts, we analyzed the effects of αβ-type SASP on the photochemistry of poly(dA-dG)·poly(dC-dT) labeled with [methyl-3H]thymidine. UV irradiation of this polymer produced two major photoproducts detected by paper chromatography of trifluoroacetic acid hydrolysates, one migrating at the position of UT, and one with thy(6-4)pyo (Fig. 5; note change of scale on vertical axis). The identity of UT was confirmed by its isolation and generation of T upon reirradiation (data not shown). In contrast, the material migrating at the position of thy(6-4)pyo did not generate T upon reirradiation (data not shown), consistent with it being a 6-4 photoproduct. Further proof that this material was thy(6-4)pyo was (i) that hydrolysis of the labeled polymer with formic acid generated three new photoproducts on chromatograms (Fig. 6; note change of scale on vertical axis), as has been reported for the 6-4 addition product between T and C (14), and (ii) that thy(6-4)pyo formation as a function of UV fluence proceeded with 30 to 40% the efficiency of UT formation, similar to what has been found by others (Table 2) (13, 14). As further evidence of 6-4 photoproduct formation in poly(dA-dG)·poly(dC-dT), a large amount of fluorescing material was generated by UV irradiation of this polymer (Fig. 7).

As noted above with poly(dA-dG)·poly[3H]dC-dT), SspC binding suppressed all UV-induced photoproduct formation in poly(dA-dG)·poly[3H]dC-dT, again with no appearance of a significant new photoproduct (Fig. 5 and 6). Formation of fluorescing photoproducts in poly(dA-dG)·poly(dC-dT) was also abolished by SspC binding (Fig. 7). Binding of other wild-type αβ-type SASP to poly(dA-dG)·poly(dC-[3H]dT) also suppressed photoproduct formation (Table 1), although as noted above, two proteins that bound weakly to this polymer were not as effective as those that bound more tightly (see below). Two proteins, a γ-type SASP (SASP-B) and SspC<sup>Gln</sup>, had no effect on the photochemistry of poly(dA-dG)·poly(dC-[3H]dT) (Table 1). Analysis of the DNase sensitivity of SASP-poly(dA-dG)·poly(dC-[3H]dT) complexes was consistent with the photochemistry results as SASP-B and SspC<sup>Gln</sup> gave no DNase protection, SspC and SASP-α and -β from C. bifermentans gave almost complete protection, while the other proteins gave only slight protection (Table 3). Since the
DNase protection given DNA by α/β-type SASP is due to the binding of these proteins to the DNA (11, 17), this suggests that C. bifermentans SspC and SASP-α and -β bind tightly to poly(dA-dG)·poly(dC-[3H]dT) irradiated with or without SspC

FIG. 5. Chromatogram of trifluoroacetic acid-derived UV photoproducts from poly(dA-dG)·poly(dC-[3H]dT) irradiated with or without SspCTyr. Aliquots of poly(dA-dG)·poly(dC-[3H]dT) (6 µg) with or without SspCTyr (30 µg) were incubated, irradiated, and hydrolyzed with trifluoroacetic acid, and photoproducts were analyzed as described in Materials and Methods. Symbols: ○, irradiated without SspCTyr; □, irradiated with SspCTyr. Thy(6-4)pyo is abbreviated 6-4. Note the change in scale on the vertical axis.

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DISCUSSION

There are a number of conclusions which can be drawn from the data presented in this communication. First, it appears that binding of α/β-type SASP to DNA can prevent UV-induced formation not only of TT, but also of a number of other photoproducts formed between adjacent pyrimidines, including CT, CC, and bipyrimidine photoadducts. While the mechanism by which α/β-type SASP binding causes this change in DNA photochemistry is not clear, protein binding is required for this effect because α/β-type SASP that do not bind to DNA have no effect on DNA photochemistry, with weakly binding proteins having intermediate effects. As discussed previously (12), α/β-type SASP cannot influence DNA’s UV photochemistry by light absorption by the protein moiety, but must do so by altering DNA structure. Indeed, a number of studies have shown that changes in DNA structure alter its UV photochemistry (3, 14, 23). Structural changes in DNA taking place upon α/β-type SASP binding have been studied to date only with several chromosomal DNAs, and this work has shown that SASP binding causes the DNA to change to an A-like conformation (10). While the structural effects of SASP binding on poly(dG)·poly(dC) and poly(dA-dG)·poly(dC-dT) have not yet been determined, we predict that a change to an A-like structure will be induced in poly(dA-dG)·poly(dC-dT). However, poly(dG)·poly(dC) may already be in an A-like conformation in solution, at least in part (15), which may explain the high affinity of α/β-type SASP for this polymer (17). Since poly(dG)·poly(dC) also shows striking changes in its photochemistry upon α/β-type SASP binding, there may be features of the α/β-type SASP-DNA complex other than an A-like conformation of the DNA which are responsible for the DNA’s low UV photoactivity. The identity of these other features is not clear, but one candidate is the exclusion of crucial water molecules

