Archaebacterial Histone-like Proteins

PURIFICATION AND CHARACTERIZATION OF HELIX STABILIZING DNA BINDING PROTEINS FROM THE ACIDOThERMOPHILE SULFOLOBUS ACIDOCALDARIUS*

(Received for publication, April 3, 1989)

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Four DNA binding histone-like proteins have been purified from the nucleoid of the acidothermophilic archaebacterium Sulfolobus acidocaldarius to homogeneity employing DNA-cellulose chromatography and carboxymethylcellulose chromatography. The molecular weights of these proteins are in the range 8,000–12,500. Immunoblotting results suggest that a few antigenic determinants are common among these proteins which could not be detected by immunodiffusion. Spectroscopic properties of the proteins have been studied. The amino acid compositions of these proteins show both similarities and differences with histones and prokaryotic histone-like proteins. All of the four proteins bind native and denatured DNAs and single stranded DNA since low intracellular salt concentration, high temperature of growth, and a low G+C content of DNA would be associated with intracellular DNA. Hence, we wanted to isolate the archaebacterial nucleoid (chromatin) to study its isolation. In a separate study, several types of DNA binding proteins have been isolated with DNA has been studied by electron microscopy (Lurz et al., 1986). Two major "chromosomal" proteins have been isolated and characterized from (sheared DNA-protein complexes of Sulfolobus acidocaldarius by Green et al. (1983) in their search for isolating proteins that might stabilize DNA against thermal denaturation. However, the proteins (M, 36,000 and 14,500) could not afford protection to DNA against thermal denaturation. In all of the above cases, the DNA binding proteins have been isolated from cell extracts of these organisms and their association with nucleoid has not been documented.

S. acidocaldarius lacks a rigid cell wall (Brock et al., 1972) and probably is in osmotic equilibrium with its environment. Therefore, the intracellular salt concentrations are expected to be low (Green et al., 1983). It is speculated that compensatory factors may be present to stabilize the intracellular DNA since low intracellular salt concentration, high temperature of growth, and a low G+C content of DNA would destabilize the helical structure of DNA. Such factors should be associated with intracellular DNA. Hence, we wanted to isolate the archaebacterial nucleoid (chromatin) to study its structure and protein composition and to look for factors responsible for the thermal stability of DNA. Recently, we have shown that the nucleoid of this organism contains four Acid-soluble, histone-like DNA binding proteins. Three of these proteins strongly protect DNA against thermal denaturation (Reddy and Suryanarayana, 1988). The purification and physico-chemical and nucleic acid binding properties of these proteins are reported here.

EXPERIMENTAL PROCEDURES

Bacterial Strains—The strains used in this study were S. acidocaldarius DSM 639 obtained from Deutsche Sammlung von Mikroorganismen, Gottingen, West Germany and E. coli A19 from the laboratory of Dr. A. R. Subramanian, Max-Planck-Institut for Molecular Genetics, Berlin, West Germany.

* This work was supported by the University Grants Commission, New Delhi, India. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1714 solely to indicate this fact.
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**Bacterial Growth**—S. acidocaldarius was grown at 75 °C for 40–48 h with vigorous aeration in a medium containing 0.1% yeast extract, 0.1% bactotryptone, 0.05% casamino acids, 0.1% glucose, 0.02% NaCl, 0.13% (NH₄)₂SO₄, 0.03% KH₂PO₄, 0.025% MgSO₄, 0.07% CaCl₂, and 4 ml of 1 M H₂SO₄ to adjust the pH to 3.0 (Kikuchi and Asai, 1984). Growth was followed by measurement of absorbance at 650 nm. Bacterial cultures were harvested after neutralizing the culture with 1 M Tris base (4–5 ml/liter). Yeild of the cells was about 15 g/liter culture. The cell pellets were stored frozen at −80 °C.

E. coli was grown in enriched medium at 37 °C with aeration (Minks et al., 1978). The midlogarithmic phase cultures were chilied, harvested by centrifugation, and stored frozen at −80 °C.

