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Mode of DNA binding by SopA protein of *Escherichia coli* F plasmid

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The binding of SopA to the promoter region of its own gene, in which four copies of SopA's recognition sequence, 5′-CTTTGC-3′, are arrayed asymmetrically, was examined *in vitro*. Titration using electrophoretic mobility shift assay showed that the stoichiometry of SopA protomers to the promoter-region DNA is 4 and that the binding is highly co-operative. The co-operativity was corroborated by EMSA and DNase I footprinting for a number of mutant DNA fragments in which 5′-CTTTGC-3′ was changed to 5′-CTTACG-3′. EMSA in the style of circular permutation showed that SopA bends DNA. Mutation at either outermost binding site had a different effect on DNA bending by SopA, reflecting the asymmetry in the arrangement of the binding sites, for which the results of DNase I footprinting were in agreement. Gel filtration chromatography and analytical ultracentrifugation of free SopA showed that the protein can exist as a monomer and oligomers in the absence of ATP. Hence, the results indicate that the co-operativity in SopA's DNA binding is based on its intrinsic protein–protein interaction modified by DNA interaction.

Keywords: asymmetric recognition sites/co-operativity/DNA bending/DNA–protein interaction/plasmid partitioning.

The F plasmid of *Escherichia coli* is stably inherited despite its small copy number. One of the mechanisms that help achieve this stability is active partitioning of the plasmid DNA by *sop* gene of the plasmid (*1*). The *sop* locus consists of two protein coding regions, *sopA* and *sopB*, and a *cis* element, *sopC*, located downstream (*2*). SopA, a 44-kDa protein, binds to the promoter region of the *sopAB* operon as a repressor and is also an ATPase that forms a multimer upon hydrolysing ATP (*3–7*). SopB, a 35-kDa protein, binds to *sopC* and also interacts with SopA (*3*, *7–12*). Although the detailed mechanism of the active partitioning has remained elusive, oscillatory movement of SopA inside cell, which is postulated to be based on SopA's polymerization, SopA–SopB interaction and interactions of SopA and SopB with DNA, is believed to be important (*7*, *12–14*).

DNase I footprinting has shown that the SopA protein binds to the four 5′-CTTTGC-3′ sequences in the promoter region of *sopAB* (*3*; Fig. 1). The arrangement of the recognition sites does not conform to the classic palindromic structure most commonly seen for bacterial operators. The three upstream recognition sequences are arrayed in a direct repeat with 13-bp intervals; the fourth is located further downstream by 28 bp and inverted. In one example of a repressor that binds to direct and inverted DNA repeats, the DNA-binding unit of the ω protein of *Streptococcus pyogenes* plasmid pSM19035 is composed by a dimer of a small DNA-binding domain and binds to one non-palindromic element (*15*). The binding of the ω protein molecules to the recognition sequences, which are seemingly random in direction but adjacently arrayed, is mediated by one same dimer–dimer interface. This scheme, however, cannot accommodate the separation of sites 3 and 4 in the *sopAB* promoter region (Fig. 1). Thus, studying the DNA binding by SopA may uncover a novel mode of DNA–protein interaction.

These considerations led us to carry out experiments *in vitro* on SopA–DNA interaction, the results of which are presented below. While this work was in progress, the X-ray structures of ParA protein of P1 and P7 plasmids were reported (*16*). ParA is a close homologue of SopA and also functions as a repressor. In contrast to SopA, the binding site of ParA is a simple palindrome and ParA is believed to bind to the operator as a dimer (*16*, *17*). Another difference between SopA and ParA is that ParA's binding to its specific site is enhanced by binding of ADP to the protein, whereas no such enhancement is observed for SopA (*7*, *18*). Delineation of SopA–DNA interactions, therefore, is also important in understanding the evolution of these related plasmid partition systems.

Materials and Methods

Purification of SopA

The *sopA* gene was overexpressed in *E. coli* BL21(DE3) by use of the pET11 plasmid system (*19*). Briefly, SopA was purified as follows. Frozen induced cells were thawed and disrupted with chicken hen egg white lysozyme and Triton X-100, and the lysate was cleared by ultracentrifugation. The SopA protein was purified on an open column of cellulosephosphate (P11, Whatman) with a sodium chloride gradient in 20 mM sodium succinate, pH 6.5, 1 mM EDTA, 10 mM 2-mercaptoethanol and 10% glycerol and subsequently on a medium-pressure quaternary-amine column (Bio-Scale Q5, BioRad Laboratories) with a sodium chloride gradient in 20 mM Tris–HCl, pH 8.0, 1 mM EDTA and 5 mM 2-mercaptoethanol.

