ISSN : 0974 - 7427 Volume 6 Issue 1

Trade Science Inc.

BIOCHEMISTRY
An Indian Journal

Regular Paper

BCAIJ, 6(1), 2012 [29-40]

Characterization of chromatin (nucleoid) from extreme halophilic archaeon *Halobacterium salinarium H3* shows no nucleosomal organization

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Received: 2nd January, 2012 ; Accepted: 28th January, 2012

ABSTRACT

Halobacterium salinarium, a euryarchaeote, is a member of haloarchaea requiring at least 3.5 M NaCl for optimal growth. We intended to biochemically analyze the chromatin from this organism because of the reported nucleosomal structure of the chromatin. Chromatin (nucleoid) was isolated from H. *salinarium* H3. Nucleoid fractions isolated from the mid logarithmic and late logarithmic phase cells were separated into two peaks (peaks I and II) by sucrose density gradient centrifugation. Growth cycle dependent variation in the two chromatin Peaks was observed as reported earlier. DNA and protein compositions were determined for the isolated chromatin factions from both mid logarithmic and late logarithmic cells. Micrococcal nuclease digestion and agarose gel electrophoresis of the different chromatin factions showed absence of repeating units (nucleosome-like) of DNA protein complexes and DNA fragments did not resolve into DNA ladder pattern up on gel electrophoresis. Our results suggest that the chromatin of *H. salinarium* is more like bacterial and crenarchaeal chromatin and unlike that of other euryarchaeal chromatin from thermophilic methanogens which is organized like mini nucleosomes with the help of histone fold proteins (e.g. HMf).

KEYWORDS

Haloarchaea;
Nucleoid;
Chromatin;
Micrococcal nuclease;
Nucleosome.

INTRODUCTION

Eukaryotic chromatin consists of DNA-protein complexes containing nucleosomal repeating units where DNA is wound around an octamer of histones to form chromatin fiber[1]. Chromatin organization in archaea has both eukaryal and eubacterial characteristics. Some euryarchaea (thermophilic methanogens) contain histones that have primary sequences which form a histone fold that facilitate DNA wrapping into nucleosome-

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like structures. Nucleosome in *Methanobacterium Thermoautotrophicum* and *Methanobacterium fervidus* comprise an archaeal histone tetramer wound around by about 80 bp of DNA and is compacted into nucleosome-like particles by proteins of HMf family[2-4]. In comparison, Crenarchaea (mostly thermoacidophiles) do not contain histone homologues but contain abundant amounts of DNA binding proteins belonging to Sac 7 and sac10 family proteins[5-7]. In thermophilic archaea, the organization of intracellular DNA is more like bacterial nucleoid with no apparent eukaryotic chromatin like organization[8, 9]. In bacteria, several factors contribute to DNA compaction and organization which include entropic effects, supercoiling and DNA–protein interactions (involving at least ten different DNA binding proteins) and macromolecular crowding with no apparent nucleosomal organization[10-12]. The chromatin in *Halobacterium salinarium* has been reported to consist of regions of DNA associated with protein and regions of DNA free of protein. Electron microscopy of the two types of the nucleoid fractions showed more nucleosome like structure (chromatin) of protein associated DNA fraction chromatin[13]. Takayanagi *et al.*[14] further reported that sheared chromosomes obtained from the late exponential phase cells resolved into two peaks on a sucrose density gradient. The peak I consists of protein free DNA and peak II consists of rugged fibers consisting of nucleosome like structures as seen under microscope. Most of the DNA is in peak I form during exponential phase and in peak II form during late logarithmic phase. The transition between the two forms occurs during the late exponential phase.

In the present study, biochemical characterization of Halobacterium salinarium H3 chromatin (nucleoid) fractions was under taken with respect to its associated proteins and the mode of compaction, whether it is like nucleosomal type as in methanogenic archaea or non nucleosomal type as in mesophilic bacteria and. thermophilic archaea. We found growth phase dependent variation in nucleoid fractions on sucrose gradient centrifugation as reported earlier[14]. The two types of nucleoproteins differ in terms the amount of protein associated with DNA. Micrococcal nuclease digestion of nucleoid fractions followed by agarose gel electrophoresis showed that both are non-nucleosomal type as indicated by the electrophoretic pattern of the digests. Our results are contrary to what was reported before[13, 14] based on electron microscopic studies of nucleoid fractions. We suggest that the condensed chromosomal structure in Halobacteria may be referred as nucleoid as it lacks nucleosomal organization.

MATERIALS AND METHODS

DNase I, bovine serum albumin, Nonidet P-40, sucrose, agarose, Coomassie brilliant blue R-250, pancreatic RNase, polyethylene glycol-20,000, sodium deoxycholate and ethidium bromide were obtained from Sigma-Aldrich chemical company. TritonX-100 was from Fluka chemicals, Germany. Sephacryl S-200, Acrylamide and Tris hydroxyl methyl aminomethane (Tris) were obtained from Amersham Pharmacia chemical company, U.S.A. Sodium dodecylsulfate (SDS), N-N' methylene bis acrylamide were purchased from Serva chemical company, Germany. Yeast extract, casein acid hydrolysate were obtained from Hi-media chemical company Mumbai, India. Protein Molecular weight markers, Micrococcal nuclease (MNase) and Lambda DNA Hind III/Eco I digest were purchased from Bangalore Genei, India. *Halobacterium salinarium H3* strain was obtained from National Collection of Industrial Microorganism (NCIM) Pune, India.

Growth and cell harvesting

H. Salinarium was grown in a medium containing 0.75 % casein acid hydrolysate, 0.2% KCl, 2 % magnesium sulfate, 0.005 % ferrous sulfate, and 40 µg of manganese chloride and 20 % sodium chloride, pH adjusted to 7.0 with 2N NaOH. The cells were harvested by centrifugation at 6000 rpm at 4 ºC for 20 minutes. The cell pellet was washed by resuspending in solution containing 50 mM KCl and 3.14 M NaCl followed by centrifugation at 6000 rpm at 4 ºC. Cells grown up to 5 days (mid logarithmic phase) and 7 days (late logarithmic phase) were used for the isolation of nucleoid.

