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Synthesis and characterization of a 29-amino acid residue DNA-binding peptide derived from α/β-type small, acid-soluble spore proteins (SASP) of bacteria

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A 29-amino acid residue peptide (SASP-peptide) derived from the sequence of the putative DNA-contacting portion at the carboxyl terminus of an α/β-type small, acid-soluble spore protein (SASP) of *Bacillus subtilis* has been synthesized by automated solid-phase methods and tested for its ability to interact with DNA. Circular dichroism (CD) spectroscopy reveals an interaction between this SASP-peptide and DNA, both by an increase in α-helix content of the peptide (which alone has a mostly random conformation) and by enhancement of the 275-nm CD band of the DNA. In contrast to results with intact α/β-type SASP, however, the peptide does not induce a B → A conformational transition in the DNA. The SASP-peptide also binds to poly(dG)·poly(dC) and protects this polynucleotide against DNase I digestion and UV light-induced cytosine dimer formation, parallel to findings made previously with native α/β-type SASP. The results confirm the hypothesis that the carboxyl-terminal region of the α/β-type SASP directly contacts DNA and possesses some, but not all, of the functional characteristics of the intact molecule.

DNA-binding peptide; SASP; Circular dichroism; DNase protection; DNA photochemistry

1. INTRODUCTION

In every case investigated, spores from the *Bacillus* or *Clostridium* lines of Gram-positive bacteria contain large amounts of α/β-type small, acid-soluble spore proteins (SASP) [1]. These molecules consist of single polypeptide chains of 65–75 amino acid residues (M_r = 5–7 × 10^3) which bind to double-stranded DNA [2]. They occur only in the spore core, closely associated with the DNA [1]. All species examined contain a number (up to seven) of homologous α/β-type SASP (Fig. 1) which together may comprise up to 20% of the total spore protein. Vegetative cells lack α/β-type SASP which are only synthesized midway in sporulation; spore germination leads to rapid SASP degradation, thereby providing a supply of amino acids for the nascent cell. α/β-Type SASP also have another, more vital function in dormant spores; they greatly increase the resistance of spores to the lethal effects of UV radiation [1,6]. Mason and Setlow [7] demonstrated this conclusively by deleting the genes for first one and then both of the major α/β-type SASP from *B. subtilis*. UV resistance fell dramatically, the double-mutant spores actually showing greater sensitivity to UV than vegetative cells.

A number of studies have shown that the major DNA lesion formed by UV irradiation of spores differs from the well-known cyclobutane-type thymine dimer which predominates in most UV-irradiated DNA systems both in vivo and in vitro [6,8]. Instead, upon UV irradiation spores accumulate 5-thyminyl-5,6-dihydrothymine ('spore photoproduct') [8]. The less error-prone repair of this lesion compared with that of thymine dimers appears to account for the enhanced resistance of spores to UV [9]. Since UV irradiation of purified DNA under conditions of low humidity also forms a significant amount of spore photoproduct [10], the altered DNA photochemistry in spores has been attributed to an alteration in DNA conformation: when the DNA undergoes a change from B-form (at high relative humidity) to A-form (at lower humidity) its photoproduct distribution switches from one consisting mostly of thymine dimers to one dominated by spore photoproduct. This leads to the obvious inference that α/β-type SASP may provide a UV-protective effect to spores by causing a B → A conformation change upon binding to DNA [11,12]. In vitro studies by Mohr et al. [13] have borne out this supposition: both circular dichroism (CD) and Fourier-transform infrared (FTIR) spectroscopy demonstrate the proposed conformation change upon α/β-type SASP binding.

Abbreviations: SASP, small, acid-soluble spore protein(s); CD, circular dichroism; Fmoc-, *N*-(9-fluorenylmethoxycarbonyl)-; PepSyn KA, polyacrylamide kieselguhr modified for amino acid attachment – Trademark of Milligen/Biosearch, Inc.; TFA, trifluoroacetic acid; TFE, 2,2,2-trifluoroethanol.

