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DNA aggregation by an archaeal DNA binding protein Sac10b and its novel DNA nicking activity

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The solution structure of an archaeal DNA binding protein Sac10b (DBNP-B) by cross-linking with formaldehyde and its interaction with DNA were studied. Results indicated that Sac10b existed as oligomeric structure in solution and the oligomerization was greatly stimulated by Mg^{2+} ions and elevated temperatures. In light of our earlier observation that Sac10b interacts with DNA forming different types of complexes with DNA at different protein concentrations, its DNA-binding properties were also studied. Results demonstrated that the protein formed rapidly sedimentable co-aggregation complexes with both native and denatured DNA in a protein concentration-dependent manner. These protein DNA complexes were fluid-like crystalline material at a protein DNA ratio (3-6:1 w/w). Gel mobility shift assays carried out to study the interaction of the protein with plasmid DNA indicated possible DNA nicking by the protein. The DNA nicking activity of Sac10b was optimal in *p*H range of 7-8.5 and was dependent on Mg^{2+} ions. It was maximal at protein to DNA ratio of (8:1, w/w) and very little activity was observed above and below this ratio. Nicking of DNA at this ratio indicated structure-specific DNA nicking by the protein. The protein might have important multi-functional role in the DNA metabolism in this organism.

Keywords: *Sulfolobus acidocaldarius*, Sac10b, DNA aggregation, DNA nicking

Several small, basic DNA-binding proteins have been reported from thermoacidophilic archaea *Sulfolobus acidocaldarius*[1] and have been grouped into three classes based their molecular mass (7, 8 and 10 kDa). Four low molecular weight DNA binding proteins from *S. acidocaldarius* nucleoid named helix-stabilizing nucleoid proteins HSNP-A (11 kDa), HSNP-C (8 kDa) and HSNP-C' (7 kDa), and a DNA binding nucleoid protein DBNP-B (10 kDa) have been identified in our laboratory. HSNP proteins strongly protect DNA against thermal denaturation[2,3]. HSNP-C' and DBNP-B are found to be identical to Sac 7d and Sac10b respectively. Sso 10b from *S. solfataricus* is one of the Sac10b family protein which is conserved in most archaeal genomes that have been sequenced (both from euryarchaeota and crenarchaeota)[4]. A general review on archaeal chromosomal proteins is presented elsewhere[5].

Sac10b is major protein (4-5% of total soluble protein) of *S. acidocaldarius* and binds both double and single-stranded DNA strongly[1,3]. It forms different complexes with DNA at different protein to DNA ratios, as indicated by electron microscopic studies[6,7] as well as susceptibility of DNA to DNase I digestion[7]. Ssh10b, a Sac10b family protein from *S. shibate* affects DNA supercoiling in a temperature-dependent manner and may exist predominantly as a dimer with some oligomeric aggregation[8]. Several archaeal species have two copies of the Sso10b genes (*Sso10b1* and *Sso10b2*). Alba is identified as a protein tightly bound to Sir2 protein, and is shown to be identical to Sso10b1[9].

The crystal structures of Sso10b1 at 2.8 Å (tetragonal) and 2.6 Å (hexagonal) resolution[10] and that of related DNA binding protein from the hyperthermophilic euryarchaeon *Methanococcus jannaschii* Mja10b have been determined[11]. However, the crystal structure of Sso10b2 from *S. solfataricus* has been determined at a substantially higher resolution (1.85 Å) than that of Sso10b1[12]. The crystal structures indicate that the protein exists as dimer.

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Solution structure of Sso10b2, as determined by NMR data indicates predominantly dimeric structure[13]. Biophysical studies have shown that Ssh10b is extremely heat stable in the temperature range 25-85°C[14].

In the present investigation, the solution structure of Sac10b, an archaeal DNA binding protein has been studied by cross-linking experiments using HCHO. Its interaction with DNA has also been studied to understand the physiological role. The protein oligomerizes at physiological temperatures and oligomerization is stimulated by Mg^{2+} ions. It forms rapidly sedimentable co-aggregation complexes with DNA and nicks circular DNA in the presence of Mg^{2+} in a concentration-dependent manner.

Materials and Methods

Sulfolobus acidocaldarius DSM 639 was obtained from Deutsche Sammulung Von Mikroorganisemen, Gottingen, Germany. It was grown at 75°C for 40-48 h with vigorous aeration[2]. The cells were harvested after neutralizing the culture with 1 *M* Tris base. Sac10b (DBNP-B) was purified as described previously[3].