Fig. 1 Arrangement of the four SopA binding sites. The nucleotide sequence of the promoter region of the *sopAB* operon is shown. The SopA recognition sequence, 5′-CTTTGC-3′, and its complement are indicated by arrows. The promoter sequence and the initiation codon of *sopA* are boxed. The published sequence contained an extra G immediately downstream of the fourth binding site (*2*, *3*). The absence thereof, in agreement with another sequence in the database (accession M12987), was confirmed by sequencing pXX704 provided by Dr Hiraga.

The purified protein was dialysed against 50 mM sodium chloride, 20 mM Tris–HCl, pH 8.0, 1 mM EDTA, 5 mM dithiothreitol and 50% glycerol and stored at −20°C. The concentration of the protein was determined by A_{280} in 6M guanidium chloride according to Pace *et al.* (*20*).

Plasmid construction

DNA cloning was carried out by standard methods (*21*). pXX704N was a derivative of a mini-F plasmid pXX704 (*22*) in which 5′-CTAT CCGC-3′, ∼320 bp upstream of the initiation codon of *sopA*, was replaced with the NotI site, 5′-GCGGCCGC-3′, by use of polymerase chain reaction (PCR). A set of pXX704N derivatives was constructed by substituting 5′-CTTTGC-3′ with 5′-CTTACG-3′ by PCR. pBS-PsopAB was constructed by inserting a ∼230-bp PCR fragment comprising the promoter region of *sopAB*, made with primers 5′-GCGCGGCCGCTAGTGATAATAAGTGACTG-3′ (NotI site underlined) and 5′-CGCTCGAGATTTCATGACCAGCGTTT ATG-3′ (XhoI site underlined), in between the NotI and the XhoI sites of pBluescript KS(+) (Stratagene). A set of its derivatives with substitution of 5′-CTTTGC-3′ with 5′-CTTACG-3′ was also made. The sequences of the PCR segments were confirmed.

Electrophoretic mobility shift assay

For electrophoretic mobility shift assay (EMSA), DNA fragments were prepared by PCR with pXX704N-series or pBS-PsopAB-series plasmids as template. Primers used were: 5′-TTAAACAACTTTGCG GTTTTTTGA-3′ and 5′-AATCATTGCTTTGCGTTTTTTTATT-3′ for 94-bp fragments; 5′-CTCTTCTTATCTCCTTTTGTAGT-3′ and 5′-AAGTGTTTCCATGAGTTTCATTC-3′ for 164-bp fragments; 5′-TCCTTCTCTTTCCCTTCGTC-3′ and 5′-AATCATTG CTTTGCGTTTTTTTATT-3′, 5′-GTTTATTACTCTGAATTG GG-3′ and 5′-ATAGCCTTCGTCATTTCATG-3′, 5′-CTGAGCG TAAGAGCTATCTG-3′ and 5′-CCGCTGCTTCACCTATTC TC-3′, 5′-CTGCGGCGAGCGCTAGTGAT-3′ and 5′-AATTTC CATATCCGGGTGCG-3′, and 5′-TTAAACAACTTTGCGGTT TTTTGA-3′ and 5′-ATCGCGTACCAAACACATCA-3′ for sets of five 400-bp fragments. The PCR products were purified with agarose gel electrophoresis and High Pure PCR Purification Kit (Roche Diagnostics) and the concentrations were determined by fluorescence of Hoechst 33258 on a fluorimeter (BioRad Laboratories). For titration experiments, the DNA fragments were further purified by phenol/chloroform extraction and ethanol precipitation and the concentrations were determined by UV absorption at 260 nm. For titration experiments (shown in Fig. 2), mixtures of SopA and DNA in 10 mM Tris–HCl pH 7.6, 50 mM sodium chloride, 3 mM magnesium chloride and 10 mM dithiothreitol were incubated at 0°C for 10 min and a quarter volume of 40% glycerol was added for gel loading. Electrophoresis was carried out using 6% polyacrylamide (29 : 1) gel with 90 mM Tris, 90 mM borate and 20 mM magnesium chloride as electrophoresis buffer on Mini PROTEAN Tetra (BioRad Laboratories) at 200 V and 4°C. For the other EMSA experiments (shown in Figs 3 and 5), mixtures of SopA and DNA in 10 mM Tris–HCl pH 7.6, 50 mM sodium chloride, 5 mM calcium chloride, 3 mM magnesium chloride, 10 mM dithiothreitol and 0.1 mg/ml bovine serum albumin were incubated at 0°C for 10 min and a quarter volume of 40% glycerol was added for gel loading. Electrophoresis was carried out using 6% polyacrylamie (29 : 1) gel with 90 mM Tris, 90 mM borate and 9 mM magnesium chloride on Se600 Ruby (GE Healthare Bioscience) at 150 V and 4°C (for experiments shown in Fig. 3) or on Mini PROTEAN Tetra at 200 V and 4°C (for those shown in Fig. 5). DNA was stained with SYBR Green I (Molecular Probes), and the gels were imaged on a Typhoon scanner (GE Healthcare Bioscience).