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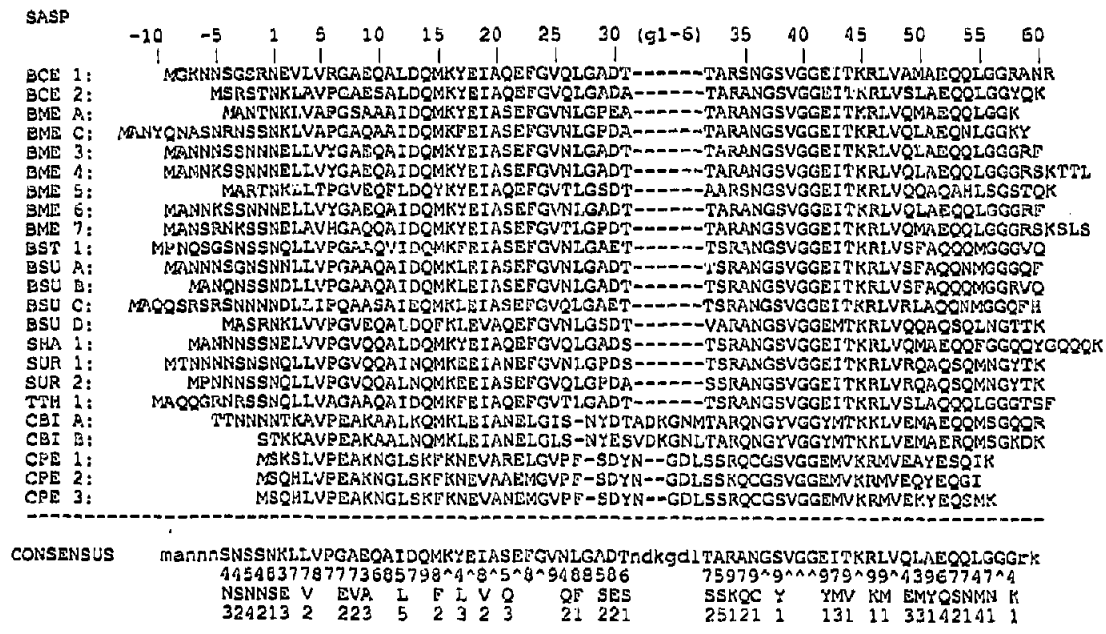

Fig. 1. Amino acid sequences of α/β-type SASP from different species. The data were taken from references [1,3–5]. The numbers below the consensus sequence indicate the frequency with which the consensus residue occurs at that position (^ = 100%, 9 = 90%, 8 = 80%, etc.). A list of secondary consensus choices (with frequencies) is also given at the bottom. Lower-case letters correspond to the consensus in regions where 5 or more SASP have no residues present. (Key: BCE, *Bacillus cereus*; BME, *Bacillus megaterium*; BST, *Bacillus stearothermophilus*; BSU, *Bacillus subtilis*; SHA, *Sporosarcina halophila*; SUR, *Sporosarcina ureae*; TTH, *Thermoactinomyces thalpophilus*; CBI, *Clostridium bifermentans*; CPE, *Clostridium perfringens*.)

We have now embarked on structural studies aimed at elucidating the mechanism of action of this novel family of DNA binding proteins. The availability of cloned genes coding for α/β-type SASP provides sufficient protein, as well as the opportunity to perform site-directed mutagenesis [14,15]. Analysis of several such mutants [15] as well as the analysis of conserved α/β-type SASP sequence features (Fig. 1) and comparison of these with the distantly related HBs protein of *Bacillus stearothermophilus* has allowed us tentatively to identify the carboxyl-terminal part of the α/β-type SASP as containing the region which contacts DNA directly [16]. On the basis of this identification we have now synthesized a 29-residue peptide corresponding to the carboxyl-terminal portion of SspC, an α/β-type SASP from *Bacillus subtilis*, and tested it for the ability to interact with DNA and influence DNA properties.

2. MATERIALS AND METHODS

2.1. *Nucleic acids*

Genomic DNA from *E. coli* was isolated by standard methods and purified by phenol extraction. Plasmid pUC19 labeled with [*methyl*-^{3}H]thymidine was isolated from *E. coli* JM83 and purified by CsCl density gradient centrifugation [14]. Pharmacia supplied poly(dA-dG)·poly(dC-dT) and this polymer was labeled with [*methyl*-^{3}H]dTTP as previously described [17]. Poly(dT)·poly(dC) came from Sigma and was tritium-labeled according to the following protocol: 11 μg of polymer was incubated for 1.0 h at 37°C in 200 μl of a reaction mixture containing 50 mM Tris-HCl/100 mM HEPES (*N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid)) (pH 8.0), 5 mM $MgCl_2$, 1 mM dithiothreitol, 50 mM KCl, 10 μM [5-^{3}H]dCTP (50 μCi) and ca. 10 units of the large (Klenow) fragment of *E. coli* DNA polymerase I. The reaction was terminated by addition of an equal volume of 0.6 M NaCl plus an additional 11 μg of unlabeled poly(dG)·poly(dC) in a 2-μl volume, followed by 1.0 ml of 95% ethanol to precipitate the DNA. The DNA was collected by centrifugation, rinsed with 70% ethanol, air-dried, and dissolved in 200 μl of 10 mM Tris-acetate, pH 7.0. We routinely obtained 15–60% incorporation of input [^{3}H]dCTP into the final polymer. Poly(dG-dC)hm1·poly(dG-dC) was also from Sigma, and was labeled as described above with the addition of 100 μM unlabeled dGTP.