Escherichia coli DNA was labeled with [^{3}H-methyl] thymidine[15] and the DNA was isolated[16]. The specific activity of DNA was 5000 cpm per µg. Supercoiled pBR322 DNA was isolated from *E. coli* DH5α cells[17]. The concentrations of nucleic acids and protein were determined by UV spectroscopy and Folin reagent[18] respectively.

Cross-linking with formaldehyde

Cross-linking of Sac10b with formaldehyde (HCHO) was performed as described previously[19], with some modifications. Sac10b (5 µg) in 20 m*M* triethanolamine.HCl (*p*H 7.5) or 10 m*M* sodium acetate (*p*H 5.0) or 100 m*M* sodium tetraborate (*p*H 8.2) was reacted with 200 m*M* HCHO, *p*H 7.5 and 5.0 respectively, in the presence or absence of 10 m*M* $MgCl_2$ at 37, 65 and 80°C. Small aliquots were taken out at different time intervals and the cross-linking was quenched by the addition of TCA to 10% final concentration. The precipitates were collected by centrifugation, washed with acetone and dissolved in electrophoresis sample buffer containing 0.1% SDS, but devoid of 2-mercaptoethanol/DTT and electrophoresed on 15% SDS-polyacrylamide gels[20]. Cross-linking was also performed at different NaCl concentrations to investigate the effect of salt on oligomerization.

Aggregation assays

The aggregation assays were performed as described previously[21], with minor modifications. The reactions were carried out in 40 µl reaction volume in 10 m*M* Tris-HCl (*p*H 7.6), 1 m*M* DTT or 10 m*M* sodium acetate (*p*H 5.0), and 1 m*M* DTT in the presence or absence of $MgCl_2$ with both non labeled and ^{3}H-labeled DNA. The reaction mixtures containing constant amount of DNA (ss or dsDNA) were incubated at 65°C with increasing amounts of protein. After 15 min, the reaction mixtures were centrifuged for 3 min at 14 000 *g* in a micro centrifuge. The top 20 µl and bottom 20 µl were removed carefully and added to 100 µl of 0.1% SDS in water and the radioactivity in these fractions was measured in a liquid scintillation counter. Aggregation was defined as decrease in radioactivity in top 20 µl relative to an unsedimented control mixture.

In aggregation assays, where non-labeled DNA was used, after centrifugation, the total supernatant was removed carefully without touching the walls of the micro centrifuge tube and mixed with 10 µl 5 × SDS gel loading buffer. The pellet was dissolved in 40 µl water and 10 µl 5 × SDS gel loading buffer and both samples were heated at 65°C for 15 min and analyzed by SDS-PAGE[20].

Nicking assay

The nicking activity of Sac10b was tested by incubating DNA containing both supercoiled and relaxed forms or only relaxed form in a reaction volume of 20 µl of 50 m*M* Tris-HCl (*p*H 7.5), 50 m*M* KCl, 0.1 m*M* Na_2-EDTA, 0.5 m*M* DTT and 10 m*M* $MgCl_2$ with different concentrations of protein at 37 and 65°C for indicated times as given in the legends. The reaction was terminated by the addition of SDS to 1% final concentration and the samples were digested with 2 µl of 0.8 mg/ml proteinase K for 30 min at 37°C. Then, 3 µl of loading buffer containing 0.05% bromophenol blue, 0.05% xylene cyanol, 10 m*M* Tris-HCl (*p*H 8.0) and 50% glycerol was added and the reaction products were electrophoresed on a 1% agarose gel. The same reaction was performed at different *p*Hs (as given in Fig. 4B) and also the influence of Mg^{2+} and ATP on the nicking activity was studied.

Results

Cross-linking of Sac10b

Cross-linking was employed as a tool to study the solution state of Sac10b and performed with zero length cross-linker HCHO at three different temperatures and *p*H. Cross-linking for different time intervals (as indicated in Fig. 1) was carried out at 37°C in 20 m*M* Triethanolamine (*p*H 7.5) or 100 m*M* sodium tetraborate (*p*H 8.2). Cross linking in 20 m*M* triethanolamine (*p*H 7.5) resulted in the formation of predominantly a dimer along with some trimeric, tetrameric and high molecular weight aggregates at all time intervals (Fig. 1, lanes 1-5), whereas in sodium tetraborate resulted in the formation of dimers along with tetramers, pentamers and high molecular weight aggregates (Fig. 1A, lanes 7-11). The effect of NaCl concentration on the cross-linking pattern of Sac10b was studied at 37°C and the yield of cross-linked (oligomeric) forms was found to be low.