The abbreviations used are: SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; CM-cellulose, carboxymethylcellulose; DBNP-B, DNA binding nucleoid protein-B; HSNP, helix-stabilizing nucleoid proteins.

Thomas and Kornberg (1975). The proteins were electrophoretically transferred to nitrocellulose membranes (0.45 μm) using the electrode buffer (25 mM Tris, 192 mM glycine, 20% (v/v) methanol, pH 8.3). The electrophoets were soaked in immunoblotting incubation buffer, IBIB (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% bovine serum albumin, 0.02% sodium azide), for 5 h and subsequently for 0.5 h. The membranes were dried and counted for radioactivity in a Beckman LS-1800 liquid scintillation counter.

The electroblots were soaked in immunoblotting incubation buffer, IBIB (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% bovine serum albumin, 0.02% sodium azide), for 5 h and subsequently for 0.5 h. The membranes were dried and counted for radioactivity in a Beckman LS-1800 liquid scintillation counter.

Single radial immunodiffusion was performed in 1.5% agarose gels containing 5% antisera to each nucleoid protein in barbital buffer, pH 8.3, to quantitate the amount of these proteins in isolated cell fractions.

**Determination of the Molecular Weight of the Proteins**—The native molecular weight of the proteins were determined by electrophoresis in the presence of SDS (Thomas and Kornberg, 1975). Native molecular weights of the proteins were determined by gel filtration of purified protein (100 μg each) through a Sephacyr S-200 column (5 × 60 cm), and as standards for the estimation of total nucleoid protein and purified nucleoid proteins, respectively.

**Absorption and Fluorescence Spectra**—The absorption and fluorescence spectra of the purified proteins were recorded at 25°C in a Hitachi spectrophotometer and spectrofluorimeter, respectively. Protein solutions were in 10 mM Tris-HCl, pH 7.4, and 50 mM NaCl.

**Circular Dichroic Spectra**—CD spectra of the nucleoid proteins (in 10 mM Tris-HCl, pH 7.4, 20 mM NaCl) were recorded in a Jasco 20 scanning spectropolarimeter. The instrument was calibrated using an aqueous solution of d-10-chamnosulfonic acid. The spectropolarimeter was continuously purged with dry nitrogen before and during the experiment. A cell of 1 mm path length was used. All CD spectral data were plotted as molar ellipticity (θ) using the formula:

\[
[\theta] = \left(\frac{\theta_{obs}}{c \times 100 \times MRW}\right)
\]

where \(\theta_{obs}\) is ellipticity measured in degrees, c is concentration of the protein sample in grams/ml, 1 = path length in centimeters, MRW = mean residue molecular weight. The units of [θ] are degrees cm²/mole.

**Fourth Derivative Absorbance Spectra**—Fourth derivative spectra of the nucleoid proteins (in 10 mM Tris-HCl, pH 7.4, 20 mM NaCl) were recorded in a Hitachi spectrophotometer (model 557) at 25°C.

**Concentration of Purified Proteins**—Purified proteins were dialyzed against 20 mM Tris-HCl, pH 7.4, 50 mM KCl, 1 mM Na₂EDTA, and 6 mM 2-mercaptoethanol and passed through a small CM-cellulose column (1 ml) which was equilibrated with the same buffer. The protein was eluted with the buffer containing 0.5 M KCl. Protein containing fractions in a volume of about 2–3 ml were pooled and dialyzed against 10 mM Tris-HCl, pH 7.4, and 50 mM NaCl.