2.2. *SASP-peptide*

The SASP-peptide (see Fig. 2) was synthesized on a Milligen 9050 PepSynthesizer. The starting resin was Fmoc-lys-PepSyn KA and all amino acids were initially in Fmoc-protected form; hydroxybenzotriazole served as the coupling agent. Coupling times were extended beyond those recommended in the standard protocol in order to improve peptide quality. The entire synthesis run took 80 h. After completion of the synthesis the resin was removed from the synthesizer and lyophilized. The immobilized peptide was cleaved and deblocked with trifluoroacetic acid (TFA) using anisole as a scavenger: the resin was covered to a depth of 3 mm with a mixture of 90% TFA, 5% thioanisole, 3% ethanedithiol, and 2% anisole and allowed to stand for 2 h at room temperature. The resultant solution was filtered through glass wool and evaporated to dryness to remove TFA. Ether was added at 4°C and allowed to stand overnight, then the precipitated peptide was centrifuged and washed with ether 3 times, followed by evaporation with N_2. The final product was dissolved in water and lyophilized.

The peptide was purified on a C18 μBondapak column using a Waters high-performance liquid chromatography system with UV detection at 214 nm. A linear-gradient solvent system of water (0.05% TFA, v/v) and acetonitrile (0.05% TFA, v/v) was employed for elution with a flow rate of 1.8 ml/min. The crude peptide mixture reproducibly gave three major peaks from the column which we designated *a*, *c* and *f*, in order of increasing time of elution. Amino acid analysis showed that peak *a* contained a truncated form of the projected SASP-peptide. Peaks *c* and *f* both gave analyses consistent with the target sequence. Since both N-terminal analysis and laser-desorption mass spectros-

(a) T-S-R-A-N-G-S-Y-G-G-E-I-T-K-R-L-V-R-L-A-Q-Q-N-M-G-G-Q-F-K

(b) A-S-Q-K-R-P-S-Q-R-H-G-S-K-Y-L-A-T-A-S-T-NH_2

Fig. 2. Amino acid sequences of: (a) the SASP-peptide, and (b) the control peptide.

copy further confirmed the structure of the peak *c* solute as that of the projected SASP-peptide we have employed it for all the experiments reported here and term it SASP-peptide. (Peak *f* appears to contain an incompletely deprotected product.) A control peptide (Fig. 2), corresponding to a 20-residue portion of the sequence of bovine brain myelin basic protein, was synthesized and purified by the same methods as the SASP-peptide. Note that its carboxyl-terminal residue is amidated, resulting in an overall net charge of +5 at pH 7.0.

2.3. *SASP*

α/β-Type SASP were purified as previously described [2,4,14,15]. These included SASP-α from *Clostridium bifermentans* and mutant derivatives of SspC from *B. subtilis*: $SspC^{tyr}$(L29Y), $SspC^{gln}$(K57Q), and $SspC^{ala}$(G52A)[1]. The DNA-binding characteristics of $SspC^{tyr}$ are indistinguishable from those of wild-type SspC whereas $SspC^{ala}$ lacks DNA binding properties almost entirely both in vivo and in vitro [2,15]. $SspC^{gln}$ represents an intermediate case: it binds to poly(dG)·poly(dC), but not to other DNAs [15].

2.4. *DNase protection assay*

Complexes for DNase protection analyses were formed in 25 μl of 10 mM Tris-acetate (pH 7.0), 1 mM EDTA, with 2.9 μg ^{3}H-labeled nucleic acid (except for poly(dG)·(poly(^{3}H-dC) for which 4.3 μg was used) (1–2 × 10^5 cpm) and varying amounts of SASP-peptide or intact α/β-type SASP. After incubation for 2 h at 37°C to form protein–DNA complexes, the samples were made 1.5 mM in $MgCl_2$ and 2.5 μg of pancreatic DNase I was added to each reaction. After further incubation at 37°C for 15 min the reactions were quenched and DNase-resistant DNA quantitated by determining acid-precipitable radioactivity as described previously [2].