Cross-linking of Sac10b with HCHO at 65°C in 20 m*M* triethanolamine (*p*H 7.5) resulted in the formation of dimeric, trimeric, tetrameric, pentameric, hexameric and high molecular weight aggregates (Fig. 1B). Mg^2 enhanced the cross-linking into high molecular weight aggregates with increased tetrameric form as seen in Fig. 1B, lanes 9-14 (presence of Mg^{2+}) and lanes 2-7 (absence of Mg^{2+}). Cross-linking at 65°C in 10 m*M* sodium acetate

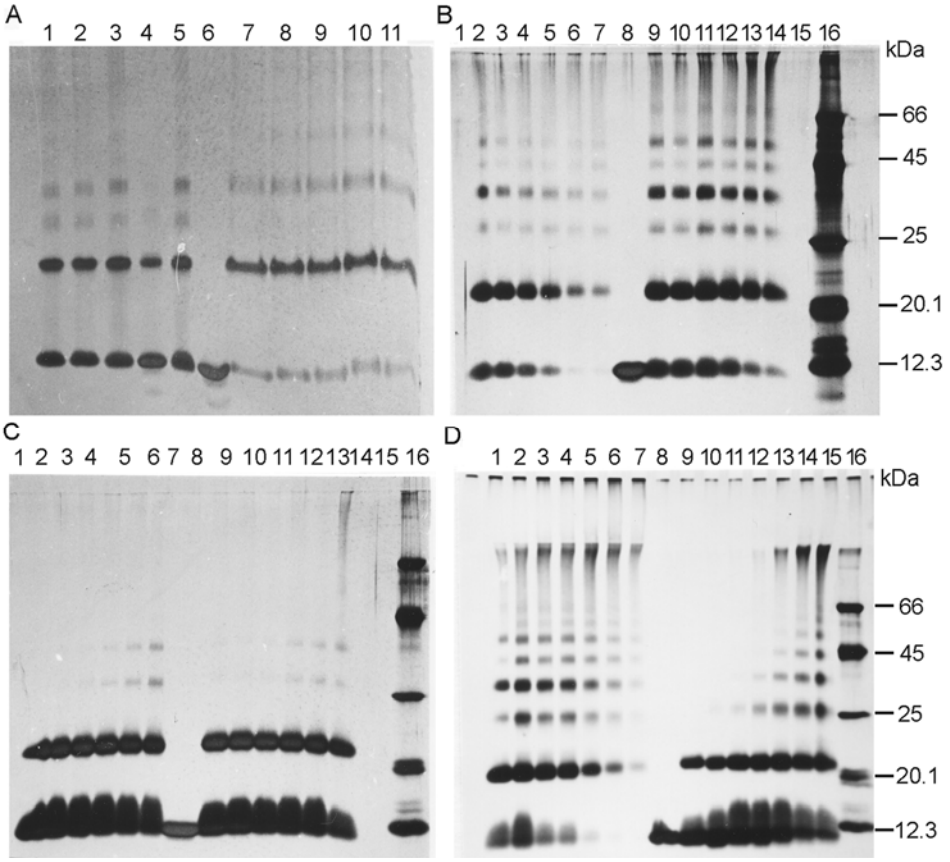

Fig.1—SDS-PAGE analysis of Sac10b cross-linked with HCHO [(A): Cross-linking at 37°C: Lane 6: Sac10b (control). lanes 1-5: cross-linking with HCHO for 1/2, 1, 2, 3 and 6 h respectively in 20 m*M* Triethanolamine (*p*H 7.5); and lanes, 7-11: Cross-linking with HCHO for 1/2, 1, 2, 3 and 6 h respectively in 0.1 *M* sodium tetraborate (*p*H 8.2). (B): cross linking at 65°C in *p*H 7.5 buffer: Lane 8, Sac10b (control); lanes 2-7, Cross linking for 10, 15, 30, 60, 120 and 180 min respectively in the absence of Mg^{2+}; lanes 9-14, Cross-linking for 10, 15, 30, 60, 120 and 180 min respectively in the presence of Mg^{2+}; and lane 16, molecular weight markers (66 kDa BSA, 45 kDa ovalbumin, 25 kDa chymotrypsinogen, 20.1 kDa trypsin inhibitor and 12.3 kDa cytochrome-c); (C): Cross- linking at 65°C in *p*H 5.0 buffer: Lane 8, Sac10b (control); lanes 2-7; cross-linking for 10, 15, 30, 60, 120 and 180 min respectively in the absence of Mg^{2+}; lanes 9-14, cross- linking for 10, 15, 30, 60, 120 and 180 min respectively in the presence of Mg^{2+}; lane 16, molecular weight markers as in Fig. 1B; (D): cross-linking at 80°C: Lane 8, Sac10b (control); lanes 1-7, cross-linking for 2, 5, 10, 15, 30, 60 and 120 min respectively in 20 m*M* triethanolamine (*p*H 7.5); lanes 9-15, cross-linking for 2, 5, 10, 15, 30, 60 and 120 min respectively in 10 m*M* sodium acetate (*p*H 5.0); and lane 16, molecular weight markers as in Fig. 1B]

(*p*H 5.0) (Fig. 1C) resulted in the formation of a 22 kDa dimeric form along with negligible amounts of trimeric and tetrameric forms progressively with time. Presence of Mg^{2+} did not enhance the formation of high molecular weight aggregates (Fig. 1C, lanes 9-14).

