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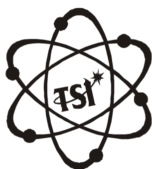

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Characterization of the nucleoid from themophilic crenarchaeon sulfolobus acidocaldarius

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ABSTRACT

Nucleoid of Sulfolobus acidocaldarius, a hyperthermophilic crenarchaeon, was analyzed to determine the composition of its nucleoprotein complexes. The nucleoid was found to have two components, a tightly aggregated membrane associated component and the membrane free component. The two components differ with respect to DNA, RNA and protein compositions. Analysis of micrococcal nuclease digest of the nucleoid suggests that the S. acidocaldarius chromatin lacks nucleosomal structure and its DNA is bound by different sets of proteins in different (domains) regions of varying length. © 2007 Trade Science Inc. - INDIA

KEYWORDS

Archaea;
Nucleoid;
Micrococcal nuclease;
S-layer.

INTRODUCTION

In bacteria, DNA is a tightly condensed into supercoiled structure called, nucleoid[1,2]. Several factors may contribute to the compaction of bacterial nucleoid like macromolecular crowding, polyamines, DNA binding proteins etc[3,4]. Among archaea, Euryarchaeal (Methanobacterium thermoautotrophicum and Methanobacterium fervidus) chromatin shares similarities with eukaryal chromatin in having histone homologues that wrap DNA into nucleosome-like structures[5,6]. In comparison, Crenarchea(mostly thermo acidophiles) do not contain histone homologues but contain abundant amounts of DNA binding proteins belonging to Sac 7 and sac10 proteins[7]. Work from our laboratory identified four acid soluble low molecular weight proteins referred to as HSNP (Helix stabilizing nucleoid protein) A, C and C' and DBNP B from Sulfolobus acidocaldarius[8,9]. HSNP-A and HSNP-C' were shown to be present exclusively on the genomic DNA[10]. HSNP C' and DBNP B, the most highly abundant among these proteins were found to be identical to Sac 7d and Sac 10b respectively[11,12,13]. Sac 10b binds DNA forming different types of complexes at different protein to DNA ratios[13,14]. See ref. 15 for a review on archaeal chromatin proteins. Sac7d has been extensively studied both with respect to its structure and its interaction with DNA[16-20].

In this study, *S.acidocaldarius* nucleoid (chromatin) was isolated and its composition was analyzed. The

nucleoid is shown to contain two distinct components with different protein composition one which is membrane associated and the other membrane free nucleoid. Analysis of micrococcal nuclease digest of the nucleoid suggests that the S.acidocaldarius chromatin lacks nucleosomal structure and its DNA is bound by different sets of proteins at different regions of DNA of varying length.

MATERIALS AND METHODS

Bacteriological reagents were purchased from Himedia. Other reagents and chemicals used were DNase(Boehringher-Mannheim), Sephacryl S-1000 (Pharmacia), Acrylamide (Pharmacia), Agarose (Bangalore genei), ethidium bromide(Sigma), Diphenylamine (Sisco Research Laboratories), Orcinol(SRL), Folin-Ciocalteau reagent(SRL), protein and DNA markers (Bangalore Genei) and Micrococcal nuclease (Fermentas)

Sulfolobus acidocaldarius strain DSM 639 was a gift from Prof Rolf Bernander, Dept of Microbiology, Uppsala University, Sweden. S acidocaldarius was grown according to[21]. Sulfolobus acidocaldarius was grown at 75 0C for 40-48 hr with vigorous aeration in a medium containing 0.1% yeast extract, 0.1% bacto tryptone, 0.5% casamino acids, 0.1%, glucose, 0.2% sodium chloride, 0.13% ammonium sulfate, 0.03% potassium dihydrogen phosphate, 0.025 magnesium sulfate, 0.07% calcium chloride and pH was adjusted to 2.8 with 1 M sulfuric acid. The cells were harvested by centrifugation at 3000 rpm at 4^0C for 15 minutes. The cell pellet was washed by resuspending it in 10 mM Tris–Cl (pH 7.6), 50mM KCl, 10 mM Magnesium acetate, 7mM β-mercaptoethanol followed by centrifugation at 5000 rpm at 4^0C.