2.5. *CD spectroscopy*

CD spectra were taken on an Aviv 62-DS spectrometer at 25°C using 1-mm or 10-mm cuvettes. Spectra were routinely run with 1.0-nm bandwidth, and points were taken at 0.5-nm intervals with 0.4 s averaging time. Smoothing was performed with the software package supplied by Aviv. Ellipticity and wavelength were periodically calibrated with (1*S*)-(+)-10-camphorsulfonic acid and benzene vapor.

2.6. *UV photoprotection assays*

Analyses of the photoproducts formed by UV irradiation of poly(dG)·poly(^{3}H-dC) with or without the SASP-peptide or intact α/β-type SASP were performed as described previously [17–19]. Complexes between polynucleotide and proteins or SASP-peptide were incubated for 2 h at 37°C before exposure to UV light mostly at 254 nm (fluence: 10 kJ/m^2). The DNA was then treated, hydrolyzed, and photoproducts analyzed by paper chromatography as described previously [17–19].

3. RESULTS

3.1. *CD spectra of SASP-peptide and SASP-peptide/DNA complexes*

Fig. 3 shows the CD spectrum in the 'protein region' (below 250 nm) of the SASP-peptide alone (curve a) and in the presence of saturating amounts of *E. coli* DNA (curve b). The free SASP-peptide has a CD spectrum similar to that of α/β-type SASP (Mohr, S.C., et al., manuscript in preparation) – a deep trough at 204 nm,

[1] Numbering for these mutants corresponds to the SspC sequence, not to the consensus numbering given in Fig. 1.

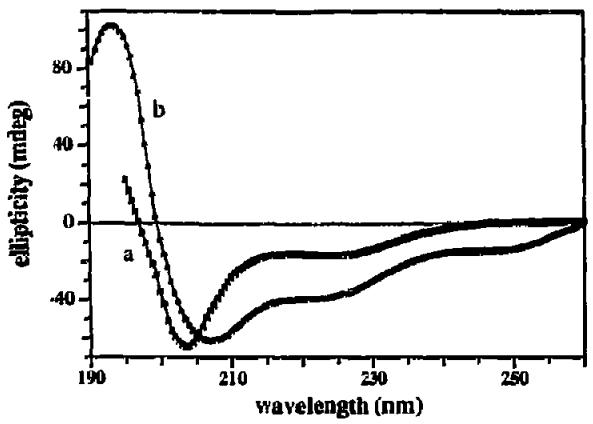

Fig. 3. CD spectrum of SASP-peptide in the absence (a) and presence (b) of *E. coli* genomic DNA. Peptide concentration: 0.22 mg/ml (7.2 × 10^{-5} M) in 10 mM Tris-acetate, pH 7.0. DNA concentration: 0.078 mg/ml. Pathlength: 1.0 mm.

and only weak ellipticity at 222 nm – indicating a largely random-coil conformation, with little secondary structure. By contrast, addition of DNA induces spectral features characteristic of α-helical structure (extrema at 191 nm and 208 nm, significant ellipticity at 222 nm)[2]. The control peptide, on the other hand, displayed no change in its CD spectrum upon the addition of comparable amounts of DNA (data not shown). (The peptide from peak *f* had virtually the same behavior as the SASP-peptide.) Based on the mean residue ellipticity at 222 nm (-1.9×10^4 deg·cm^2·dmol^{-1}) for the SASP-peptide complexed with DNA, we estimate a helix content of roughly 50% under these conditions (compared with other peptides of similar size [20]). CD experiments mixing SASP-peptide with poly(dG)·poly(dC) gave results comparable to those of Fig. 3 (data not shown), though in the case of the synthetic polymer the increase in α-helix content (as judged by θ_{222}) was slightly greater. Fig. 4 gives the result upon transferring SASP-peptide from 10 mM Tris-acetate (pH 7), to 95% trifluoroethanol (TFE). By comparison with Fig. 3, one can see that the extent of helix formation in this solvent slightly exceeds that induced by DNA.

The SASP-peptide alters the CD spectrum of DNA, but this effect differs significantly from that observed with intact α/β-type SASP [13]. As shown in Fig. 5, the amplitude of the long-wavelength CD band of the DNA increases by about 40%, but there is no indication of a shift in λ_{max} towards shorter wavelengths as occurs in the case of a B → A transition [13].

3.2. *Protection of DNA against DNase digestion*

Since the changes in CD spectra upon mixing SASP-peptide and DNA indicated an interaction between these molecules with some similarity to that between

[2] We have not subtracted the DNA contribution from these spectra because it is small (<20% at 222 nm) and may be affected to an undetermined extent by peptide binding (cf. Fig. 5).