As the cross-linking with HCHO was enhanced by elevated temperature and Mg^{2+}, it was performed at 80°C in the presence of Mg^{2+} both at *p*H 5.0 (10 m*M* sodium acetate) and 7.5 (20 m*M* triethanolamine). At *p*H 7.5, formation of dimers, trimers, tetramers, pentamers, hexamers and high molecular weight aggregates was observed within 2 min and longer incubation (>30 min) resulted in the formation of high molecular weight aggregates with disappearance of lower molecular weight aggregates (Fig. 1D, lanes 1-7). In 10 m*M* sodium acetate (*p*H 5.0) buffer at 80°C, cross-linking resulted in the formation of trimers, tetramers, pentamers, hexamers and high molecular weight aggregates with incubation for longer time periods (>30 min) (Fig. 1D, lanes 9-15).

Cross-linking with HCHO at 37°C and *p*H 5 also showed formation of only dimeric product at all time intervals. Addition of Mg^{+2} did not change the pattern as only dimers were formed both in the presence and absence of Mg^{2+}. These results indicated that Sac10b existed predominantly in a dimeric state in *p*H 5.0 buffer (10 m*M* sodium acetate) at 37°C and 65°C and in different oligomeric forms at physiological conditions (*p*H 7.5 and growth temperature).

As a control, cross-linking with HCHO was performed with a low molecular weight histone-like DNA binding protein H-NS from *E. coli*. Cross linking of H-NS with HCHO at 37°C in 20 m*M* triethanolamine-HCl (*p*H 7.5) for 15, 30, 60 and 120 min resulted in the formation of only a tetrameric form, the native aggregation state of the protein (not shown).

DNA aggregation by Sac10b

While studying the nucleic acid binding properties of Sac10b, appearance of crystalline gel-like material was observed in the reaction tubes under certain conditions. The gel mobility shift assays indicated aggregation of DNA by Sac10b at high protein concentration (greater than 3:1 protein/DNA ratio) as indicated by the retardation of DNA in the wells of agarose gels[7]. The reaction mixtures containing constant amount of DNA and increasing amounts of Sac10b at different *p*H conditions were centrifuged at 10,000 *g* for 3 min and the supernatant and pellet were analyzed for DNA. Results in Fig. 2A indicated aggregation of ssDNA and dsDNA by Sac10b at *p*H 7.6 buffer. Aggregation was also observed in *p*H 5.0 buffer (not shown).

Aggregation of DNA was dependent on protein concentration and increased with increasing protein concentration. Aggregation at *p*H 7.6 required about 2-fold higher protein concentration than at *p*H 5 and maximum aggregation occured at a protein ssDNA