**Binding of the Proteins to [H]DNA**—E. coli cells were labeled with [H]methylythymine as described by Mahler (1967), and DNA was isolated according to Marmur (1961). The specific activity of DNA was 8530 cpm/μg. Binding of proteins to [H]DNA was carried out as described by Labonne and Dumas (1983) with some modifications. Binding mixtures (100 μl) contained 20 mM Tris-HCl, pH 7.4, 150 mM KCl, 3 μg of native or denatured [H]DNA, 20 μg of ovalbumin, and 50 μl of CM-cellulose column fractions. After incubation at 30°C for 10 min the assay mixtures were diluted with 1 ml of buffer solution (same salt composition as the assay mixture) and filtered through nitrocellulose filters (previously boiled for 10 min in 20 mM Tris-HCl, pH 7.4, and 6 mM 2-mercaptoethanol). The filters were washed with additional 1 ml of buffer, dried, and counted for radioactivity in a Beckman LS-1800 liquid scintillation counter.

**Binding of the Purified Proteins to DNA-Cellulose**—Prewollen DNA-cellulose (0.2 ml) was packed and equilibrated with 20 mM Tris-HCl, pH 7.4, 50 mM KCl, 1 mM Na₂EDTA, and 6 mM 2-mercaptoethanol. Purified protein (10 μg each) was loaded on the column and eluted with increasing salt concentrations. The column fractions were spotted on Whatman 3MM filter paper strips (presoaked in 7.5% acetic acid),
stained with Coomassie Blue (0.1%) in 50% methanol, 7.5% acetic acid, and destained with a solution containing 5% methanol and 7.5% acetic acid.

**Binding of Proteins to Poly(U)**—A small column of poly(U)-Seph- 
arose (0.2 ml) was packed and equilibrated with 20 mM Tris-HCl, pH 7.4, and 100 mM KCl. 10 µg of each protein was loaded onto the column and the column was washed with increasing salt concentra-
tions to elute the bound protein. Elution of the protein was monitored as described above under binding to the DNA-cellulose column.

**Thermal Denaturation of DNA**—DNA from S. acidocaldarius and E. coli was isolated according to Marmur (1961). Thermal denaturation profiles of DNA in the absence and presence of the purified nucleoid proteins were obtained by heating different DNAs in 300 µl of 10 mM Tris-HCl, pH 7.4, and 25–50 mM NaCl or 10 mM Tris-HCl, pH 7.4, and 1 mM Na2EDTA. The rate of heating was 1 °C/min. Increase in absorbance at 260 nm was measured continuously in a Gifford spectrophotometer (model 250) equipped with thermopro-
grammer (model 2527). The melting curves were simultaneously recorded. Before heating had started, protein was added to DNA, gently mixed, and incubated for 5 min at the starting temperature. Separately, buffer blank and protein solutions were also heated to see any temperature-dependent variation in absorbance. This was found to be negligible.

**Binding of the Proteins to DNA as Measured by Intrinsic Fluores-
ce Quenching**—Binding of DNA to the nucleoid proteins was measured by this assay in a reaction mixture of 1 ml containing 10 
mM Tris-HCl, pH 7.4, 25–50 mM NaCl and 20–40 µg of each protein and increasing amounts of either native or denatured DNA (190 µg/ml) added in 5-µl portions. The decrease in the fluorescence intensity at emission maximum was measured at 25 °C in a Hitachi spectro-
fluorimeter. The data were corrected for dilution (about 5% of initial volume with the last addition). Parallel titrations of a solution of N-
acytetyltryptophanamide at the same absorbance were conducted to 
rule out artifacts due to inner filter effects.

**RESULTS**

**Isolation of Nucleoid**—Gentle lysis of S. acidocaldarius using 
nionic detergents and centrifuging the lysate through su-
crose gradients separates the rapidly sedimenting DNA-pro-
tein complex, the nucleoid (Fig. 1). Relative amounts of DNA, 
RNA, and protein in the nucleoid were estimated and were 
found to be in the ratio of 1:0.2:7. Fractions from the nucleoid 
gradient were also analyzed by SDS-PAGE (Fig. 1B). 
Fractions corresponding to the nucleoid peak contain several 
proteins in the molecular weight range from 8,000 to 150,000; 
out of these, low molecular weight proteins are present in 
abundant amounts.