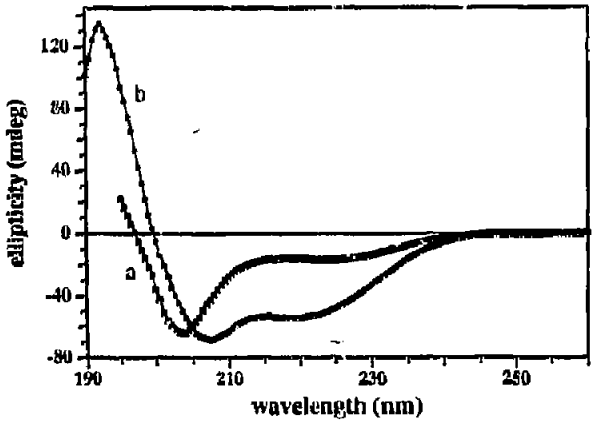

Fig. 4. CD spectrum of SASP-peptide in the absence (a) and presence (b) of 95% TFE. Peptide concentration: 29.5 μg/ml in 10 mM Tris-acetate, pH 7.0. Pathlength 10.0 mm.

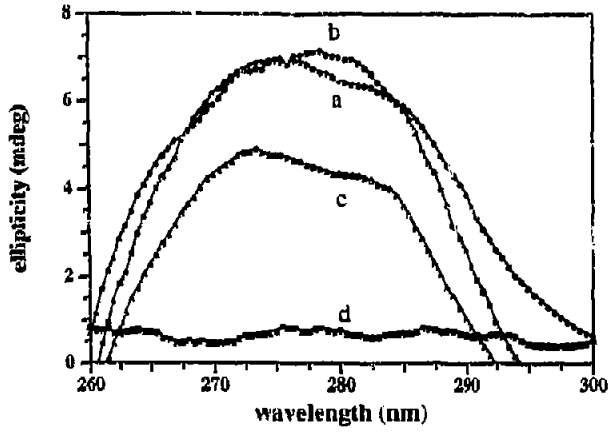

Fig. 5. CD of SASP-peptide/DNA complexes in the 'DNA region'. Peptide/DNA ratios (w/w): (a) 30, (b) 5, (c) 0. Curve d is the spectrum of the peptide alone. Pathlength 10.0 mm.

α/β-type SASP and DNA, we analyzed the system further by DNase protection assays. As shown previously [2], intact α/β-type SASP (e.g. $SspC^{tyr}$) provide almost full DNase resistance to a random sequence DNA such as pUC19, as well as to poly(dA-dG)·poly(dC-dT), poly(dG-dC)·poly(dG-dC) and poly(dG)·poly(dC). By contrast, the SASP-peptide only gave significant DNase resistance to poly(dG)·poly(dC), with slight protection of poly(dG-dC)·poly(dG-dC) (Table I). Even with poly(dG)·poly(dC), maximal protection by the SASP-peptide (i.e. saturation of the nucleic acid) required 2- to 3-fold more material (on a molar basis) than did protection by $SspC^{tyr}$ or other α/β-type SASP (Table I, also cf. [2]). Furthermore, under the DNase digestion conditions used, the saturated SASP-peptide/poly(dG)·poly(dC) complex was only 50% DNase-resistant, while the saturated complex with $SspC^{tyr}$ was ~90% resistant (Table I) as has been found with other intact α/β-type SASP [2]. The control peptide gave no DNase resistance to any of the polynucleotides tested, including poly(dG)·poly(dC) (data not shown). The lack of full DNase resistance of a saturated SASP-peptide/poly(dG)·poly(dC) complex is undoubtedly a reflection of the weak binding between the SASP-peptide and this nucleic acid, which allows significant access to DNase. Indeed, extended DNase digestion even of saturated $SspC^{tyr}$/DNA complexes results in eventual solubilization of the nucleic acid [2], and in one experiment using 1/4 the usual amount of DNase, the saturated SASP-peptide/poly(dG)·poly(dC) complex was ~85% DNase resistant (data not shown).

3.3. *Effect of SASP-peptide on poly(dG)·poly(dC) photochemistry*

The good DNase protection afforded poly(dG)·poly(dC) by the SASP-peptide indicated that the peptide interacts with this polynucleotide much like an intact α/β-type SASP. Consequently, we tested the effect of SASP-peptide on the UV photochemistry of poly(dG)·poly([^{3}H]dC). As found previously [17], cyclobutane-type dimers between adjacent cytosine residues (CC dimers) were the major photoproduct in UV-irradiated poly(dG)·poly([^{3}H]dC), and CC dimer formation was suppressed upon α/β-type SASP binding (Table II). Binding of the SASP-peptide also reduced CC dimer formation in poly(dG)·poly(dC) over 7-fold, a reduction similar to that obtained with several intact α/β-type SASP (Table II) [17]. Note, however, that $SspC^{ala}$, a mutant version of SspC which has lost all DNA-binding activity, has virtually no effect on poly(dG)·poly(dC) photochemistry (Table II).