DNase I footprinting

For DNase I footprinting, pBS-PsopAB-series DNA was digested with NotI or XhoI, and the ends were dephosphorylated with heat-labile alkaline phosphatase. After heat inactivation of the phophatase, the ends were radiolabelled with T4 polynucleotide kinase and [γ-^{32}P] ATP. After heat inactivation of the kinase, the DNA was digested with SacI or KpnI, respectively, and short DNA fragments and un-incorporated nucleotide were removed by gel filtration with a spin column (Microspin S-200 HR, GE Healthcare Bioscience). About 35 fmol of DNA and 35 pmol of SopA were mixed in 20 mM Tris–HCl, pH 8.0, 100 mM sodium chloride, 3 mM magnesium chloride, 3 mM calcium chloride, 0.1 mM EDTA, 0.1 mM dithiothreitol, 50 mg/ml bovine serum albumin, incubated on ice for 20 min, reacted with 0.4 U of DNase I (New England Biolabs) for 20 min on ice in a total volume of 20 µl and extracted with phenol. The products were analysed against Maxam–Gilbert A + G ladder (*21*) on 7 M urea sequencing gel with 5% Long Ranger (Lonza). Autoradiography was carried out with an ImagePlate (Fuji photo film) and a Typhoon scanner.

Gel filtration column chromatography

Gel filtration was carried out on a Superdex 200 10/300 GL column (GE Healthcare Bioscience) at a flow rate of 0.5 ml/min. The eluant used was 20 mM Tris–HCl, pH 8.0, 0.1 mM EDTA and sodium chloride at concentrations indicated in Fig. 6. SopA solutions were dialysed against buffer containing no glycerol prior to chromatography. Molecular weight markers were obtained from GE Healthcare Biosciences.

Analytical ultracentrifugation

SopA was dialysed against 20 mM Tris–HCl, pH 8.0, 0.1 mM EDTA and sodium chloride at 0.1 M, 0.3 M or 0.5 M, and diluted to $A_{280} = 0.9$ (∼16 µM). The solutions were spun in an An50Ti roter at 20°C and 50,000 rpm on a Beckman Optima XL-I analytical ultracentrifuge. Sedimentation profiles measured by A_{280} were recorded without time intervals between successive scans. Sedimentation coefficient distribution function, $c(s)$, was obtained by using SEDFIT program with the partial specific volume calculated by Sednterp (*23–25*). Molecular mass distribution, $c(M)$, was obtained by converting $c(s)$ to $c(M)$ on the assumption that the frictional ratio, f/f_0, was common to all the molecular species (as implemented in the SEDFIT program).

Results

Four SopA protomers bind to the promoter region of sopAB

With EMSA and DNase I footprinting, Mori *et al.* (*3*) showed that the SopA protein binds to the promoter region of the *sopAB* operon and that its recognition sequence was 5′-CTTTGC-3′. This showed that the number of SopA protomers directly in contact with the recognition sequences was to be 4. However, this number of 4 only set the lower limit of the stoichiometry of SopA to DNA in the complex, because additional SopA protomers could stabilize the complex *via* protein–protein interaction. In order to determine the stoichiometry, we carried out a titration experiment using a 94-bp promoter DNA fragment by EMSA at high concentrations of DNA and SopA. The fragment contained 8 bp on the upstream side of site 1 and 8 bp on the downstream side of site 4 and spanned the entire region of DNase I footprints (*3*; also *v.i.*). The result, shown in Fig. 2, showed that all the DNA was shifted at the SopA : DNA ratio of 4. More SopA, at the SopA : DNA ratio of 44, caused no further shift

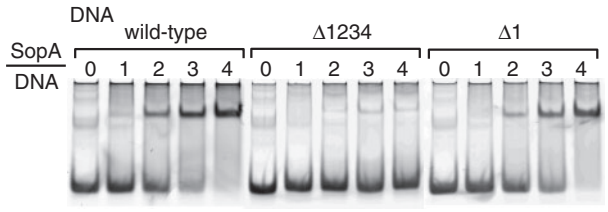

Fig. 2 Titration of promoter-region DNA with SopA. EMSA was carried out for a 94-bp wild-type DNA fragment, a mutant DNA fragment with 5′-CTTTGC-3′ substituted with 5′-CTTACG-3′ at all the binding sites (designated Δ1234), and a mutant DNA fragment with the substitution at site 1 (Δ1); 6.6 pmol DNA was mixed with SopA protein at the indicated SopA protomer : DNA ratios in 10 μl.