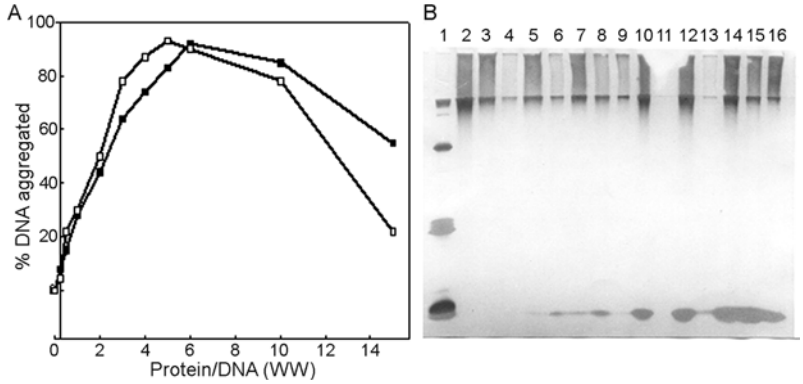

Fig. 2—Aggregation of DNA by Sac10b [(**A**): Assay was carried out using ^{3}H-labeled *E. coli* DNA (native or denatured) Aggregation was defined as decrease in the radioactivity of top 20 µl relative to an unsedimented control mixture. (-□-) denatured DNA; (-■-) Native DNA, and (**B**): SDS-PAGE analysis of aggregation reaction products. Aggregation of *S. acidocaldarius* native DNA (1 µg) in 10 m*M* Tirs-HCl (*p*H 7.6), 12 m*M* $MgCl_2$ and 1 m*M* DTT in a reaction volume of 40 µl was incubated with different concentrations of Sac10b. After incubation at 65°C for 15 min, reaction mixtures were centrifuged at 10,000 × *g* for 30 min. Total supernatant was collected carefully and mixed with 10 µl of 5 × SDS-PAGE gel loading buffer. 40 µl water and 10 µl of gel loading buffer were added to the pellets and the samples were heated at 65°C for 15 min and electrophoresed on 15% gels. Lane 1, molecular weight markers (as in Fig. 1B) and Sac10b; lane 2, DNA without centrifugation (control); lane 3, supernatant (control); lane 4, pellet (control); and lanes 5, 7, 9, 11, 13, 15, supernatant fractions from reaction mixture incubated with 0.5, 1.0, 2.0, 3.0, 6.0, and 12.0 µg Sac10b respectively; and lanes 6, 8, 10, 12, 14, 16, pelleted fractions from the reaction mixtures incubated with 0.5, 1.0, 2.0, 3.0, 6.0 and 12.0 µg Sac10b respectively]

ratio of 6.0 (filled squares). In case of dsDNA, maximum aggregation was observed at a protein to DNA ratio of about 5 (open squares) at *p*H 7.6. But, at higher protein to DNA ratios (>8) aggregation decreased with both ss and dsDNA at both *p*Hs. Aggregation of DNA (either ss or dsDNA) was not dependent on Mg^{2+} as same pattern was obtained in the absence and presence of Mg^{2+} (results not shown).

Aggregation was also assayed by using non-labeled DNA isolated from *S. acidocaldarius*. Immediately after centrifugation, the total supernatants were removed carefully and mixed with gel loading buffer and electrophoresed. The gels were silver stained to visualize both dsDNA and Sac10b (Fig. 2B). Most of the dsDNA (seen as a smear on the top portion of gel) was completely sedimented by Sac10b, as all DNA was in the pellet (Fig. 2B, lanes 12 and 14). The protein was also pelleted along with dsDNA as the supernatant did not contain either protein or DNA (Fig. 2B, lanes 11 and 13)). At higher protein concentration (>8 protein to DNA ratio), DNA aggregation was inhibited as seen by the presence of DNA in the supernatant along with Sac10b (Fig. 2B, lane 15). Non-labeled ssDNA (denatured *S. acidocaldarius* DNA) exhibited similar aggregation behavior in the presence of Sac 10b as analyzed by SDS-PAGE (data not shown).

Nicking activity of Sac10b

During mobility shift experiments with pBR322 DNA, there was a gradual increase in the intensity of relaxed DNA band, suggesting possible nicking of supercoiled DNA by Sac10b (data not shown). Nicking activity of Sac10b was tested by incubating pBR322 DNA as described in 'Materials and Methods'. DNA was converted to a form migrating to the position of linear form at a DNA: protein ratio of 1:5 to 1:8 (Fig. 3A, lanes 5-7). The optimum concentration of Sac10b for conversion of pBR322 DNA to linear form was 1:6-8 of DNA: Sac10b. Above this ratio, no conversion of DNA to linear form was observed. The nicking activity was observed both at 37 and 65°C with more activity at 65°C.

The nicking activity was also tested by incubating pBR322 DNA in the assay buffer with increasing concentrations of Sac10b in the absence of Mg^{2+}. As can be seen from Fig. 3B, both supercoiled and relaxed forms of DNA were intact at all Sac10b concentrations, implying that Mg^{2+} was essential for