Isolation of nucleoid from H. salinarium by sucrose density gradient centrifugation

The procedure described by Takayanagi et al[14]

was used for the isolation of nucleoid. Freshly harvested mid logarithmic phase and late logarithmic phase cells were suspended gently in 6 ml of buffer containing 50 mM Tris-HCl pH 7.6, 1 mM Na_2-EDTA, 1 mM magnesium acetate, 7 mM 2-mercapto ethanol, 3.4 M NaCl and were allowed to lyse at 0 ºC for 30 minutes. The resulting viscous lysate (2 ml each) was gently applied on 5-20 % sucrose gradient containing the above buffer and centrifuged in Sorvall ultracentrifuge at 105,000 g / 1 hr. Fractions of 1.5 ml from the top each of the gradient tube were collected and the absorbance was measured at 260 and 280 nm.

Isolation of total Nucleoid

Nucleoid from *H. salinarium was* also isolated according to a procedure developed in our laboratory[15]. Freshly harvested cells (4 g) were suspended in 12 ml of nucleoid buffer containing 20 mM Tris-HCl pH 7.6, 0.5 M NaCl, 3.4 M KCl, 0.1 M magnesium acetate, 1 mM Na_2 -EDTA, 7 mM 2-mercaptoethanol and after the addition of 1.5 ml of lysis mixture (NP-40, 1 % Triton X-100 and 0.1 % sodium deoxycholate) the suspension was incubated for 1 hr at 4°C with constant gentle shaking. The viscous lysate was centrifuged at 1000 rpm for 20 minutes at 4°C. The supernatant was collected and loaded onto a 30 % sucrose cushion and centrifuged at 10,000 rpm for 1 hr at 4°C. The nucleoid pellet was collected and resuspended in nucleoid buffer applied again on a 20 % sucrose cushion in nucleoid buffer and centrifuged at 30,000 rpm for 1hr. The nucleoid pellet thus obtained was collected and suspended in nucleoid buffer and used for further studies.

Micrococcal nuclease (MNase) digestion of nucleoid from H. salinarium

Nucleoid fractions were digested with MNase following the procedure of Owens-Hughes and Workman[16]. Nucleoid was dialyzed against the MNase digestion buffer containing 20 mM Tris-HCl pH 7.6, 50 mM NaCl, 50 mM $MgCl_2$ and 1 mM $CaCl_2$. After dialysis, nucleoid fraction (1-2 mg/ml DNA) was incubated with MNase (0.1 unit /µg DNA) in digestion buffer for different time intervals (1min, 3 min, 5 min, 10 min, 20 min, and 30 min). Reaction was stopped by adding SDS and EDTA to a final concentration of 1% and 25 mM respectively. The reaction products were analyzed by electrophoresis on a 0.8 % agarose gel.

Isolation of MNase digested products of nucleoid by Sephacryl S-200 column:chromatography

Sephacryl S-200 was packed to a bed volume of 100 ml in a column (55 cm x 1.6 cm) and equilibrated with the buffer containing 20 mM Tris-HCl pH 7.6, 250 mM NaCl, 1 mM EDTA, 7 mM 2-mercaptoethanol. Nucleoid fractions (2 ml of peak I and peak II obtained through sucrose density gradient centrifugation of mid logarithmic, late logarithmic phase cells and total nucleoid from mid logarithmic phase and late logarithmic phase cells) were digested with MNase (0.1 U/1µg DNA) as described above and dialyzed against buffer containing 20 mM Tris-HCl pH 7.6, 250 mM NaCl, 1 mM Na_2-EDTA, 7 mM 2-mercaptoethanol were chromatographed on separate Sephacryl S-200 columns. Elution of the columns were carried out with 2 bed volumes of the buffer and 3 ml fractions were collected at the rate of 24 ml per hour. Fractions were measured for absorbance at 260 and 280 nm and analyzed by SDS-PAGE and 0.8% agarose gel electrophoresis.

Agarose gel electrophoresis

DNA and nucleic acid: protein complexes were electrophoresed on 0.8 % agarose gels. Agarose gels were formed in 1X TAE (40 mM Tris-acetate pH 7.8 and 1 mM sodium EDTA) buffer. Samples were mixed with 6X buffer containing 0.25 % bromophenol blue, 0.25 % xylene cyanol and 30 % glycerol to a final concentration of 1 X. Electrophoresis was carried out with 1X TAE as electrode buffer at 40 V till the bromophenol blue dye reached 3 quarters of the gel. The gel was stained with 0.5 µg/ml ethidium bromide for 30 minutes, destained with water for 1 hour and visualized by UV illumination.

SDS - polyacrylamide gel electrophoresis

SDS-PAGE was performed using 18 % gel as described by Thomas and Kornberg[17].

Other methods

Protein concentration was estimated by the method of Lowry *et al*[18]. DNA and RNA contents were measured with calf thymus DNA and yeast RNA as standards by the methods of Burton[19] and

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Ceriotti[20] respectively.

RESULTS AND DISCUSSION

Isolation of nucleoid fractions by sucrose density gradient centrifugation

Centrifugation of lysates obtained from mid logarithmic phase and late logarithmic phase cells on sucrose gradient resulted in resolution of nucleoid into two components, peak I and peak II (rapidly sedimenting fraction). The overall yield of the two peaks showed growth phase dependent variation. Peak I was higher than peak II in mid logarithmic phase (Figure 1A) whereas peak II was higher than peak I in late logarithmic phase (Figure 1B) i.e. there was a decrease in peak I in late logarithmic phase as compared to peak I in mid logarithmic phase as reported earlier[14]. Both nucleic acid and protein content in the two fractions were analyzed. There was reduction of both protein and nucleic acid (DNA) content in peak I obtained from late logarithmic phase cells and there was an increase in both protein and nucleic acid (DNA) contents in peak II obtained from late logarithmic phase cells.

The patterns of proteins from isolated nucleoid peak fractions were analyzed by SDS- PAGE. Protein profiles of peak I and peak II nucleoid fractions of mid logarithmic phase (5 days) cells collected from the gradient showed presence of both high and low molecular weight proteins ranging from 10 KDa to 110 KDa in both the peak fractions of nucleoid (Figure 1C & Figure 1D). The peak I fractions contain high molecular weight proteins and low amount of low molecular weight proteins, followed by fractions towards bottom corresponding to peak II which have similar protein banding pattern as seen in peak I. SDS-PAGE analysis of the nucleoid or chromosomal fractions of peak I and peak II obtained from the late logarithmic phase cells also showed both high and low molecular weight protein. Peak I from late logarithmic phase contained fewer proteins in comparison to Peak II fractions (Figure 1E & Figure 1F). Overall, there was decrease in protein in both Peak I and Peak II fractions obtained from late logarithmic phase nucleoid. A 29 kDa protein is consistently present in all fractions in substantial amount.