**Presence of Histone-like Proteins in the Nucleoid**—Histones 
were isolated from eukaryotic chromatin by dilute acid treat-
ment. We also treated the nucleoid with dilute acid which 
resulted in the precipitation of most of the proteins. The 
acid extract obtained contained four low molecular weight poly-
peptides as analyzed by SDS-PAGE (Fig. 2). The four proteins 
have been named HSNP-A, DBNP-B, HSNP-C, and HSNP-
C' based on their DNA binding properties (HSNP, helix 
stabilizing nucleoid protein; DBNP, DNA binding nucleoid 
protein). The amount of these acid-soluble proteins in the 
nucleoid was estimated to be 90% of the weight of DNA. 
Because these low molecular weight proteins are acid-soluble, 
basic, and are associated with the archaeabacterial chromatin, 
it was thought that these proteins may correspond to histone-
like proteins in this archaeabacterium. Therefore, we developed 
purification procedures for these proteins for their character-
ization.

**Purification of Acid-soluble Proteins of the Nucleoid**—Be-
cause the acid-soluble proteins are associated with the nu-
cleoid and are presumably with intracellular DNA, we used 
DNA-cellulose chromatography for the isolation of the four 
proteins.

**DNA-Cellulose Chromatography**—Concentrated nucleoid 
fraction (2 ml) obtained from 1 g of S. acidocaldarius was 
dialyzed against 0.2 M H2SO4 as described under "Experimen-
tal Procedures." The clear acid extract obtained after centrif-
gulation was dialyzed against DNA-cellulose column buffer 
and passed through a 1-ml column equilibrated with the above 
buffer. The column was washed successively with buffers of 
increasing KCl concentrations (0.15, 0.3, 0.6, and 2 M). Fractions 
(1 ml) were collected and analyzed for protein. No 
protein was eluted with 0.15 and 2 M KCl buffer. The peak 
fractoins of 0.3 and 0.6 M KCl eluates were analyzed by SDS-
PAGE (Fig. 2). The proteins denoted by HSNP-A and HSNP-
C' were eluted together in 0.3 M salt buffer, and DBNP-B 
and HSNP-C were eluted with 0.6 M salt buffer. Both these 

![Fig. 1.Sucrose density gradient profile of nucleoid from S. acidocaldarius lysate (A): SDS-PAGE of gradient fractions (B)]. A, the lysate was layered onto a sucrose gradient and centrifuged as described in the text. Fractions of 2 ml were collected by aspiration. The fractions were analyzed for UV absorption (●) by diluting aliquots (0.1 ml) with 10 mM Tris-HCl pH 7.4, and 3 mM magnesium acetate, and the DNA content (○) in the fractions was determined using 15 µl of the fractions for ethidium bromide fluorescence assay. 10 fluorescence units correspond to 0.1 µg of DNA. Fractions 1–31 are from top to bottom. B, samples (50 µl) of fractions were treated with 1% SDS and 1% 2-mercaptoethanol at 65 °C for 10 min and electrophoresed on 18% slab gels. Lane 1, cell lysate; lanes 2–15 correspond to fractions 1, 3, 7, 9, 13, 17, 19, 21, 23, 24, 26, 28, 30, and 31, respectively; lane 16, molecular weight standard proteins (from top to bottom bovine serum albumin (M, 65,000), ovalbumin (M, 45,000), myoglobin (M, 17,000), and cytochrome c (M, 12,000).
fractions were further chromatographed on CM-cellulose columns.