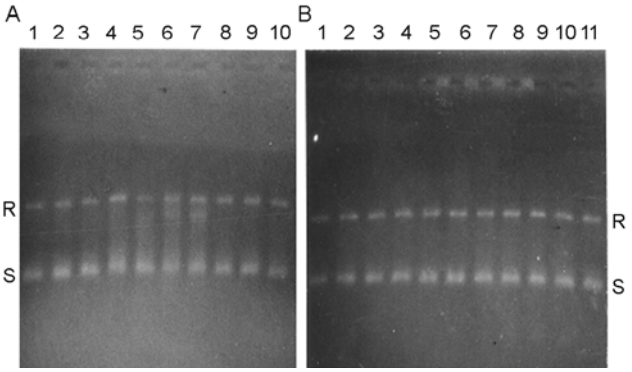

Fig. 3—Nicking activity of Sac10b [(**A**): Sac10b was assayed for nicking activity by incubating pBR322 DNA (300 ng) containing both supercoiled and relaxed forms as described in 'Materials and Methods' with increasing concentrations of protein. The reaction products were deproteinised with 1% SDS, subjected to proteinase K digestion at 37°C for 30 min and electrophoresed on a 1% agarose gel. Lane 1, DNA incubated without Sac10b (control); lanes 2-10, DNA incubated with 0.15, 0.3, 0.6, 0.9, 1.8, 2.4, 3, 6 and 9 µg Sac10b respectively; and (**B**): Nicking activity of Sac10b in the absence of Mg^{2+}: pBR322. DNA (300 ng) was incubated with increasing concentrations of Sac10b in buffer without Mg^{2+}. The reaction products were deproteinised with 1% SDS and electrophoresed on a 1% agarose gel. Lane 1, DNA without Sac10b (control); lane 2-11, DNA incubated with 0.15, 0.3, 0.6, 1.2, 2.4, 3, 6, 9, 12 and 15 µg Sac10b respectively]

the nicking activity. The influence of ATP on the nicking activity was tested by incubating pBR322 DNA with increasing concentrations of Sac10b in 2 m*M* ATP containing buffer. No increase was observed in the conversion of DNA to linear form at all concentrations of Sac10b, suggesting that ATP was not required for the nicking activity (not shown)

pBR322 DNA, which is mostly relaxed was incubated in the nicking assay buffer with increasing concentrations of Sac10b and the reaction products were electrophoresed (Fig. 4A). In this also, the relaxed DNA was efficiently converted to linear form in the range of protein to DNA ratios of 4-8. Nicking assay was carried out at different *p*H conditions to determine the optimum pH for Sac10b to convert pBR322 DNA (mostly relaxed) to linear form. As can be seen from Fig. 4B (lanes 6 and 7), optimum *p*H was found to be 7.0 to 7.5 at which all the supercoiled form present is converted to relaxed and or linear form.

To determine the optimum time, pBR322 DNA was incubated with DNA: Sac10b at a ratio of 1:8 for different time intervals in the nicking assay buffer as given in 'Materials and Methods'. As can be seen in Fig. 4C, by 5 min (lane 2), linear form appeared with a progressive increase in the conversion of relaxed to

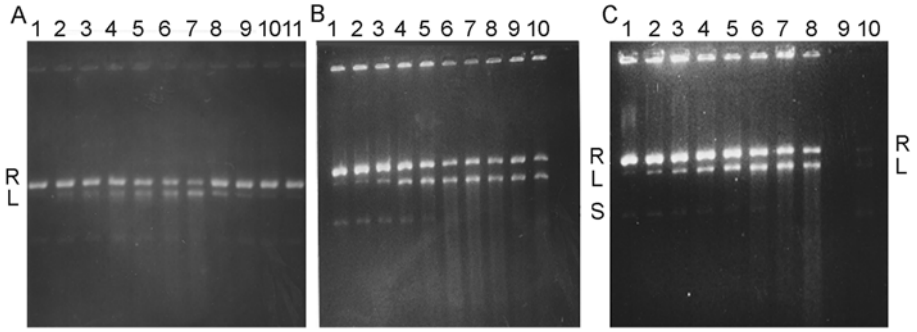

Fig. 4—Nicking of relaxed pBR322 DNA [(**A**): pBR322 relaxed DNA (300 ng) was incubated with increasing concentrations of Sac10b for 30 min. The reaction products were deproteinised with 1% SDS and electrophoresed on a 1% agarose gel. Lane 1, DNA without Sac10b (control); lanes 2-11; DNA incubated with 0.15, 0.3, 0.6, 0.9, 1.2, 2.4, 3, 6, 9 and 12 µg Sac10b respectively; (**B**): Influence of *p*H on the nicking activity of Sac10b: pBR322 relaxed DNA (300 ng) was incubated for 30 min with Sac10b at a DNA:protein ratio of 1:8 in buffers of different *p*Hs. The reaction products were deproteinised with 1% SDS and electrophoresed on a 1% agarose gel. Lane 1, DNA without Sac10b in 50 m*M* Tirs-HCl (*p*H 7.5) (control); lanes 2-5, DNA incubated with Sac10b in 50 m*M* Na acetate (*p*H 5.0, 5.5, 6.0 and 6.5 respectively); and lanes 6-10, DNA incubated with Sac10b in 50 m*M* Tris-HCl (*p*H 7.0, 7.5, 8.0, 8.5 and 9.0 respectively); and (**C**): Time course of nicking of relaxed pBR322 DNA. pBR322 DNA (300 ng) was incubated with 2400 ng of Sac10b in 50 m*M* Tirs-HCl (*p*H 7.5), 50 m*M* KCl, 0.1 m*M* EDTA, 0.5 m*M* DTT and 10 m*M* $MgCl_2$ for different time intervals. The reaction products were deproteinised with 1% SDS and electrophoresed on a 1% agarose gel. Lane 1, DNA incubated without Sac10b (control); lanes 2-8, DNA incubated with Sac10b for 2, 5, 10, 15, 30, 60 and 120 min respectively; and lane 10, pBR322 *Hind* III digest containing relaxed, linear and supercoiled forms. R, L & S denote relaxed, linear and supercoiled forms]