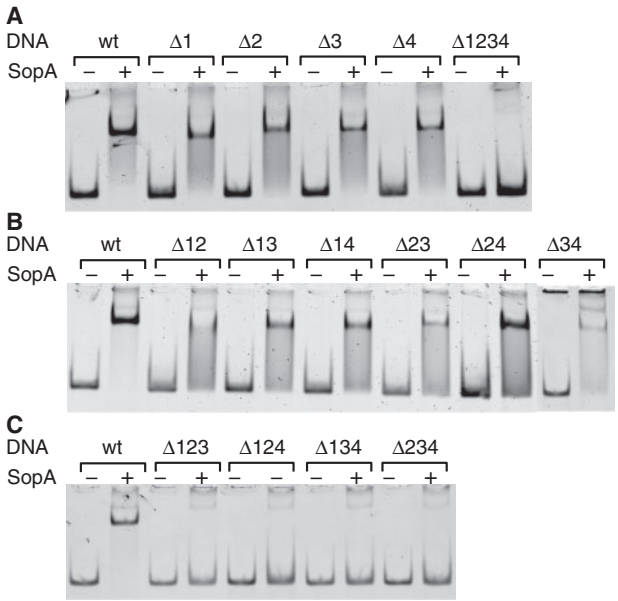

Fig. 3 Binding of SopA to mutant DNAs. EMSA was carried out for 164-bp DNA fragments with 5′-CTTTGC-3′ changed to 5′-CTTACG-3′; 90 fmol DNA in 20 μl with or without 35 pmol SopA protomer was electrophoresed. (A) Fragments with one or four substitution(s). The number(s) after Δ indicates the substitution site(s) shown in Fig. 1. (B) Fragments with two substitutions. (C) Fragments with three substitutions.

(data not shown), indicating that the shifted species was fully occupied with SopA. When all the DNA molecules are bound with SopA, there can be free SopA molecules. Therefore, the result set the upper limit of the stoichiometry at 4. Given the lower limit of 4, set by the DNA sequence, the stoichiometry was concluded to be 4. The result in Fig. 2 also indicated that the binding of SopA to DNA was highly co-operative, because only one shifted species was observed, confirming the observation by Mori *et al.* (*3*). When 5′-CTTTGC-3′ was substituted with 5′-CT TACG-3′ at all the four binding sites, no binding of SopA was observed, demonstrating that 5′-CTTT GC-3′ is indeed the recognition sequence of SopA (Fig. 2).

SopA binding is highly co-operative

In order to examine the co-operative nature of SopA binding, EMSA was carried out for 164-bp promoter DNA fragments in which 5′-CTTTGC-3′ was substituted with 5′-CTTACG-3′ at one or more binding sites. The fragments contained 43 bp on the upstream side of site 1 and 43 bp on the downstream side of site 4. The results are shown in Fig. 3. When the substitution was at any one of the sites, mobility shift was seen in the same manner as the wild-type DNA (Fig. 3A). The binding of SopA to these mutant DNAs was further examined by DNase I footprinting. The result with the wild-type DNA for the coding strand of *sopAB* (Fig. 4A) confirmed the results by Mori *et al.* (*3*): footprints as well as hyper-sensitive sites were observed extensively. We carried out footprinting for the non-coding strand as well (Fig. 4B). Footprints were observed as extensively, and a very strong hyper-sensitive site was observed between sites 3 and 4. Effects of substitution depended on the site of substitution. When the substitution was at site 1, footprints at the site on both strands disappeared. Footprints at sites 2–4 and hyper-sensitive sites remained, though with altered strengths. The number of SopA protomers in this altered complex was very likely to be 4, because the result of SopA titration was the same as for the wild-type DNA (Fig. 2). When the substitution was either at site 2 or 3, the footprints became weaker at the substitution site, but not at the other sites. The results showed that the binding equilibrium shifted towards dissociation only at the substitution site, while four SopA protomers were binding to the DNA. When the substitution was at site 4, footprint was no longer observed on the coding strand. However, on the non-coding strand, the DNase I cleavage pattern for the mutant DNA (lane Δ4) was different from that for the DNA with the substitution at all the four sites (lane Δ1234), suggesting some interaction at the site. Thus, four SopA protomers must have been in contact with this mutant DNA. These results also show high co-operativity in the binding of SopA to DNA.