**CM-Cellulose Chromatography**—Pooled 0.3 and 0.6 M eluates obtained from the DNA-cellulose column were separately chromatographed on two CM-cellulose columns. The 0.3 M KCl eluate was diluted to bring the salt concentration to 0.1 M and passed through a CM-cellulose column (1 ml bed volume) equilibrated with 20 mM Tris-Cl, pH 7.4, 100 mM KCl, 1 mM Na₂EDTA, and 6 mM 2-mercaptoethanol. The column was washed in a stepwise manner with equilibrating buffer, 0.25 M KCl buffer, and 0.4 M KCl buffer. Peak fractions from each salt elution were analyzed by SDS-PAGE. No protein was eluted with the equilibrating buffer. The proteins HSNP-A and HSNP-C' were eluted with 0.25 M KCl buffer, and 0.4 M KCl buffer. Peak fractions from each salt elution were analyzed by SDS-PAGE. No protein was eluted with the equilibrating buffer. The proteins HSNP-A and HSNP-C' were eluted with 0.25 M salt, HSNP-A eluting in the earlier fractions and HSNP-C' eluting in the later fractions (Fig. 3A). Fractions containing each protein were pooled separately and concentrated. The 0.6 M eluate from the DNA-cellulose chromatography was also chromatographed on CM-cellulose exactly as described above for the 0.3 M eluate. The protein HSNP-C was eluted in 0.25 M KCl buffer whereas DBNP-B was eluted in the 0.4 M salt buffer (Fig. 3B). The pooled peak fraction of each protein was concentrated. HSNP-A and HSNP-C' were rechromatographed separately on CM-cellulose to remove traces of cross-contamination.

**Partition of the Proteins from Post-ribosomal Supernatant**—In order to purify reasonably large amounts of the proteins and to avoid repeated nucleoid isolation for purification, we also developed a purification method for these proteins from acid-treated post-ribosomal supernatant (S-100). Treatment of S-100 (obtained from DNase-treated cell extracts) with dilute acid and chromatography of the acid extract on DNA-cellulose was performed in the same manner as described above for the chromato-}

graphy of the acid extract of the nucleoid. The elution patterns obtained for the four proteins were also like those obtained before. The elution pattern obtained after chromatography of the acid extract of S-100 on DNA-cellulose is given in Fig. 4 which is quite similar to that obtained earlier with acid extract of the nucleoid (Fig. 2).

**Single Step Separation of the Proteins by CM-Cellulose Chromatography**—In order to purify the proteins from large amounts of S-100 another purification procedure was developed which is routinely used for the isolation of these proteins. In this, the chromatographic steps were reversed, i.e. first CM-cellulose followed by DNA-cellulose chromatography. Acid extract of S-100 (20 ml) was passed through a CM-cellulose column of 20 ml bed volume (1.2 x 30 cm) equilibrated with 0.05 M KCl containing 20 mM Tris-Cl, pH 7.4, 1 mM Na₂EDTA, and 6 mM 2-mercaptoethanol, and the column was washed successively with 0.05, 0.1, 0.2, and 0.4 M KCl buffers. Fractions (3 ml) were collected and analyzed for protein absorption at 280 nm and DNA binding by filtration (Fig. 5). The profile showed four protein peaks (peaks I–IV) out of which three showed binding to DNA. Electrophoretic analysis revealed the presence of pure HSNP-A in the I peak, HSNP-C' in the II peak, a mixture of HSNP-C and -C' in
the III peak, and DBNP-B in the IV peak (Fig. 5B). Three proteins are already obtained in pure form by this chromatography. The yield of the proteins by different methods was estimated and is given in Table I.

### Physical Properties of the Proteins

The molecular weights of the proteins as determined by SDS-PAGE were 12,000, 11,500, 10,500, and 8,000 for HSNP-A, DBNP-B, HSNP-C, and HSNP-C', respectively. Cross-linking experiments with dimethyl suberimidate and gel filtration on Sephacryl S-200 indicated that the proteins HSNP-A, DBNP-B, and HSNP-C' exist as dimers in solution, whereas HSNP-C exists as a monomer (results not shown).

### UV absorbance

Proteins HSNP-A, DBNP-B, HSNP-C, and HSNP-C' showed absorption at 280 nm with a lower absorbance at 250 nm. The spectrophotometry of HSNP-A showed contributions of all the three aromatic amino acids to absorption. In the case of DBNP-B the minimum around 290 nm which is characteristic of tryptophan was missing, indicating that tryptophan is missing in this protein. The spectra of HSNP-C and HSNP-C' showed contribution of all three amino acids to different extents (Fig. 7).