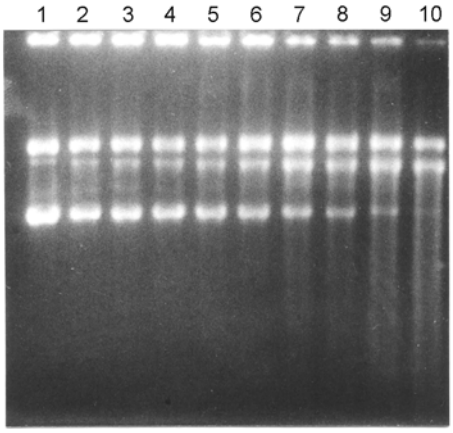

Fig. 5—Nicking activity with SV40 DNA [SV40 DNA (1.2 µg) was incubated with increasing concentrations of Sac10b in *p*H 7.5 buffer containing 10 m*M* $MgCl_2$ for 1 h. The reaction products were deproteinised with 1% SDS and electrophoresed on a 1% agarose gel. Lane 1, DNA incubated without Sac10b (control); lanes 2-10, DNA incubated with 0.15, 0.3, 0.6, 0.9, 1.2, 2.4, 3, 6 and 9 µg Sac10b respectively]

linear DNA up to 1 h. Thereafter, the linear form further degraded to lower fragments seen as a smear in the gel (lane 8). However, not all the relaxed DNA was converted to linear form.

Incubation of SV40 DNA with increasing concentrations of Sac10b at 37°C in the nicking assay buffer (Fig. 5) showed the linear form increased and the supercoiled form decreased with increasing concentrations of Sac10b. At higher Sac10b concentration (>3 protein to DNA ratio), most of the supercoiled DNA was converted to relaxed or linear forms with faint smear in the gel (Fig. 5 lanes 9, 10). Nicking activity of Sac10b was also tested by incubating the λDNA with increasing concentrations of the protein under similar conditions as in Fig 4A. The DNA appeared intact and no nicking activity was detected on λDNA (not shown).

Discussion

One of the interesting aspects reported in this paper was oligomeric aggregation of Sac10b. The protein appeared to exist in a very high molecular weight aggregated state, as indicated by increase in aggregation pattern at 80°C (optimum temperature for growth of *S. acidocaldarius*). The multimeric state identifiable was clearly hexameric aggregate with a Mr of about 60 kDa (at *p*H 7.6, in presence of Mg^{2+} ions), apart from very high oligomers However, under most of the cross-linking conditions, dimeric, tetrameric and hexameric species were dominant over odd numbered aggregates (trimer, pentamer *etc*).

Sac10b readily formed oligomers, but did not exist in any specific oligomeric form. At *p*H 5, only dimers of Sac10b were observed up on cross-linking. Dimeric form observed in crystal structures could be due to formation of crystals in acidic *p*H conditions. Our results strongly indicated oligomeric aggregation

of the protein in solution under physiological conditions. NMR studies also indicated oligomeric aggregation in *p*H 7.0 buffer[13]. The cross-linking of Ssh10b with two different cross-linkers showed low concentration of cross-linked species from dimers to hexamers, which might be due to the fact that cross-linking in this study was carried out only at 25 and 45°C[8].

Another physical property of Sac10b was variability in the environment of aromatic amino acids as a function of the solution conditions. The protein contains two tyrosine and one phenylalanine residues[22]. Its emission fluorescence enhanced significantly in the presence of Mg^{2+} and stimulated by moderate concentration of Na^{+} (our unpublished results, Jaya KS, Ph.D. thesis, University of Hyderabad, 1998). These results indicated that conformational changes in the protein and aromatic amino acids were highly accessible in the presence of these ions. Results on nucleic acid binding properties[7], DNA aggregation (present study) which occurred optimally in the presence of Mg^{2+} and the observed protein fluorescence increase (or exposure of aromatic amino acids) suggested functional involvement of these amino acids in the interactions of Sac 10b.