When the substitution was made at two binding sites in any combination, mobility shifts similar to that for wild-type DNA were observed, although smearing indicated weakening of the binding (Fig. 3B). Under the experimental conditions, no shift was observed for DNAs with a single 5′-CTTTGC-3′ (Fig. 3C). Thus, it is concluded that no pair is particularly important in SopA's DNA binding, although the binding strength may vary. In addition, the number of SopA protomers in the complexes was likely to be the same despite the variety of the arrangement of the two recognition sequences, because the positions of the shifted bands were the same as that for the wild-type DNA.

DNA bending by SopA

DNase I footprining revealed the presence of a very strong hyper-sensitive site and a quasi-periodic appearance of weak hyper-sensitive sites in the upstream half on the non-coding strand (Fig. 4). On both strands, sites susceptible to DNase I digestion had intervals of 8–11 bp. These observations led us to suspect that the DNA was bent by SopA. In order to examine this possibility, EMSA in the style of circular permutation was carried out (*26*). Five DNA fragments of 400 bp in length, each shifted by 76 or 77 bp in position along the

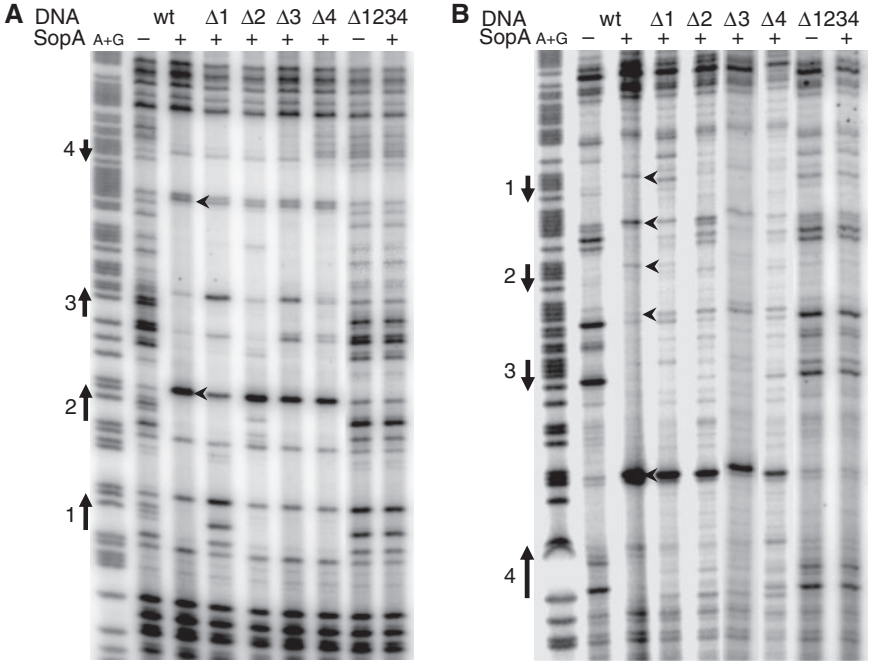

Fig. 4 DNase I footprinting of SopA bound to mutant DNAs. DNase I footprinting was carried out for wild-type and mutant DNAs on the coding strand (A) and the non-coding strand (B). The designations of the mutants are the same as in Fig. 3A. Arrows indicate the binding sites as shown in Fig. 1, on the basis of the 'A + G' lanes loaded with Maxam–Gilbert A + G reactions of the wild-type DNA. Arrowheads indicate hyper-sensitive sites for wild-type DNA (for panel A, in accordance with ref. 3).

F-plasmid DNA, were prepared (Fig. 5A). Although the DNA fragments had nearly the same mobility by themselves (Fig. 5B), they migrated differently, when bound with SopA (Fig. 5B). The fragment with the promoter region in the middle had the smallest mobility and the fragments with the promoter region at either end had the largest mobilities. The results demonstrated that the SopA binding did bend DNA. The mobility of the shifted DNA relative to that of the free DNA was plotted against the fragment position and tentatively fitted with a quadratic function (Fig. 5D). The centre of the curve was at position 163 ± 20, which was 5 bp downstream of site 3.