4. DISCUSSION

Examination of the aligned sequences of all the known α/β-type SASP (Fig. 1) leads to some strong inferences about the structure and function of the members of this protein family. Most striking is the very high degree of sequence conservation. According to current estimates, Clostridia diverged from Bacilli more than

Table I

Protection of various polydeoxynucleotides against DNase I by intact α/β-type SASP or the SASP-peptide[a]

		DNase-resistant nucleic acid (%)[b]				
		$SspC^{tyr}$		SASP-peptide		
DNA	Protein added (μg):	12	38	13	26	48
[^{3}H]pUC19		74	93	–	<5	<5
poly(dA-dG)·poly(dC-[^{3}H]dT)		49	89	–	<5	<5
poly(dG-[^{3}H]dC)·poly(dG-[^{3}H]dC)		83	93	–	<5	7
poly(dG)·poly([^{3}H]dC)		60	92	14	50	48

[a] DNase protection assays were carried out with various ^{3}H-labeled nucleic acids as described in section 2.

[b] The amount of DNase-resistant nucleic acid in the absence of protein was always <5% of the total.

10^9 years ago [21], yet their α/β-type SASP have a dozen identical residues and many more highly conserved substitutions. This suggests that these proteins confront some very demanding structural/functional constraints and that portions of their sequence must specify significant functional properties.

A second point concerns the division of the sequences into two roughly equal parts by the gap imposed between consensus positions 31 and 32 (Fig. 1). Since Clostridial α/β-type SASP behave comparable to those of *Bacillus* species in experiments measuring SASP interaction with DNA [2,17,18], it seems fair to assume that these proteins share the same overall conserved tertiary structure (at least when complexed with DNA). For this to be true the 'gap' residues in Clostridial proteins must constitute a protruding loop bridging two more essential structural elements, defined by the sets of highly conserved residues centered around positions 19–20 and around position 41 (Fig. 1). The question then arises, which of the most conserved elements in the α/β-type SASP amino acid sequence directly interacts with DNA? Several pieces of evidence point to the carboxyl-terminal fragment as the site of DNA contact. First, it has a net charge of 2+, compared with 2− for the amino-terminal portion of the molecule (calculated for the consensus sequence at pH 7.0). Second, it contains the site of the most functionally disruptive site-directed mutation yet produced: $SspC^{ala}$ has a gly → ala substitution at position 40[3] and lacks all of the signature properties of α/β-type SASP – conferring UV resistance on spores [15], promotion of plasmid negative supercoiling [15], protection of DNA against DNase I digestion [2,15], and alteration of DNA photochemistry in vitro [17,18]. For such a chemically minor change to have such profound functional consequences it seems most probable that the change directly disrupts the protein/DNA interface. Finally, the argument based on presumed structural homology between α/β-type SASP and the known structure of the histone-like (HU) protein from *B. stearothermophilus* places the DNA-contacting portion of α/β-type SASP in the carboxyl-terminal part of the molecule [16].

Table II

Effect of intact α/β-type SASP and SASP-peptide on UV photochemistry of poly(dG)·poly(dC)[a]

SASP added	CC dimer[b] formed (% of control)
None	100[c]
C. bifermentans α	17
$SspC^{lys}$	14
$SspC^{gln}$	15
$SspC^{ala}$	87
SASP-peptide	16

[a] Complexes between poly(dG)·poly([^{3}H]dC) (6 μg ≈ 8×10^4 cpm) and various proteins (30 μg) were formed in 70 μl of 10 mM Tris-acetate (pH 7.0) and 1 mM EDTA, irradiated, processed and the photoproducts quantitated as described in section 2.

[b] Note that CC dimers are detected on chromatograms as uracil dimers [17].

[c] This value was set at 100%, but ranged between 1.4% and 3.7% of total cytosine for different polymer preparations. However, a no-protein control was run simultaneously with the samples in each case, and the percentages given are relative to these controls.