Nucleoid isolation

Nucleoid was isolated as described earlier for the isolation of nucleoid associated proteins[9] with some modifications. Cell pellet (2g) was resuspended in 4ml of buffer containing 10 mM Tris-Cl (pH 7.6), 150 mM KCl. Lysis buffer (4 ml) containing 10mM Tris-Cl (pH 7.6), 1% NP-40, 2mM spermidine-HCl, 10mM sodium EDTA(pH 8) was added to the cell suspension and incubated at 10^0C for 1hr. The lysate was centrifuged at 1500rpm at 4°C for 10 minutes. The supernatant was collected and loaded on to a 30% sucrose cushion and centrifuged at 10,000rpm at 4^0C for 1hr. The nucleoid pellet was resuspended in 2-3ml of buffer containing 20 mM Tris-Cl(pH 7.6), 150mM KCl, 2mM sodium EDTA and 6mM β-mercaptoethanol.

(nucleoid buffer)

Sephacryl S-1000 column chromatography

Nucleoid (2ml) was mildly sonicated and clarified by centrifugation at 1500rpm at 4^0C for 10 min and loaded onto the S-1000 column (100ml). Elution was carried out with 2 bed volumes of buffer and 3ml fractions were collected at 24 ml per hour. Fractions were analyzed for absorbance at 260 and 280nm, protein, DNA and RNA content. Fractions were also analyzed by SDS-PAGE and agarose gel electrophoresis.

Micrococcal nuclease digestion of chromatin

This was performed according to[22]. Nucleoid (1μg) was incubated with 1 unit of micrococcal nuclease in 20 mM Tris-Cl pH 8.8, 50mM NaCl, 50 mM MgC12 and 1mM CaC12 at 37^0C for 1hr. Reaction was stopped by addition of SDS and EDTA to 1% and 25mM final concentrations respectively.

Sephacryl S-200 column chromatography

Micrococcal nuclease digest of the nucleoid was loaded onto the Sephacryl S-200 column (50cm× 1.6cm). Elution was carried out with 2 bed volumes of nucleoid buffer and 3 ml fractions were collected. Fractions were measured for absorbance at 260 and 280nm and analyzed by electrophoretic techniques.

Isolation of S-layer

S-layer was isolated as described earlier[23]. Cells harvested at mid logarithmic phase were suspended in 10mM NaCl, 1mM PMSF, 20mM magnesium sulfate and 0.5% sodium lauroyl-sarcosine (Solution A). 2μg DNase I was added per ml suspension and incubated for 20min at 45 ºC. The suspension was centrifuged for 10 min at 14000 rpm, the supernatant was discarded and the pellet was resuspended in solution A and the above steps were repeated. The white layer (S layer) on top of the pellet was suspended carefully in buffer B containing 10mM NaCl, 20 mM magnesium sulfate and 0.5% SDS, incubated for 20 minutes at 45^0C and cen-