When 5′-CTTTGC-3′ at site 1 was substituted with 5′-CTTACG-3′, the mobility shift diminished except for the most downstream fragment and the centre of the curve moved to position 200 ± 33, indicating that the bend became smaller due to loss of interaction at the site of substitution (Fig. 5C and D). This result was in agreement with the results of DNase I footprinting (Fig. 4). In contrast, when the substitution was at site 4, the mobility shift was very similar to that for the wild-type DNA, with the centre at 164 ± 29 (Fig. 5C and D). This also agreed with the footprinting results, which indicated some DNA–protein interaction at the site despite the substitution (Fig. 4).

Oligomeric states of SopA

The results described above pointed to a highly co-operative nature of SopA's binding to DNA. In particular, the results of EMSA for DNA with two substitutions suggested strong SopA–SopA interaction. In order to examine this protein–protein interaction, gel filtration chromatography was carried out for free SopA protein (Fig. 6). At a sodium chloride concentration of 0.5 M, the protein eluted at a position of ~75 kDa with some tailing. As the salt concentration was lowered, species of higher molecular weight increased.

Sedimentation velocity was also carried out for free SopA protein at three salt concentrations. The data for 0.5 M sodium chloride are shown in Fig. 7. At this salt concentration, three major molecular species of $s_{20,w} = 3.4$ S (37% of total absorbance), 5.1 S (47%) and 14.1 S (11%) were found to be present. The molecular weights were estimated to be 53, 100 and 510 K, respectively. At 0.3 M, three major species of $s_{20,w} = 3.5$ S (25%; molecular weight = 46 K), 5.3 S (28%; 86 K) and 15.3 S (43%; 410 K) were present. At 0.1 M, one major species of $s_{20,w} = 14.8$ S (79%; 430 K) was present. The frictional ratio, obtained by assuming that it was the same among the molecular species, was 1.43 for 0.5 M sodium chloride, 1.17 for 0.3 M and 1.33 for 0.1 M.

Discussion

Although SopA was shown to bind to the four 5′-CTT TGC-3′ sequences in the promoter region of *sopAB* over 20 years ago (*3*), the asymmetric arrangement of the four sequences has posed a conundrum regarding the SopA–SopA interaction in the SopA–DNA complex, because importance of symmetry in oligomeric proteins has long been recognized (*27*). The present study re-examined the DNA–protein interaction by using a number of mutant DNAs and by examining the states of free SopA. Although the conundrum has not been fully solved, the results suggest that the