Agarose gel analysis of deproteinized samples of the peak I and peak II from mid logarithmic (5 days) phase cells showed presence of high molecular weight DNA bands along with low molecular weight smear of DNA in the both peak I and peak II fractions (Figure 1G & Figure H)). The results obtained indicate that peak I has relatively more high molecular weight DNA than peak II from mid logarithmic phase cells. DNA content of the gradient peak I fraction from late logarithmic phase cells showed very low concentrations of high molecular weight DNA bands whereas peak II fractions contained higher concentrations of both high and low molecular weight DNA bands (Figure 1I) & Figure 1J). We did not observe any difference in agarose gel electrophoretic pattern after treatment with RNase indicating lack of RNA in the isolated nucleoid fractions.

MNase digestion studies of nucleoid. Since Takayanagi et al.[14] have used chromatin fractions isolated from late exponential phase we have used nucleoid fractions isolated from late logarithmic phase. The Different fractions of nucleoid analyzed by MNase digestion are Sucrose gradient Peak I and peak II fractions from late logarithmic phase cells, total nucleoid isolated mid logarithmic phase cells and late logarithmic phase cells.

Isolation of MNase digested products of nucleoid by Sephacryl S-200 column chromatography

Peak I and peak II fractions obtained by sucrose density centrifugation of nucleoid from late logarithmic phase cells were individually treated with MNase. The MNase digested products were chromatographed on Sephacryl S-200 columns. Sephacryl S-200 column chromatography of *H. salinarium* nucleoid (or) chromosomal peak I fraction digested with MNase from late logarithmic phase resolved into two peaks, a smaller peak in the initial fractions and a bigger peak in the later fractions (Figure 2A). SDS-PAGE analysis (Figure 2B) showed presence of proteins in range of ~ 60 kDa to 20 kDa in the peak I fractions. Sephacryl S-200 peak II mainly comprised of abundant amount of 29 kDa protein and a few other proteins in the molecular weight range of 20 KDa and 14 KDa. Agarose gel analysis (Figure C) showed that Sephacryl S-200 peak I fraction contained much less DNA than peak II which contained DNA fragments of about 200 bp.

Sucrose gradient chromosomal peak II fraction from

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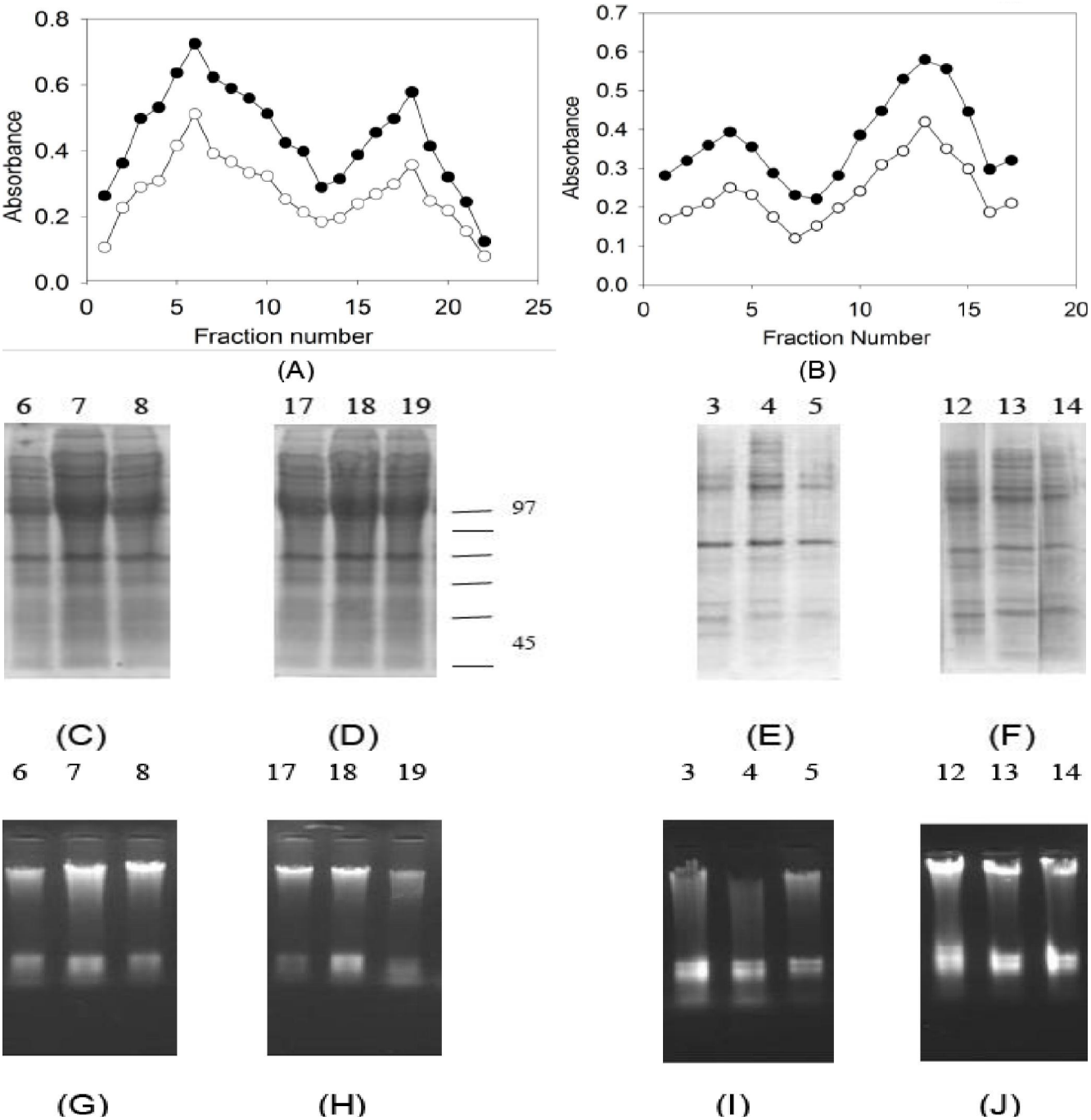