The present results strongly support the above interpretation. The SASP-peptide, chosen to span the putative DNA-interacting portion of the SspC sequence[4], obviously binds to DNA and protects poly(dG)·poly(dC) against both DNase I digestion and photochemical CC dimer formation. In the process of binding to DNA the SASP-peptide becomes much more highly ordered, paralleling the behavior of the intact α/β-type SASP themselves (Mohr, S.C. et al., manuscript in preparation). While we have attributed this solely to increased α-helix content, caution should be exercised when interpreting CD spectral changes in terms of specific conformational changes, and this assignment must be considered tentative. The TFE result (Fig. 4) shows that the SASP-peptide very likely has the capacity to acquire an even greater degree of α-helicity than observed on binding to DNA (the amplitude of $[\theta]_{222}$ increases by a factor of 2.9, compared with an increase of 2.4 times upon binding to DNA). It is possible that in the complex between intact α/β-type SASP and DNA, the amino-terminal part of the protein further extends the helical structure formed by the carboxyl-terminal (SASP peptide) part. This could come about through disruption of some helix-inhibiting intrapeptide hydrophobic interactions in a fashion analogous to the postulated mode of action of TFE [22].

Obviously, the SASP-peptide does not have a high binding affinity for ordinary DNAs. Significantly, we only obtained DNase resistance and photoprotection with the peptide complexed to poly(dG)·poly(dC), the most A-like of all the simple synthetic DNA polymers. This is consistent with the behavior of the intact α/β-type SASP themselves; in all situations they interact more strongly with poly(dG)·poly(dC) than any other nucleic acid [2]. We can surmise that the pre-existing A-like conformation of this polymer allows the SASP-peptide to assume a complementary α-helical structure and then bind with reasonably high affinity. On the other hand, in the absence of a pre-existing A-like conformation of the nucleic acid, the SASP peptide becomes more α-helical (Fig. 3), but does not have sufficient binding energy to promote the B → A conformation change (Fig. 5).

This interpretation meshes with results obtained for the $SspC^{ala}$ protein (Mohr et al., manuscript in preparation) and leads to the hypothesis that α/β-type SASP/DNA binding occurs via a two-step process [16], viz. the

[3] Using the consensus numbering scheme given in Fig. 1, the residue is no. 52 in the SspC sequence.

[4] The SASP-peptide corresponds to residues 44–72 of SspC except for the substitution of lysine for histidine at position no. 72 to facilitate the synthesis. Five native α/β-type SASPs have lysine at this position (it is the residue found there most frequently).

mostly disordered protein (or peptide) encounters the polynucleotide in solution and binds to it forming a complex (I) in which the protein (or peptide) acquires significantly increased α-helical structure; subsequently the polynucleotide undergoes a conformation change (essentially a B → A transition [13]) and a new, tighter complex (II) forms. Further increase in ordered secondary structure of the protein may occur in the step from complex I to complex II. Presumably $SspC^{ala}$ is incapable of the conversion to form II, while the SASF-peptide can only accomplish this with poly(dG)·poly(dC).

A number of reports of DNA-induced conformation changes in DNA-binding proteins have appeared recently. The complexes between the bZIP class of 'leucine zipper' proteins and their specific DNA binding loci have been suggested to involve the formation of more highly ordered protein secondary structures [23–28], though in these cases there appear to be no significant DNA conformation changes. As shown by a synthetic peptide model, the amino-terminal 24 residues of RecA protein shift from being largely unstructured in water to being highly α-helical in the presence of DNA [29]. Histones H1 and H5 have carboxyl-terminal domains which, though randomly coiled in aqueous solution, can be induced to become highly α-helical by suitable solvent conditions and are thought to become so upon complexing with DNA [30]. An NMR study of the solution structure of the *E. coli trp* repressor has shown that in the uncomplexed protein, the putative DNA binding region is much more flexible than the core of the molecule [31]. Anthony-Cahil et al. [32] have recently shown that $MyoD_{res}$, a 60-residue peptide containing the presumed DNA-binding portion of the helix–loop–helix protein MyoD, undergoes a significant increase in α-helical structure upon complexation with a 25-base pair oligonucleotide containing its specific binding site. In this context the α/β-type SASP (Mohr et al., manuscript in preparation) and SASP-peptide display behavior typical of many DNA-binding protein domains (or subdomains).

The successful synthesis of the SASP-peptide and the demonstration that it possesses some of the signature characteristics of the intact proteins opens the door to analysis of the functional roles of their key amino acid residues. Solid-phase peptide synthesis affords a much more rapid and economical means of producing molecules of this size with defined amino acid changes than does site-directed mutagenesis. Thus, by suitable substitutions it may be possible to design a 'super SASP-peptide' with significantly increased DNA-binding activity. It would also be of interest to synthesize a peptide corresponding to the highly conserved portion of the α/β-type SASP closer to the amino terminus; this would allow experiments to test further the hypothesis that the present SASP-peptide comprises the principal DNA-contacting part of the protein as well as allow in vitro complementation experiments with the two peptides.