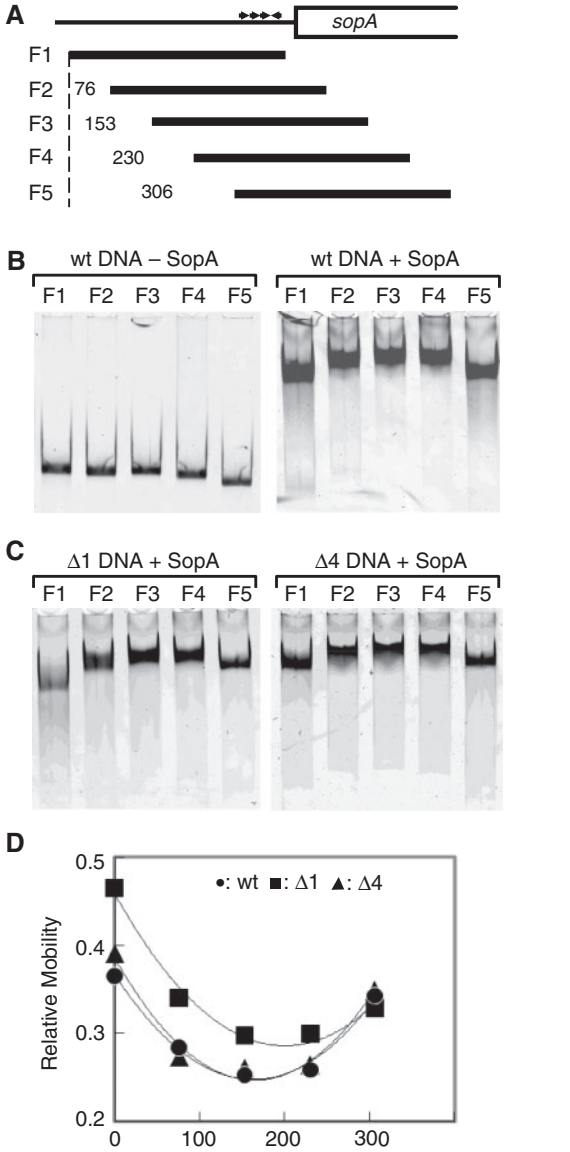

Fig. 5 DNA bending by SopA. EMSA in the style of circular permutation was carried out for sets of DNA fragments. (A) The fragments used, all of them 400 bp in length, are shown in relation to the SopA binding sites (arrows) and the *sopA* gene. The numbers show the positions of F2–F5 relative to F1. F1 and F5 contained 8 bp outside sites 4 and 1, respectively. Note it was not a *sensu stricto* circular permutation experiment, because the sequences upstream and downstream of the binding sites were different. (B) 180 fmol of each of wild-type F1–F5 fragments in 20 μl was electrophoresed with or without 18 pmol SopA protomer. (C) Each of the F1-F5 fragments with substitution at site 1 (Δ1) or at site 4 (Δ4) were electrophoresed with SopA. (D) The ratio of the migration distance with SopA to that without SopA was calculated for each fragment and plotted against the position of the fragment. For each set, the F1–F5 fragments without SopA and the F5 fragment with SopA were electrophoresed side-by-side for calibration. Lines show best-fit quadratic functions.

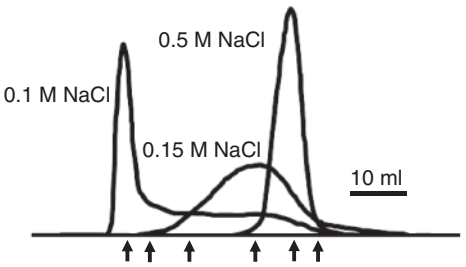

Fig. 6 Gel filtration chromatography of free SopA. Sodium chloride at the indicated concentrations was included in the eluant; 100 μl of SopA at the protomer concentrations of 17 μM (for 0.5 M NaCl), 27 μM (for 0.15 M NaCl) and 26 μM (for 0.1 M NaCl) were applied to a Superdex 200 column, and elution was monitored by absorption at 280 nm. The chromatograms are adjusted to give one same area. The arrows indicate the positions of the molecular markers (from left, void, blue dextran; 669 K, thyroglobulin; 440 K, ferritin; 158 K, aldolase; 65 K, conalbumin; 43 K, ovalbumin), which were independent of the salt concentration.

SopA–SopA interaction is multiple and/or flexible and that the interaction is modified by SopA–DNA interaction, as discussed below.

One theoretical possibility of the SopA–DNA interaction was that SopA would bind to the operator region as a dyadic dimer: one protomer would bind to site 4 and the other protomer, alternately to one of the other sites. As shown above, substituting the most downstream site did not abrogate DNA binding, which made this mechanism implausible. Another possibility was that a symmetric SopA oligomer larger than a tetramer would bind to the four sites. However, the results of this study showed that the stoichiometry of SopA to DNA was 4 and that the SopA protein binds to its four binding sites in a highly co-operative manner. Even when two of the four sites were substituted, the remaining two, in any combination, were able to bind SopA and the complex had the same electrophoretic mobility as the complex with the wild-type DNA.

The results of EMSA and DNase I footprinting showed difference in SopA–DNA interaction between sites 1 and 4, presumably reflecting the asymmetric arrangement of the recognition sequences. When 5′-C TTTGC-3′ was substituted with 5′-CTTACG-3′ at site 1, DNase I footprints disappeared at the site and the bend of the poromoter-region DNA decreased, indicating loss of interaction at the site. In contrast, when the substitution was at site 4, DNase I footprinting on the non-coding strand showed some interaction and the bend of the DNA was the same as in the wild-type DNA. Thus, whereas the interaction at site 1 is sequence-specific, the interaction at site 4 is less so. SopA has been shown to interact with non-specific DNA in the presence of ATP (*25*). It also was reported that SopA mutants that failed to bind specifically to the *sopAB* promoter region still bound DNA non-specifically (*25*). Therefore, the conformation of the SopA protomer binding to site 4 may be more relevant to the conformation that binds DNA non-specifically. The sequence specificity at site 2 or 3 cannot be assessed on the basis of the present data, because structural alteration due to substitution should be limited at these inner sites, as the DNA is 'stapled' to the SopA tetramer at the other three sites.