Figure 1 : (A) Sucrose density gradient (5-20%) centrifugation profile of chromatin fractions from mid logarithmic phase cells (5 days culture). (B) Sucrose density gradient (5-20%) centrifugation profile late logarithmic phase cells (7 days culture). A_{260} (-•-) and A280 (-o-). SDS-PAGE analysis of peak I and Peak II fractions obtained from sucrose density gradient centrifugation of chromatin fractions from mid logarithmic (C, D) and late logarithmic phase cells. (E, F). Fractions (100 µl in each case) were TCA precipitated and analyzed on an 18% SDS-Polyacrylamide gel followed by Coomassie blue staining. Numbers given on the top of the gels represent the peak fractions analyzed. Positions of the molecular weight markers are indicated. Agarose gel electrophoresis of DNA from of peak I and Peak II fractions obtained from sucrose density gradient centrifugation of chromatin fractions from mid logarithmic (G, H) and late logarithmic phase cells (I, J). Peak fractions (50 µl) from density gradient centrifugation were phenolized; nucleic acids were precipitated with ethanol and analyzed by 0.8% agarose gel electrophoresis. The gels were stained with ethidium bromide. Numbers given on the top of the gels represent the peak fractions analyzed.

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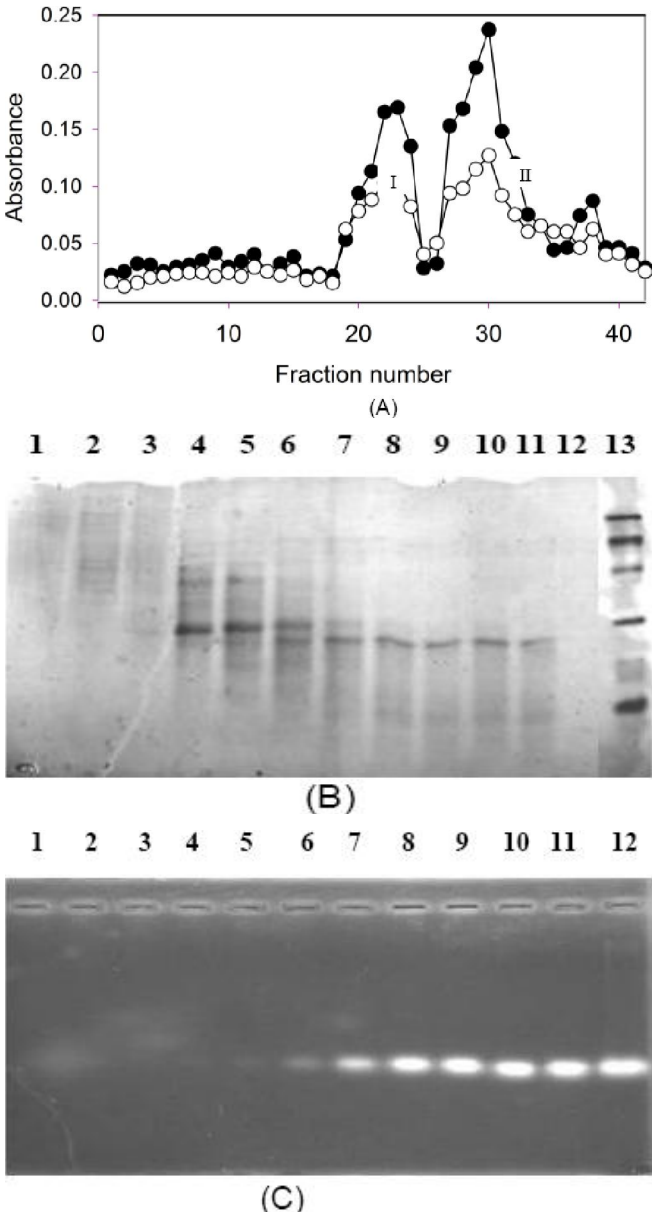

Figure 2 : (A) Sephacryl S-200 column elution profile of *H salinarium H3* gradient chromatin peak I fraction (from late logarithmic phase, Fig. 1B) digested with micrococcal nuclease. Absorbance of fractions was measured. A_{260} (-•-) and A_{280} (-o-). (B) SDS-PAGE analysis of peak I and Peak II fractions obtained from Sephacryl S-200 column. Fractions (60 µl) were analyzed on 18% polyacrylamide gels. Lanes 1 and 2, fractions 6 and 19; Lanes 3 -6, Peak I fractions 20, 21, 22 and 24 respectively; Lanes 7-12, fractions 28-33; Lane 13 molecular weight markers. (C) Agarose gel electrophoresis of DNA extracted from Peak I and peak II Fractions obtained from Sephacryl S-200 column Fractions (60 µl) were phenolized and with ethanol precipitated DNA was analyzed on 0.8% agarose gels. Lanes 1-3, fractions 4, 8 and 16 respectively; Lanes 4 - 7: peak I fractions 20, 21, 22 and 24 respectively; Lanes 8-11: Peak II fractions 29, 30, 32 and 33 respectively; Lane 12: Total DNA extracted from peak I pool fraction.