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REFERENCES

[1] Setlow, P. (1988) Annu. Rev. Microbiol. 42, 319–338.
[2] Setlow, B., Sun, D. and Setlow, P. (1992) J. Bacteriol. 174, 2312–2322.
[3] Magill, N., Loshon, C.A. and Setlow, P. (1990) FEMS Microbiol. Lett. 72, 293–298.
[4] Cabrera, R.M., Mason, J.M., Setlow, B., Waites, W.M. and Setlow, P. (1989) FEMS Microbiol. Lett. 61, 139–144.
[5] Cabrera, R.M. and Setlow, P. (1991) FEMS Microbiol. Lett. 77, 127–132.
[6] Setlow, P. (1988) Comments Mol. Cell. Biophys. 5, 253–264.
[7] Mason, J.M. and Setlow, P. (1986) J. Bacteriol. 167, 174–178.
[8] Donnellan Jr., J.E. and Setlow, R.B. (1965) Science 149, 308–310.
[9] Munakata, N. and Rupert, C.S. (1972) J. Bacteriol. 111, 192–198.
[10] Rahn, R.O. and Hosszu, J.L. (1969) Biochim. Biophys. Acta 190, 126–131.
[11] Nicholson, W.L. and Setlow, P. (1990) J. Bacteriol. 172, 7–14.
[12] Stafford, R.S. and Donnellan Jr., J.E. (1968) Proc. Natl. Acad. Sci. USA 59, 822–829.
[13] Mohr, S.C., Sokolov, N.V.H.A., He, C. and Setlow, P. (1991) Proc. Natl. Acad. Sci. USA 88, 77–81.
[14] Nicholson, W.L., Setlow, B. and Setlow, P. (1990) J. Bacteriol. 172, 6900–6906.
[15] Tovar-Rojo, F. and Setlow, P. (1991) J. Bacteriol. 173, 4827–4835.
[16] Rao, H., He, C., Tzertzinis, G., Setlow, P. and Mohr, S.C. (1991) J. Biomolec. Struc. Dyn. 8, a175.
[17] Fairhead, H. and Setlow, P. (1992) J. Bacteriol. 174, 2874–2880.
[18] Nicholson, W.L., Setlow, B. and Setlow, P. (1991) Proc. Natl. Acad. Sci. USA 88, 8288–8292.
[19] Setlow, B. and Setlow, P. (1987) Proc. Natl. Acad. Sci. USA 84, 421–423.
[20] Yang, J.T., Wu, C.-S.C. and Martinez, H.M. (1986) Methods Enzymol. 130, 208–269.
[21] Wilson, A.C., Ochman, H. and Prager, E.M. (1987) Trends Genet. 3, 241–247.
[22] Osterhout Jr., J.J. (1992) FASEB J. 6, A50.
[23] Vinson, C.R., Sigler, P.B. and McKnight, S.L. (1989) Science 246, 911–916.
[24] O'Neil, K.T., Hoess, R.H. and DeGrado, W.F. (1990) Science 249, 774–778.
[25] Talanian, R.V., McKnight, C.J. and Kim, P.S. (1990) Science 249, 769–771.
[26] Weiss, M.A., Ellenberger, T., Wobbe, C.R., Lee, J.P., Harrison, S.C. and Struhl, K. (1990) Nature 347, 575–678.
[27] O'Neil, K.T., Shuman, J.D., Ampe, C. and DeGrado, W.F. (1991) Biochemistry 30, 9030–9034.
[28] Saudek, V., Pasley, H.S., Gibson, T., Gausepohl, H., Frank, R. and Pastore, A. (1991) Biochemistry 30, 1310–1317.
[29] Zlotnick, A. and Brenner, S.L. (1988) J. Mol. Biol. 209, 447–457.
[30] Clark, D.J., Hill, C.S., Martin, S.R. and Thomas, J.O. (1988) EMBO J. 7, 69–75.
[31] Arrowsmith, C., Pachter, R., Altman, R. and Jardetzky, O. (1991) Eur. J. Biochem. 202, 53–66.
[32] Anthony-Cahill, S.J., Benfield, P.A., Fairman, R., Wasserman, Z.R., Brenner, S.L., Stafford III, W.F., Altenbach, C., Hubbell, W.L. and DeGrado, W.F. (1992) Science 255, 979–983.