Gel filtration chromatography and analytical ultracentrifugation showed that SopA can be in oligomeric forms in the absence of ATP. The results of analytical ultracentrifugation indicated that the major molecular species of SopA were very likely to be monomeric,

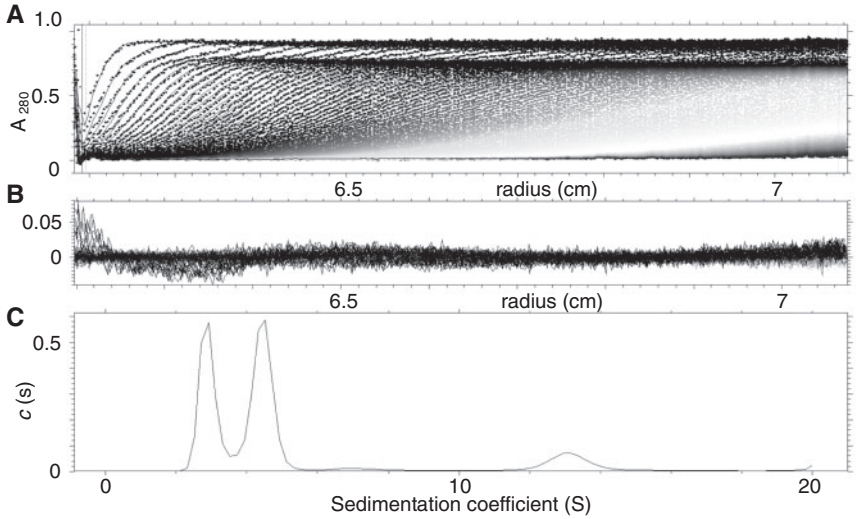

Fig. 7 Sedimentation velocity of free SopA at 0.5 M sodium chloride. (A) Sedimentation profiles at 50,000 rpm. Fitting curves were finite-element solutions of the Lamm equation by SEDFIT program (*24*, *25*). (B) Residuals of the fit. (C) The distribution of the sedimentation coefficient obtained by the Lamm equation analysis. The coefficient is not corrected for the density and viscosity of the solution, while the $s_{20,w}$ values given in the text are corrected.

dimeric and decameric. The typical frictional ratio of a globular protein is 1.2, and the numbers obtained for SopA at three salt concentrations indicate that the SopA species have more or less elongated shapes (*28*). The molecular weight estimates by gel filtration chromatography were larger presumably for the shape factors. Although the distributions of molecular species obtained by the two methods are not in total agreement, they are consistent in that species of higher molecular weights increased at lower salt concentrations. Castaing *et al*. (*29*) stated that a small fraction of SopA was dimeric in the absence of ATP, when analysed by gel filtration chromatography. Although the discrepancy between their and the present results may be due to differences in the experimental conditions, such as the concentration of SopA, the experimental details were not given in their report.

From the present results, the distribution of molecular species of free SopA cannot be deduced for the protein concentrations and the salt concentration employed in EMSAs, which were much lower. Lowering the protein concentration will favour disassembling of oligomers, while the present results show that lowering the salt concentration should facilitate formation of species of higher molecular weights. If raising the salt concentration parallels lowering the protein concentration, free SopA is likely to exist as a heterogeneous mixture of molecular species under the conditions of EMSAs, whether the results of gel filtration chromatography or those of analytical ultracentrifugation may be referred to. However, the results of EMSAs using wild-type and mutant DNAs showed that SopA bound to the promoter-region DNA highly co-operatively as a tetramer. Thus, the results of this study show that SopA–SopA interaction is inherent in the absence of ATP and strongly suggest that it is modified by SopA–DNA interaction.

The discussion above argues for flexibility and/or multiplicity in the SopA–SopA interaction. The following lends support to this notion. ParA, a close homologue of SopA, binds to a 42-bp pseudopalindrome, when bound with ADP, and the X-ray structure of an ADP-bound ParA dimer could be modelled to interact with 40-bp DNA, when the DNA was bent (*16*, *17*). ParA was shown to be dimeric without ADP, and X-ray crystallography revealed as many as four structures for dimeric apoParA, which were quite different from the ADP-bound structure (*16*). Although the dimer interfaces shared one interaction, they were diverse. Thus, ParA has a large degree of variability in its protein–protein interfaces as well as a large flexibility in its conformation (*16*). It would not be implausible to assume that SopA is as flexible. Analytical ultracentrifugation showed the presence of a decameric SopA species. A likely configuration for a stable decamer is a ring, the formation of which requires two distinct protein–protein interfaces.

Lastly, we would like to make a technical comment on the circular permutation assay, which has been extensively used to detect DNA bending by protein (*26*, *30*). The present results showed that the technique was effective for a large protein–DNA complex. In the course of the experiment, we did not detect clear shift differences until lengthening the DNA to 400 bp. Longer 'arms' presumably amplified the differences. We have not tested longer DNAs. However, we do not think that further lengthening would have improved the results very much, because the persistence length of the DNA is only ~130–150 bp (*31*).

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Conflict of interest
None declared.

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