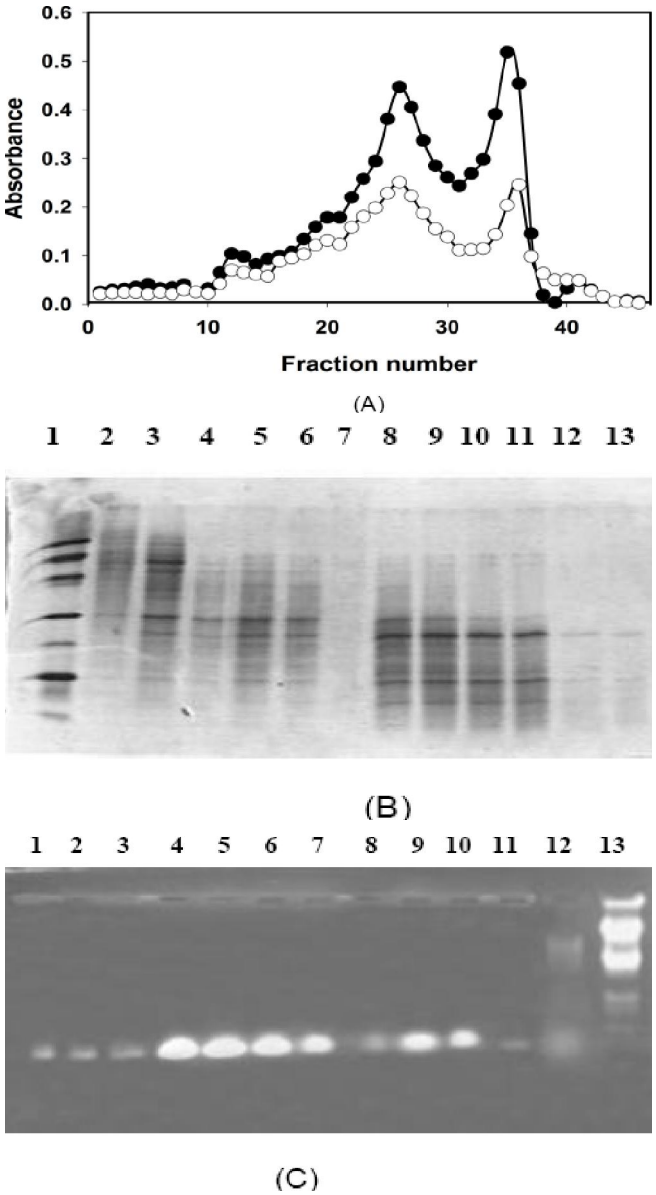

Figure 3 : (A) Sephacryl S-200 column elution profile of *H. salinarium H3* chromatin peak II fraction from sucrose gradient centrifugation of late logarithmic phase (Fig 1B) digested with MNase. Absorbance of fractions was measured, A_{260} (-•-) and A_{280} (-o-) (B) SDS-PAGE analysis of fractions obtained from Sephacryl S-200 column chromatography. Fractions (60 µl) were analyzed on 18% SDS- Polyacrylamide gels. Lane 1: molecular weight markers, Lanes 2 and 3: fractions 12, 15; Lanes 4-7: Peak I fractions 22, 24, 26 and 28 respectively; Lanes 8-13: Peak II fractions 33-38 respectively. (C) Agarose gel electrophoresis of DNA extracted from Peak I and peak II Fractions obtained from Sephacryl S-200 column Fractions (60 µl) were phenolized and ethanol precipitated DNA was analyzed on 0.8% agarose gels. Lanes 1-3: fractions 5, 10 and 15 respectively; Lanes 4-7: Fractions 22, 24, 26 and 28 respectively; Lanes 8-11, fractions 35, 36, 37 and 38 respectively; Lane 12, Total DNA extracted from Peak II pool fraction; Lane 13: Lambda DNA Hind III/Eco I digest.

late logarithmic phase cells was also digested with MNase and the digest was chromatographed on Sephacryl S-200. Chromatography resulted in the elution of two distinct peaks (Figure 3A). SDS-PAGE analysis (Figure 3B) showed similar protein pattern as in Figure (2B) in the Sephacryl S-200 peak I fractions. The fractions of Sephacryl S-200 peak II mainly comprised of abundant amount of 29 kDa protein along with a few proteins of 20 KDa and 14 KDa. Agarose gel analysis of extracted DNA (Figure 3C) in the Sephacryl S- 200 peak I and peak II fractions showed presence of DNA fragments of the size of 200 bp migrating towards the bottom of the gel.

Micrococcal nuclease sensitivity of isolated total nucleoid and analysis of the products

MNase digestion of total nucleoid from both mid logarithmic phase and late logarithmic phase cells was carried out and digestion products were analyzed by agarose gel electrophoresis. Agarose gel analysis of MNase digested products of nucleoid from 5 days cells showed smear like DNA bands with decreasing size with increasing incubation time (Figure 4A). After deproteinization of MNase digested products, DNA bands of approximately 200 bp were observed in all fractions (Figure 4B). The smear like appearance in Figure 4A could be the result of binding of the protein. MNase digested products of nucleoid from late logarithmic phase cells also showed similar results as in mid logarithmic phase cells. No ladder pattern of DNA was observed with all the chromatin fractions.

Sephacryl S -200 column chromatography of MNase digested products of total nucleoid

Sephacryl S -200 column chromatography of MNase digested products of total nucleoid isolated mid logarithmic phase also resolved into two peaks (Figure 5A). Protein and DNA were analyzed by SDS-PAGE and agarose gel electrophoresis respectively. SDS-PAGE analysis of peak I fractions obtained from Sephacryl S-200 column chromatography of total nucleoid digested with MNase showed (Figure 5B) high molecular weight proteins in the range of ~100 kDa to ~ 35 kDa and a few low molecular weight proteins. Peak II fractions showed presence of a few high mo-