### Circular Dichroic Spectra

The CD spectra of the DNA binding proteins from *S. acidocaldarius* were recorded as described under “Experimental Procedures.” HSNP-A, DBNP-B, and HSNP-C' have CD spectra suggestive of moderate amounts of α-helix (Fig. 8). HSNP-A showed the two characteristic minima at 208 and 222 nm for a protein with a helical structure and the spectrum resembles that of protein NS from *E. coli* (Dijk and Reinhardt, 1986). Calculations showed that the protein DBNP-B has highest content of ordered structure. The CD spectrum of HSNP-C' after calculation according to Chen et al. (1974) gave a value of 25% α-helix, 22% β-sheet structure for this protein (Table III). Protein HSNP-C has a CD spectrum with a rather unusual shape and a very low signal intensity and it shows some resemblance to fd gene 5 protein (Day, 1973).

### Amino Acid Compositions of the Proteins

The amino acid compositions of all four proteins have been determined and are given in Table IV. The compositions show a preponderance of basic amino acids. The mole % of basic amino acids is in the range 20-30%. Methionine is absent from all the proteins. Proline is either absent (HSNP-A and HSNP-C') or present only in trace amounts (DBNP-B and HSNP-C), unlike eukaryotic histones which have a relatively higher content of this amino acid. All the proteins contain phenylalanine and tyrosine. Fluorescence emission spectra indicate the presence of tryptophan in HSNP-A, HSNP-C, and HSNP-C'. DBNP-B, however, does not contain tryptophan. The proteins are relatively rich in dicarboxylic amino acids, glycine, valine, and leucine. The amino acid composition of these proteins shows both similarities and differences with eukaryotic histones and prokaryotic histone-like proteins.

### Binding of the Proteins to Nucleic Acids

The fact that the proteins are associated with nucleoid and could be purified by DNA-cellulose chromatography suggests that they have affinity to DNA. The ability of the purified proteins to bind native DNA and the strength of the binding was measured by passing 10 μg of each protein through a small DNA-cellulose column and eluting with buffers of increasing salt concentrations. Purified proteins bound to DNA-cellulose in a manner similar to that observed during their purification. The proteins also

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**Fig. 4. SDS-PAGE analysis of fractions obtained after DNA-cellulose chromatography of S-100 acid extract.** Acid extract of S-100 was passed through a DNA-cellulose column as described in the text. 50-μl peak fractions were treated with sample buffer and electrophoresed as in legend to Fig. 1. Lanes 1 and 16, molecular weight standard proteins (top to bottom, bovine serum albumin, ovalbumin, chymotrypsinogen, myoglobin, and cytochrome c); lanes 2, S-100 acid extract; lanes 3-5, 0.15 M KCl peak fractions; lanes 6-10, 0.3 M KCl peak fractions; lanes 11-15, 0.6 M KCl peak fractions. The positions of the nucleoid proteins are indicated by arrows 1-4 (HSNP-A, DBNP-B, HSNP-C, and HSNP-C', respectively).
showed binding to single stranded RNA as measured by binding to a poly(U)-Sepharose column. DBNP-B and HSNP-C bound strongly to poly(U) and could be eluted with only high salt concentrations (0.5–0.8 M NaCl). Binding of the individual purified proteins to [3H]DNA was measured by nitrocellulose filtration techniques. HSNP-A, DBNP-B, and HSNP-C bound to radiolabeled native and denatured DNAs to different extents, whereas HSNP-C', which is one of the most abundant proteins, did not show any binding to native or denatured DNA by this assay (Reddy and Suryanarayana, 1988).

Intrinsic fluorescence of proteins due to aromatic amino acids is quenched upon binding of nucleic acids to proteins if the residues are in the vicinity of the nucleic acid binding
proteins and measuring the diameter of the precipitin rings obtained.

The amount of the proteins in cell extract, post-ribosomal super-
natant, and ammonium chloride wash of ribosomes was
determined by single radial immunodiffusion using 1% agarose gels
followed by DNA-cellulose.