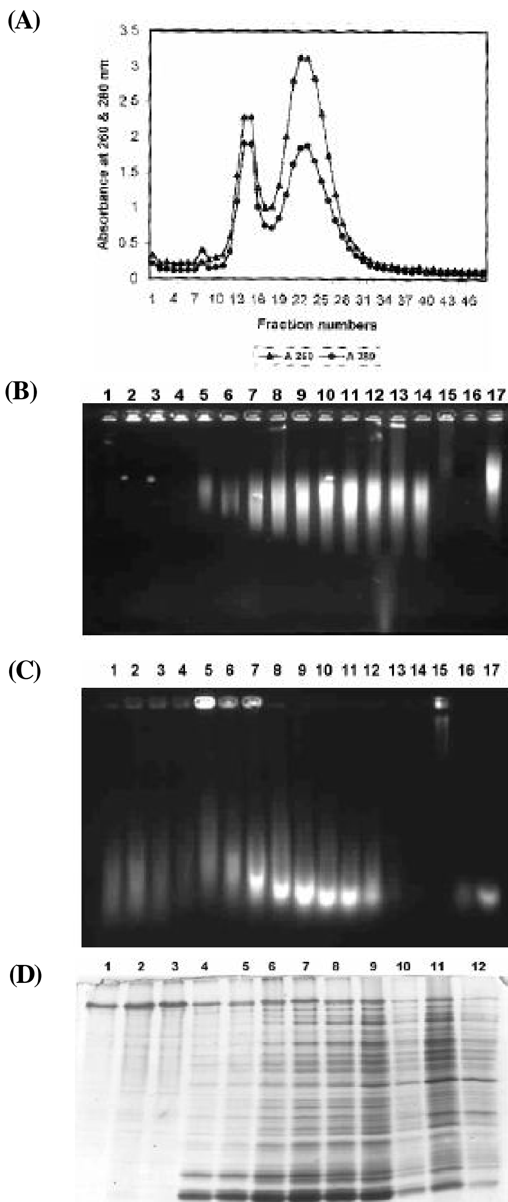

Figure1 : (A) Sephacryl S-1000 column elution profile of Sulfolobus acidocaldarius nucleoid; (B) Agarose gel electrophoretic analysis of the nucleoprotein complexes in fractions from Sephacryl S-1000 column chromatography. The gel was stained with ethidium bromide. Lane 1-7: Peak I fractions 8-14; Lane 8-16: Peak II fractions 15-23 and Lane 17: Nucleoid; (C) Electrophoresis of extracted nucleic acid component in the column fractions. nucleoprotein complexes were deproteinized prior to electrophoresis on a 1.4 % agarose gel. Lane 1-7: Peak I fractions 8-14; Lane 8-14: Peak II fractions 15-21; Lane 15: Nucleoid and Lane 16-17: E. coli tRNA; (D) 18 % SDS- PAGE followed by staining with Coomassie blue. Lane 1-3: Peak I fractions 14, 15 and 16 respectively; Lane 4-11: Peak II fractions 21 to 28 respectively. Lane 12: Nucleoid (10μl).

trifuged at 14000 rpm for 10min. The supernatant was discarded and the pellet suspended again in buffer B and the above steps were repeated. The white pellet (S-layer) was washed thoroughly with water and suspended finally in water.

DNA cellulose column chromatography of S-layer proteins

The S-layer sample(2mg) was dialyzed against nuceloid buffer and loaded on a 5ml double stranded DNA cellulose column equilibrated with the same buffer. The column was washed with 150mM and 300mM KCl containing buffers. Fractions(1ml) were collected and protein composition was analyzed by SDS-PAGE.

SDS-PAGE was performed using 18% gels[24]. DNA was estimated by diphenylamine method[25]. Protein was estimated as described[26] using bovine serum albumin as a standard. RNA was estimated by orcinol method[27].

RESULTS AND DISCUSSION

The nucleoid isolated on a 30 % sucrose cushion was mildly sonicated and chromatographed on a Sephacryl S-1000 column as described in the methods section to isolate nucleoprotein complexes. As seen in figure 1A, nucleoid was resolved into two peaks. The total amount of protein, DNA and RNA is much higher in peak II than peak I. Peak I has 350μg of protein, 68μg RNA and 16 μg DNA per ml whereas peak II has 310-μg protein, 544μg RNA and 43μg DNA.

Nucleoprotein complexes from Sephacryl S-1000 column nucleoid fractions were analyzed by agarose gel electrophoresis (Figure 1B) Peak I fractions contained high molecular weight nucleoprotein aggregates which were retained in the wells of the gels. Nucleoprotein complexes in peak II initial fractions migrated as a smear ranging in size of 1 kbp to 5 kbp and in the later fractions of 500 bp and 1 kbp bands along with high molecular weight smear of 5 kbp to 10 kbp. Nucleic acids extracted from peak I and peak II were analyzed by agarose gel electrophoresis (Figure 1C) which migrated as a smear. In the fractions of peak I the nucleic acids migrated as a smear of 1 kbp to 3 kbp along with high molecular weight nucleic acids retained in the wells. Peak II fractions migrated as a smear of

Figure 2: Isolation of S-layer protein from peak fractions of nucleoid chromatographed on Sephacryl S-1000. S-layer protein was isolated, as described in the methods section, from exponential phase cells and from peak I and peak II obtained from chromatography of nucleoid on sephacryl S-1000. The proteins were analyzed by electrophoresis on an 18 % SDS polyacrylamide gel followed by Coomassie blue staining. Lane 1: S-layer from peak I; Lane 2: S-layer from peak II; Lane 3: S-layer from S. acidocaldarius cells; Lane 4: Molecular weight markers (94 kDa, 66 kDa, 43 kDa, 29 kDa and 21 kDa).