<table>
<thead>
<tr>
<th>UV fluence (kJ/m²)</th>
<th>Photoprod. (%) of total thymine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UT</td>
</tr>
<tr>
<td>0.2</td>
<td>0.6</td>
</tr>
<tr>
<td>0.8</td>
<td>2.5</td>
</tr>
<tr>
<td>2.8</td>
<td>3.2</td>
</tr>
<tr>
<td>10</td>
<td>3.3</td>
</tr>
</tbody>
</table>

* Poly(dA-dG)·poly(dC-[3H]dT) was irradiated, and the polymer was hydrolyzed with trifluoroacetic acid and analyzed by paper chromatography as described in Materials and Methods.
from DNA upon α/β-type SASP binding, since DNA dehydration has been shown to reduce cyclobutane dimer formation upon UV irradiation and to promote SP formation (14). A second possibility is that the DNA bases are held very rigidly in an α/β-type SASP-DNA complex, thus not allowing movement needed for reactions between adjacent bases. Possibly, detailed structural analysis of an α/β-type SASP-DNA complex may shed some light on this question.

A second conclusion drawn from this work is that a thymyl-cytosine adduct analogous to SP, a thymyl-thymine adduct, does not form appreciably in α/β-type SASP-DNA complexes. In UV-irradiated SspC-poly(dA-dG) · poly(dC-dT) complexes, no new photoproduct composed more than 0.4% of total thymine. In contrast, SspC-pUC19 complexes irradiated similarly give ~7% of thymine as SP (12), despite the fact that many thymine residues in pUC19 cannot form SP. The reason for the lack of thymyl-cytosine adduct formation in UV-irradiated SASP-DNA complexes is not completely clear but is presumably due to the decreased susceptibility of the 5 position of cytosine to attack by the methyl group of thymine, relative to the reactivity of the 5 position of thymine.

The third conclusion we can draw from our data concerns the effects of α/β-type SASP binding on DNA in vivo. Previous work has shown that α/β-type SASP binding to DNA in vivo essentially abolishes TT formation and probably CT formation (16). Since CT and TT are mutagenic and/or major lethal lesions in vivo, suppression of their formation explains in large part the role of α/β-type SASP in spore UV resistance. Although the amount of CC formed in DNA is less than that of CT and TT, CC is also very likely a mutagenic and/or lethal lesion. The fact that binding of α/β-type SASP to DNA in vitro suppresses CC formation strongly suggests that α/β-type SASP binding to DNA in vivo would have the same effect. There is also abundant evidence that bipyrimidine adducts, both between two adjacent C residues and between C and T residues, are mutagenic and/or lethal lesions (6, 8). Again the suppression of formation of these adducts by α/β-type SASP binding in vitro suggests that this is also the case in vivo. These findings then provide further explanation for the elevated UV resistance of bacterial spores, an elevated UV resistance which is due in large part to the binding of spore DNA by α/β-type SASP.

In contrast to the large suppression of 6-4 photoproduct formation upon α/β-type SASP binding to DNA in vitro, several workers have reported that UV irradiation of dormant spores of Bacillus species does generate thy(6-4)pyr (7, 13). While some of this photoproduct may be formed only in cells or germinated spores contaminating the dormant spore preparation, it seems likely that some is formed in the dormant spores themselves. This would seem to be at odds with the results of α/β-type SASP binding to DNA in vitro. However, previous work has shown that one of the differences between the UV photochemistry of DNA in spores and in an α/β-type SASP complex in vitro is that in vitro the yield of SP as a function of UV fluence is 5 to 10% of that in vivo (12). While the reasons for this difference are not clear, one contributing factor may be the huge pool of dipicolinic acid present in dormant spores which may sensitize spore DNA to UV light in some fashion (12). This may also be the case for 6-4 photoproduct formation. If this is true, then it would be consistent with the huge increase in spore UV resistance seen transiently early in spore germination, as dipicolinic acid is excreted before α/β-type SASP are degraded (12, 20). This transient UV-resistant period is accompanied by an extremely low production of all photoproducts in DNA (20).

TABLE 3. Binding of SASP to poly(dA-dG) · poly(dC-dT)*

<table>
<thead>
<tr>
<th>Addition</th>
<th>DNAse-resistant DNA (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>&lt;1</td>
</tr>
<tr>
<td>α/β-type SASP</td>
<td></td>
</tr>
<tr>
<td>SspC-Tyr (B. subtilis)</td>
<td>96</td>
</tr>
<tr>
<td>SspC-Glu (B. subtilis)</td>
<td>&lt;2</td>
</tr>
<tr>
<td>SASP-A (B. megaterium)</td>
<td>15</td>
</tr>
<tr>
<td>SASP-I (B. cereus)</td>
<td>19</td>
</tr>
<tr>
<td>SASP-α (C. bifamenti)</td>
<td>85</td>
</tr>
<tr>
<td>SASP-β (C. bifamenti)</td>
<td>99</td>
</tr>
<tr>
<td>γ-type SASP (SASP-B [B. megaterium])</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

* Complexes were formed by using 20 μg of the indicated SASP (except for 14 μg of SASP-I and 15 μg of SspC-Glu) and 3 μg of poly(dA-dG) · poly(dC-dT)[3H]. Complexes were then analyzed for DNAse-resistant DNA as described in Materials and Methods.

REFERENCES