**TABLE I**

**Recovery of the nucleoid proteins after purification**

<table>
<thead>
<tr>
<th>Starting material</th>
<th>Chromatographic steps</th>
<th>Yield of the purified protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid extract of nucleoid</td>
<td>DNA-cellulose followed by CM-cellulose</td>
<td>0.08 0.2 0.06 0.35</td>
</tr>
<tr>
<td>Acid extract of post-ribosomal supernatant</td>
<td>DNA-cellulose followed by CM-cellulose</td>
<td>0.1 0.3 0.08 0.5</td>
</tr>
<tr>
<td>Acid extract of post-ribosomal supernatant</td>
<td>CM-cellulose followed by DNA-cellulose</td>
<td>0.16 0.3 0.1 0.8</td>
</tr>
</tbody>
</table>

**Fig. 6.** Immunoblotting of individual purified proteins and a mixture of the purified proteins using antisera to individual proteins. In each case 5 μg of individual proteins and a mixture of the four proteins (5 μg each) were electrophoresed and blotted as described in the text. A, lane 1, HSNP-A; lanes 2 and 4, mixture of the four proteins; lane 3, DBNP-B. The primary antibodies used were anti-HSNP-A (lanes 1 and 2) and anti-DBNP-B (lanes 3 and 4), respectively. B, lanes 2 and 4, HSNP-C and HSNP-C', respectively; lanes 1 and 3, mixture of the four proteins. The primary antibodies used were anti-HSNP-C' (lanes 1 and 2) and anti-HSNP-C (lanes 3 and 4), respectively.

**TABLE II**

**Content of nucleoid proteins in different cell fractions**

<table>
<thead>
<tr>
<th>Cell fraction obtained from 1 g (wet weight) of cells</th>
<th>Amount of the nucleoid protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg</td>
</tr>
<tr>
<td>Cell extract</td>
<td>0.8 1.1 0.6 1.2</td>
</tr>
<tr>
<td>S-100</td>
<td>0.5 0.5 0.45 0.9</td>
</tr>
<tr>
<td>1 M NH₄Cl wash</td>
<td>0.25 0.5 0.06 0.2</td>
</tr>
</tbody>
</table>

**domain.** All the nucleoid proteins contain tryptophan and or
tyrosine, and hence binding of DNA to the proteins was
measured by this assay. All the nucleoid proteins including
HSNP-C' bind DNA to different extents. As a representation,
binding of DBNP-B and HSNP-C' to DNA as measured by
fluorescence titration is given in Fig. 9.

**Thermal Denaturation of DNA and Protection by the Iso-
related Proteins**—We performed thermal denaturation studies
using different DNAs and tested the influence of these proteins
on DNA melting. The Tₘ of *S. acidocaldarius* DNA in
0.025 M NaCl was found to be 70.5°C. On addition of
the nucleoid proteins HSNP-A, -B, and -C in increasing amounts
there was an increase in the Tₘ of all the DNAs tested. The
Tₘ increases ranged from 14 to 25°C with the three proteins
at a protein:DNA ratio (w/w) of 1:2. The data obtained from
the melting studies were plotted to show the increase in Tₘ
with increasing amounts of protein added (Fig. 10). When the
three proteins HSNP-A, HSNP-C, and HSNP-C' were added
to calf thymus DNA (Fig. 10A), *Sulfolobus* DNA (Fig. 10B),
poly(dA)-poly(dT) (Fig. 10C), and *E. coli* DNA (Fig. 10D),
there was an increase in the Tₘ of DNA with an increasing
protein:DNA ratio. However, differences were noted in the
extent of stabilization of the different DNAs with three
proteins. DBNP-B did not show any stabilizing effect as indicated
by no increase in the Tₘ of *S. acidocaldarius* DNA and
poly(dA)-poly(dT). However, addition of DBNP-B showed a
small increase in the Tₘ of calf thymus and *E. coli* DNAs.