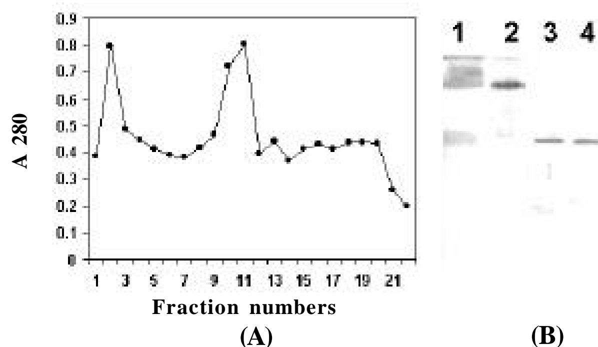

Figure 3 : (A) DNA cellulose column elution profile of S-layer proteins. Fractions 1 to 7, 0.15 M salt elution and 8 to 20 0.3 M salt elution; (B) SDS-PAGE analysis of fractions from DNA cellulose chromatography of S-layer proteins. Peak fractions (70μl) analyzed on 18% SDS polyacrylamide gels and visualized by Coomassie blue staining. Lane 1: S-layer protein from isolated from *S.acidocaldarius*, Lane 2: 150 mM wash fraction. Lane 3-4: 300 mM wash fraction

200 bp to ~2 kbp. Slower migration of nucleic acids as seen in figure 1B could be due to complex formation of nucleic acids with proteins. SDS-polyacrylamide gel electrophoresis of peak I and peak II fractions (Figure 1D) showed presence of mostly high molecular weight proteins (>60kDa) in peak I and proteins ranging from high molecular weight to low molecular weight (~100 kDa to 7 kDa) in peak II. The low molecular weight proteins, HSNP A, DBNP B(Sac 10b) and HSNP C'(Sac 7d) are present in abundance in peak II fractions.

S-1000 column fractions were deproteinized, treated with DNase I and electrophoresed to see RNA fraction in the nucleoid. RNA migrated as low molecular weight species with migration similar to tRNA (results not shown). The low molecular weight size could be due to degradation of RNA during isolation procedures. The peak I fractions showed opalescent appearance which could be due to membranous contamination. We tried to isolate S-layer protein (component of the membrane of Sulfolobus) from Peak I and Peak II pooled fractions. The Peak I contained substantial amount of 150 kDa and 60 kDa subunits of S-layer protein (figure 2) and Peak II contained very small insignificant amount which could be due contamination during pooling of the fractions. S-layer protein fraction isolated from S. acidocaldarius cells was chromatographed on a DNA cellulose column to see if any of the two proteins binds DNA. The 60 kDa S-layer protein showed strong DNA binding activity as determined by DNA cellulose chromatography (Figure 3A and figure 3B). DNA binding activity of 60 kDa s-layer protein is also interesting because earlier work has indicated that the 60 kDa subunit is facing the cytoplasmic membrane side of the cell[28]. Majority of the nucleoid DNA of heterogeneous size fragments associated with low molecular weight ac 7d and Sac 10b) eluted as Peak II. Peak II therefore represents the major component of the nucleoid and peak I membrane associated minor component

Analysis of micrococcal nuclease digested nucleoid

Total nucleoid isolated was digested with micrococcal nuclease and the digest was chromatographed on Sephacryl S-200 column. The chromatography profile of the digested nucleoid showed three distinct regions, a major peak (fractions 13 to 20), a shoulder region (fractions 21 to 29) and a small peak (fractions 30 to 37) (Figure 4A).