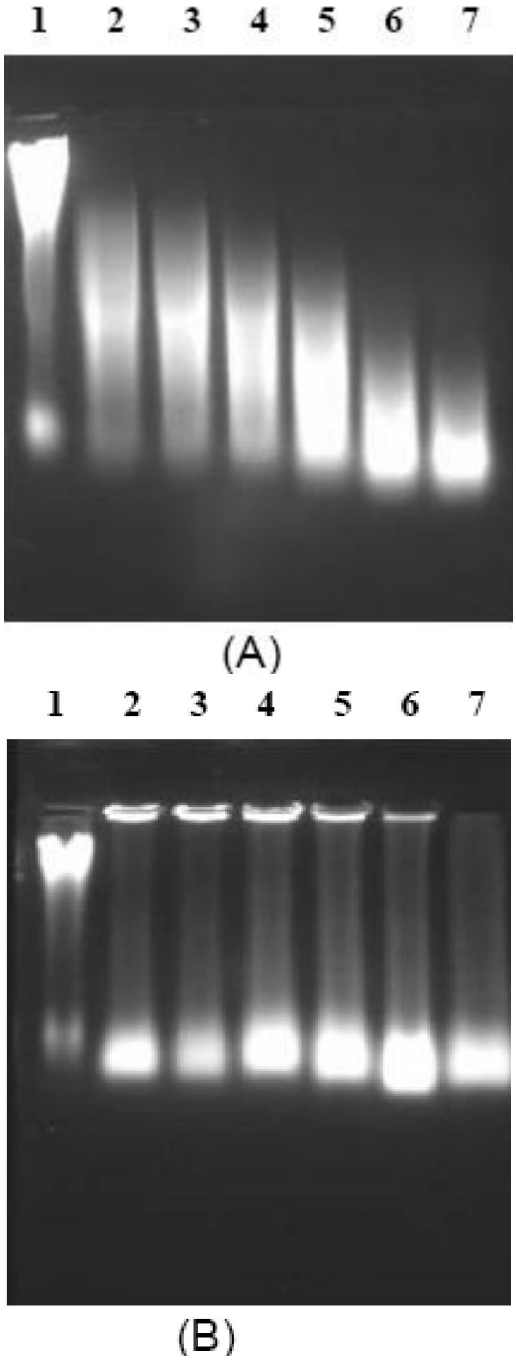

Figure 4 : (A) Agarose gel electrophoresis of MNase digest of *H. salinarium* total chromatin from mid logarithmic phase cells. Chromatin (10 µg) was digested with 1 U of MNase and nucleoprotein complexes were analyzed on 0.8% agarose gel as described. Lane 1, chromatin (control); Lanes 2-7, chromatin digested with MNase at 37 ºC for 1, 3, 5, 10, 20, 30 min respectively. (B) After digestion (as in A) the samples were deproteinized and ethanol precipitated DNA was analyzed on 0.8% agarose gel Lane 1, DNA from undigested total chromatin (control); Lanes 2-7, DNA extracted from MNase digested samples at 37 ºC for 1, 3, 5 10, 20, 30 min respectively.

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lecular weight proteins 60 kDa to 43 kDa and several low molecular weight proteins in the range of 30 kDa to 14 kDa. In all fractions of peak I and peak II, a 29 kDa protein is present predominantly. Fractions peak I and peak II were directly (i.e. DNA protein complexes) analyzed by 0.8% agarose gel electrophoresis (Figure 5C). Peak I fractions contained high molecular weight DNA protein complexes migrating as a smear from the top of the gel towards the middle of the gel (lanes 1-6) along with about 200 bp length DNA which could represent protein free DNA. However peak II fractions showed negligible amount of DNA (lanes 7-11). DNA extracted from peak I and peak II (deproteinized fractions) were also analyzed by 0.8% agarose gel electrophoresis (Figure 5D). DNA in peak I fractions (lanes 1-6) migrated as a smear of 2 to 4 kbp and ~200 bp. DNA in Peak II fractions contained very small amount of ~ 200 bp length DNA (lanes 7-10) which could not be detected in the presence of protein. Total nucleoid from late logarithmic phase cells was also subjected to MNase digestion followed by Sephacryl S-200 chromatography which resulted in resolution into two peaks as above (Figure 6A). Results of SDS-PAGE (Figure 6B), and agarose gel electrophoresis of DNA protein complexes (Figure 6C) and deproteinized DNA fractions (Figure 6D) obtained were similar to mid logarithmic phase total nucleoid except that the DNA was more susceptible to MNase digestion as indicated by presence of higher amount of 200 bp DNA fragments. This could be due to less amount of DNA bound protein. In both cases peak I fractions of Sephacryl S-200 column contained heterogeneous higher molecular weight DNA and peak II fractions has low molecular weight DNA. The low molecular weight DNA band was seen prominently only after deproteinization. Sephacryl S-200 peak I and peak II fractions showed difference in the protein profile as visualized by SDS-PAGE. These results obtained by MNase digestion of nucleoid fraction from Halobacterial cells showed that the nucleoid contained different region of DNA bound by different proteins and DNA fragments did not resolve into a ladder pattern which would be expected if DNA is organized into nucleosomal structure.

The present investigation deals with the studies on nucleoid from Halophilic euryarchaeote, *Halobacterium salinarium*. Intracellular DNA in all cell types is in condensed state with the help of several proteins. However, the type of condensation is different in different type of cells. In eukaryotic cells, the DNA is organized into chromatin with repeating nucleosomal structure. This feature is invariably present in all the eukaryotic cells studied. Intracellular DNA in prokaryotes is organized into ill defined rapidly sedimenting condensed structure called nucleoid. Several studies on *E. coli* and other bacteria showed lack of nucleosomal structure in bacterial nucleoid, but contain different domains condensed by variety of different DNA binding proteins[11, 21] and the factors contributing to DNA compaction and organization include entropic effects, supercoiling and DNA–protein interactions and macromolecular crowding[10, 12, 22, 23]. However, no defined organization can be described for bacterial nucleoid and no distinct role of different proteins in DNA condensation is reported. In case of archaea it has been reported that euryarchaeote *Methanococcus fervidus* contained proteins named HMf A and HMf B with histone domain and interact with DNA to form nucleosomal structure[3, 4]. In Crenarchaeotes like thermophilic *Sulfolobus*, no histone homologues have been found in spite of the presence of small DNA binding protein which compact DNA which were however referred as histone-like because of the basic nature of the proteins[5, 6]. The organization of intracellular DNA in thermophilic crenarchaea is nucleoid- like as in bacteria[8, 9]. Biochemical characterization of nucleoid (chromatin) from this Halophilic archaeon was carried because of the reported chromatin like structure of part of the nucleoid from this organism as studied by electron microscopy[13, 14]. Shioda et al.[13] in fact prepared DNA protein complexes directly from cell colonies isolated from agar plates on a loop for studying by electron microscopy. In the case of Peak II fractions the DNA protein complexes were highly concentrated (10 fold) and then treated with very high concentration (5%) of formaldehyde[14]. In both these cases, formaldehyde treatment could have led to non specific protein-protein and protein DNA aggregates which could have given rugged fiber images. There are no reports on the biochemical characterization of the nucleoid from halophilic archaea[24]. Hence, we investigated whether Halobacterium chromatin is of nucleosomal type seen in *Methanococcus fervidus* or the organization is more like nucleoid of bacteria and