Aggregation properties of Sac10b indicated that the protein started aggregating both single and double-stranded DNA at *p*H 7.6 and 5.0 at a concentration above 4 protein/DNA ratio. At lower *p*H, the threshold concentration was lower than at *p*H 7.6. At higher protein concentration (>8 Protein/DNA ratio), the aggregation decreased, indicating that aggregation was not due to protein-protein interaction, but was a property of the complex.

One of the novel and interesting observation during the present study was the nicking activity of Sac10b. It was observed during mobility shift experiments (see Results section) that some of the supercoiled pBR322 DNA was slowly converted to relaxed form. This led to detailed characterization of the nicking activity with respect to substrate and optimum conditions. The activity was optimal at a ratio of 4-8:1 (protein/DNA), beyond which there was inhibition. The nicking activity was optimal in the *p*H range (7-8.5) and was dependent on Mg^{2+} ions. These results clearly indicated enzyme-like nicking by Sac10b. Use of different DNAs as substrate showed lack of nicking activity with linear DNA (λDNA), indicating that the structure necessary for the nicking activity may not be formed with λDNA. Sequence-specific nicking by Sac10b could be ruled out, because a larger (48,502 bp) linear DNA like λ DNA is likely to have a target sequence which could be cleaved by the protein. Further work is necessary to analyze the mode of action, site of cleavage and the structure of DNA in the DNA-Sac10b complex that is amenable for nicking by Sac10b.

Results of earlier[7] and present studies suggested formation of at least three different types of functional complexes by Sac10b at different protein to DNA ratios. At low ratio (1-2:1), the protein bound to DNA, forming a complex holding two double-stranded DNAs and DNA in the complex was more susceptible to DNase I digestion[7]. At a ratio of about 4-8, co-aggregation complexes were formed and the protein showed DNA nicking activity. At very high ratios (>10), the protein coated uniformly along the double- stranded DNA like RecA protein, as observed in electron microscopy[6,7]. Possibly Sac10b might have a multi-functional role, facilitating several reactions connected with DNA metabolism such as DNA replication, transcription and recombination. This might be related to its ability to form distinctly different types of complexes with DNA at different concentrations

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References

1 Grote M, Dijk J & Reinhardt R (1986) *Biochim Biophys Acta* 873**,** 405-413
2 Reddy T R & Suryanarayana T (1988) *Biochim Biophy Acta* 949**,** 187-196
3 Reddy T R & Suryanarayana T (1989) *J Biol Chem* 264**,** 17298-17308
4 Forterre P, Confalonieri F & Knapp S (1999) *Mol. Microbiol* 32**,** 669-670
5 White M F & Bell S D (2002) *Trends Genet* 18**,** 621-626
6 Lurz R, Grote M, Dijk J, Reinhardt R & Dobrinski B (1986) *EMBO J* 5**,** 3715-3721
7 Sreenivas K, Jaya K S & Suryanarayana T (1998) *Biochem Mol Biol Int* 44**,** 269-282
8 Xue H, Guo R, Wen Y, Liu D & Huang L (2000) *J Bact* 182**,** 3929-3933
9 Bell S D, Botting C H, Wardleworth B N, Jackson S P & White M F (2002) *Science* 296**,** 148-151
10 Wardleworth B N, Russell R J, Bell S D, Taylor G.L & White M F (2002) *EMBO J* 21**,** 4654-4662

11 Wang G, Guo R, Bartlam M, Yang H, Xue H, Liu Y, Huang Z & Rao Z (2003) *Protein Sci* 12, 2815-2827
12 Chou C C, Lin T W, Chen C Y & Wang A H (2003) *J Bact* 185, 4066-4073
13 Biyani K, Kahsai M A, Clark A T, Armstrong T L, Edmonson S P & Shriver J W (2005) *Biochemistry* 44, 14217-14230
14 Xu S, Qin S & Pan X M (2004) *Biochem J* 382, 433-440
15 Mahler I (1967) *Meth Enzymol* 12A, 693-695
16 Marmur J (1961) *J Mol Biol* 3, 208-218
17 Wang Z & RossmanT G (1994) *BioTechniques* 16, 460-463
18 Lowry O H, Rosebrough N J, Farr A L & Randall R J (1951) *J Biol Chem* 193**,** 265-275
19 Jackson V (1978) *Cell* 15, 945-954
20 Laemmli U K (1970) *Nature* 227, 680-685
21 Krasnow M A & Cozzarelli N R (1982) *J Biol Chem* 257, 2687-2693
22 Dijk J & Reinhardt R (1986) In: *Bacterial chromatin*. (Gualerzi C & Pon C L, eds), pp 185-218, Springer-Verlag