SDS-PAGE analysis (Figure 4B) showed that the

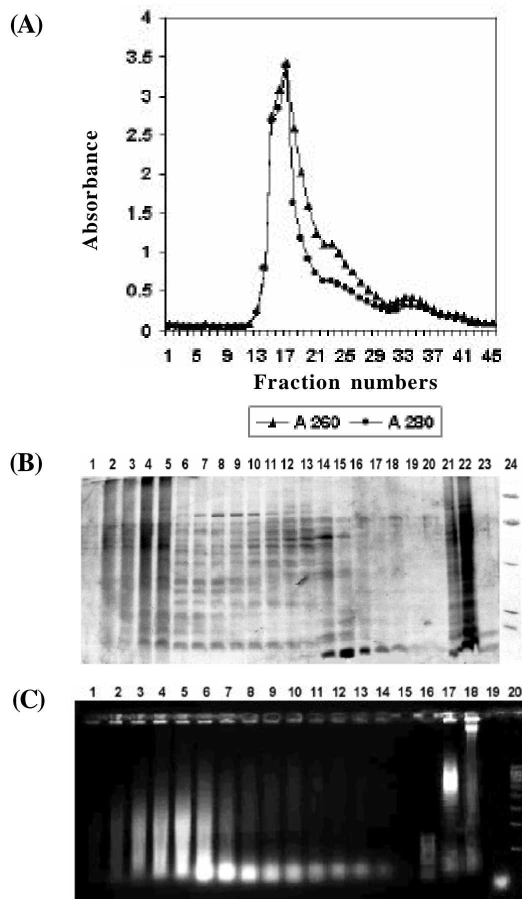

Figure 4 : (A) Sephacryl S-200 column elution profile of Sulfolobus acidocaldarius nucleoid digested with micrococcal nuclease; (B) SDS-PAGE analysis of the above column fractions. Column. fractions (200l) were electrophoresed on 18 % SDS-polyacrylamide gel. Lane 1-13: Fraction 14-26 respectively; Lane 14-20: Fraction 28,30,32,33,34,35 and 36 respectively; Lane 21: micrococcal nuclease digested nucleoid; Lane 22: Crude nucleoid; Lane 23: Nucleoid acid extract; Lane 24: Molecular weight markers (94 kDa, 66 kDa, 29 kDa, 21 kDa and 14 kDa); (C) Agarose gel analysis of the above column fractions. Fractions (500µl) were analyzed on a 0.8 % agarose gel electrophoresis Nucleic acid was stained with ethidium bromide. Lane 1-15: Fraction numbers 14-26, 28 and 30 respectively; Lane 16: Molecular weight markers; Lane 17: Nucleoid digested with micrococcal nuclease; Lane 18: Nucleoid. Lane 19: *E.coli* tRNA. Lane 20: Molecular weight markers (1kb ladder, 10 to 1kb).

major peak contains high molecular weight proteins ~ 100 to 45 kDa and a few proteins of ~30 to 20 kDa. Sac 10b was also present along with a small amount of HSNPA. The fractions in the shoulder region had Sac10b along with proteins in the molecular weight range 65 kDa to 15 kDa. The small peak contained abundant amounts of Sac 7d along with small amounts of HSNPA.

As evident from agarose gel analysis (Figure 4C) the major peak fractions of the Sephacryl S-200 column comprises nucleoprotein complexes ranging from ~500 bp to 10 kbp. Part of the complexes is also retained in the wells. Shoulder fractions contained 500bp DNA predominantly. The small peak fractions rich in HSNP C', however, show very low amounts of low molecular weight DNA (500bp).

The three distinct regions in S200 column profile showed distinctly differing composition of both nucleic acid and protein. Although Sac 7d is a major nucleoid protein it is released from nucleoprotein complexes as indicated by its elution in relatively nucleic acid free region Previous studies on the DNA binding proteins of S. acidocaldarius have shown them to form several types of complexes with DNA. HSNP C' was observed to condense large regions of DNA to form a central DNA-protein core with loops of free DNA[10,11]. Our results on the electron microscopy of complexes of HSNP-C'(Sac 7d) and HSNP-A with DNA indicated binding of these two proteins at different regions on DNA(our unpublished data). Immunogold electron microscopy has shown localization of Sac 7d and HSNPA within distinct nucleoid sub domains[10]. Therefore, the observed elution pattern of these proteins separately suggests different nucleoid proteins bind at different regions on the chromosomal DNA. The elution pattern on S-200 column also suggests that there is no evidence to indicate the occurrence of nucleosome-like repetitive DNA-protein aggregate in the Sulfolobus nucleoid unlike the chromatin in euryarchaeota.

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