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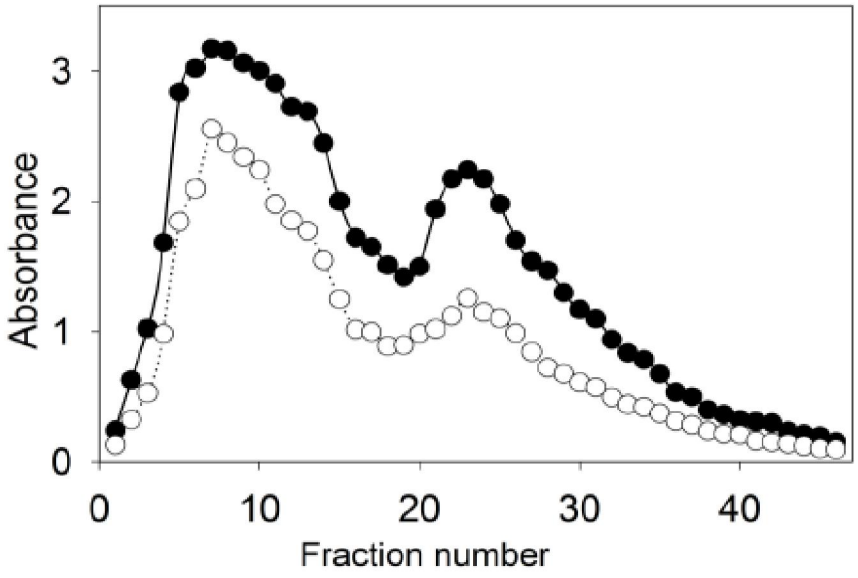

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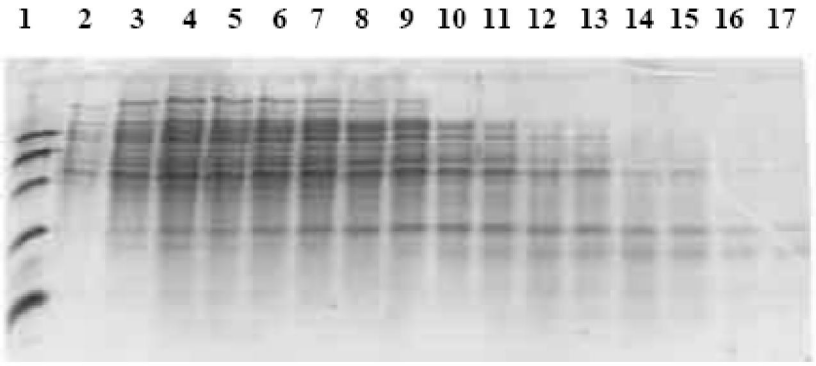

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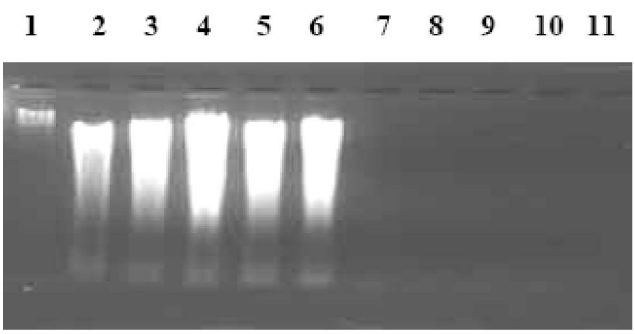

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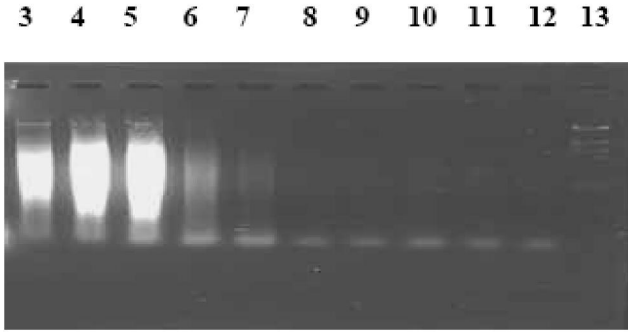

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Figure 5 : (A) Sephacryl S-200 column elution profile of MNase digested *H. salinarium* total chromatin isolated from mid logarithmic phase cells. A_{260} (- •-) and A_{280} (-o-). (B) SDS-PAGE analysis of fractions obtained from Sephacryl S-200 column chromatography. Fractions (60µl) were analyzed on an 18% SDS-polyacrylamide gels. Lane 1, protein molecular weight markers; Lanes 2-9, fractions 7-14 respectively; Lanes 10-17, fractions 21-28 respectively. (C) Agarose gel electrophoresis of Peak I and peak II Fractions (50 µl) directly loaded (DNA-protein complexes) Lanes 1-6: fractions 7 – 12 respectively; Lanes 7-11: fractions 21 -25 respectively. (D) Agarose gel electrophoresis of Peak I and peak II Fractions (50 µl) were deproteinized and loaded on 0.8% agarose gel. Lanes 1 – 6, fractions 7 – 12 respectively; Lanes 7-10, fractions 21-24 respectively; Lane 13, Lambda Hind III/Eco RI digest.

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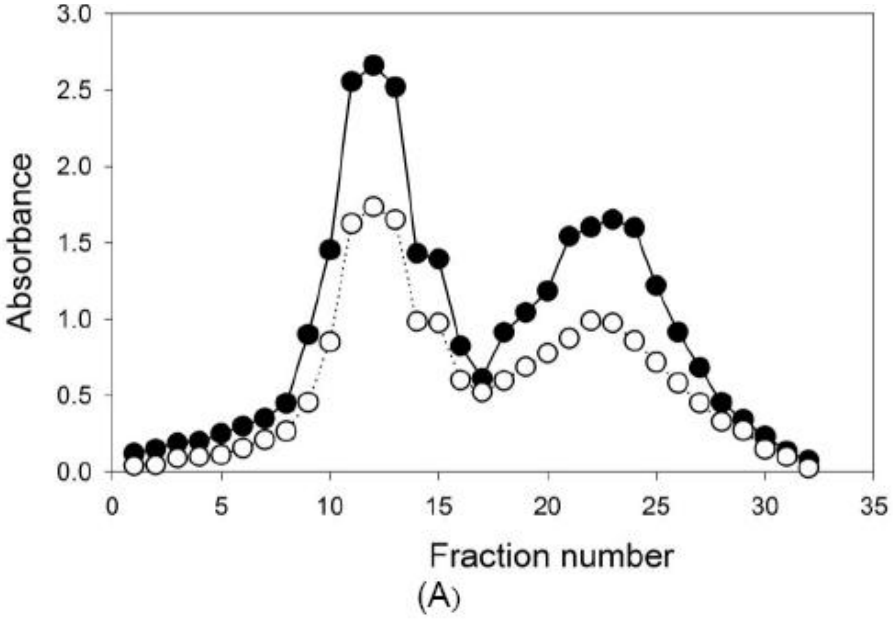

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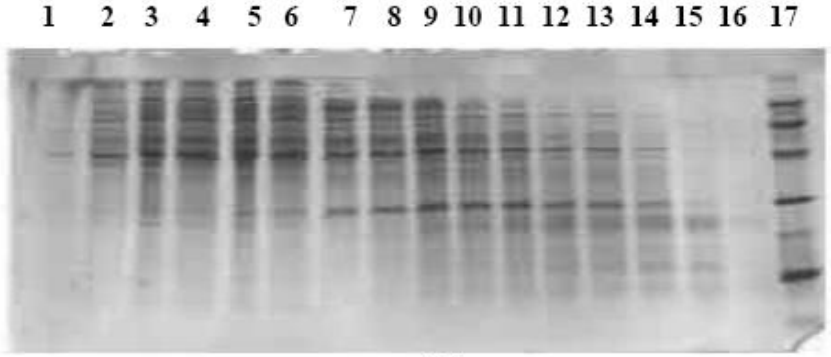

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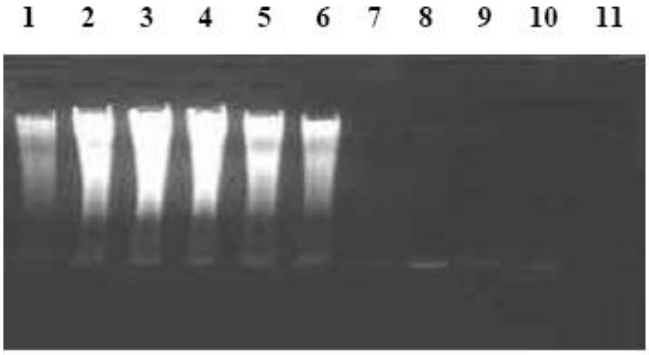

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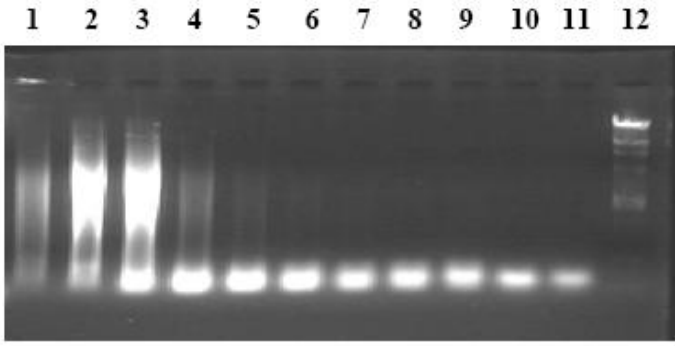

(D)

Figure 6 : (A) Sephacryl S-200 column elution profile of MNase digested *H. salinarium* total chromatin isolated from late logarithmic phase cells. A_{260} (- •-) and A_{280} (-o-). (B) SDS-PAGE analysis of fractions obtained from Sephacryl S-200 column chromatography. Fractions (60µl) were analyzed on an 18% Polyacrylamide gel followed by Coomassie blue staining. Lanes 1-8, Fraction number 8-15 respectively; Lanes 9 -16, fraction number 19 – 26 respectively; Lane 17: Protein molecular weight markers. (C) Agarose gel electrophoresis of Peak I and peak II Fractions (50 µl) directly loaded (DNA-protein complexes) Lanes 1-6: fractions 7-12 respectively; Lanes 7-11: fractions 21 -25 respectively. (D) Agarose gel electrophoresis of Peak I and peak II Fractions (50 µl) were deproteinized and loaded on 0.8% agarose gel. Lanes 1-6, fractions 10-15 respectively; Lane 7-11, fractions 20 – 24 respectively; Lane 12: Lambda DNA Hind III digest.

other thermophilic archaea. Nucleoid (Chromatin) was isolated from *H. salinarium* by the method described by Takayanagi et al[14] as well as the method developed in our laboratory for the isolation of thermophilic archaeal nucleoid[15]. These nucleoid preparations were analyzed for the protein and nucleic acid composition and by sensitivity to Micrococcal nuclease. Sucrose gradient centrifugation of lysates resolved into two nucleoid components as described before[14]. The nucleoid components showed growth phase dependent variation in nucleic acid and protein composition. Peak I fraction was more with more amount of protein and nucleic acid than peak II (rapidly sedimenting) from mid logarithmic phase cells on the contrary peak II fraction from late logarithmic phase cells was more than peak I fraction with higher amounts of the protein and DNA. Although low amounts of both protein and nucleic acids were seen in peak I fraction from late logarithmic phase cells the DNA (mostly fragmented) is not free of protein as reported by Takayanagi et al[14]. Both these peak fractions were subjected to agarose gel electrophoresis for nucleic acid analysis and SDS-PAGE for protein composition. Nucleoid from late logarithmic phase cells showed presence of some additional proteins in the lower molecular weight region (14 to 16 kDa). Nucleoid fractions obtained from the sucrose density gradient centrifugation (Peak I and Peak II) and sucrose cushion centrifugation (total nucleoid fraction) were subjected to MNase digestion. Sephacryl S-200 gel filtration chromatography of MNase digests in each case resolved into two peaks. Protein and nucleic acid analysis by SDS-PAGE and agarose gel electrophoresis showed that there was differential distribution of DNA fragments and proteins in the MNase digested products obtained from different nucleoid fractions. MNase digestion of different nucleoid fractions and analysis of DNA fragments by agarose gel electrophoresis did not show DNA ladder pattern which is characteristic of chromatin organized in nucleosomal structure. Differential distribution of DNA and proteins in the different MNase digestion products indicate that different proteins are bound to different regions of DNA in the nucleoid as indicated by elution of the MNase digestion products after Sephacryl S-200 gel chromatography. In eukaryotic cells MNase digestion of chromatin results in production of different sized DNA fragments all of which are associated with histone proteins in constant amount. Our results also suggest lack of histone like proteins. *H. salinarium* nucleoid did not contain any acid extractable low molecular weight basic proteins (results not shown).

ACKNOWLEDGEMENTS

This work was supported by University of Hyderabad, DST-FIST and UGC-SAP programs.

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