We have also tested the influence of proteins on the thermal
stability of DNA when added to a combination of two proteins.
Results presented in Fig. 11, A and B show that when the
proteins were added in combinations of two proteins there
was increase in the Tₘ of DNA. The Tₘ increase observed
when a combination of two proteins containing DBNP-B and
any of the other three proteins (HSNP-A, -C, and -C') was
found to be additive of Tₘ increase obtained with the indi-
individual proteins. For example, when 5 μg each of HSNP-A and DBNP-B were added together an increase in $T_m$ of 7.5 °C was observed. The corresponding $T_m$ increases obtained with 5 μg of HSNP-A and DBNP-B when added alone were 5 and 2 °C, respectively. Similarly, the other two protein mixtures containing DBNP-B and HSNP-C or DBNP-B and HSNP-C' also showed $T_m$ increases suggestive of an additive effect of these proteins (Fig. 11B). When any of the two protein combinations of HSNP-A, HSNP-C, and HSNP-C' was added the $T_m$ increase obtained was more than the mere additive of $T_m$ values obtained with individual proteins. For example, when 5 μg of HSNP-A was added along with 5 μg of HSNP-C' or HSNP-C, the $T_m$ increase obtained was found to be 12.5 °C. But the increases in $T_m$ observed with 5 μg of each of these proteins added alone were 5, 4.5, and 3 °C, respectively. Similarly, when the proteins HSNP-C and HSNP-C' were added together the $T_m$ increase (11.5 °C) obtained was more than the addition of the $T_m$ values obtained with the corresponding amounts of individual proteins.

In Fig. 12, the melting profile of *S. acidocaldarius* DNA in the presence of a mixture of HSNP-A, HSNP-C, and HSNP-C' (5 μg each) is presented along with that of free DNA. The combination of the three proteins had a strong synergistic stabilizing effect on the DNA. A $T_m$ increase of 23 °C was obtained when all the proteins were added together.

We have also performed thermal denaturation studies of different DNAs in the presence of very low salt (1 mM Tris-HCl and 1 mM Na₂EDTA) and studied the effect of these proteins. Also, under these salt concentration conditions all the proteins including DBNP-B showed a strong stabilizing effect. Calf thymus DNA under this salt concentration condition had a $T_m$ of 57 °C which increased to 65 °C in the presence of 20 μg of either HSNP-A or DBNP-B and about 74 °C with HSNP-C or HSNP-C'. Similarly, experiments were
**Fig. 10.** $T_m$ increase of different DNAs in the presence of the nucleoid proteins. Melting of the different DNAs was performed in the absence and presence of increasing amounts of the four nucleoid proteins added individually. From the melting profiles the $T_m$ values were obtained in each case. The amounts of the different DNAs used were 20 µg of calf thymus DNA (A), 10 µg of *S. acidocaldarius* DNA (B), 10 µg of poly(dA).poly(dT) (C), and 20 µg of *E. coli* DNA (D).

**Fig. 11.** Effect of addition of two nucleoid protein mixtures on the melting profiles of *E. coli* DNA. 10 µg of *E. coli* DNA was used in each melting study. 5 µg each of the two protein mixtures was added in each case. A, X, no protein added; Δ, HSNP-A and DBNP-B; ○, HSNP-A and HSNP-C; □, HSNP-A and HSNP-C'. B, X, no protein; △, HSNP-C and DBNP-B; ○, HSNP-C' and DBNP-B; ■, HSNP-C and HSNP-C'.

**Fig. 12.** Effect of addition of the three helix stabilizing nucleoid proteins together on the melting of *S. acidocaldarius* DNA. 10 µg of *S. acidocaldarius* DNA was used. ○, no protein; ■, 5 µg each of HSNP-A, HSNP-C, and HSNP-C'.

performed with *E. coli* DNA in the presence of individual proteins added at a protein:DNA ratio of 2. A maximum stabilizing effect resulting in the increase of the $T_m$ of *E. coli* DNA by 31 °C was observed with HSNP-C' ($T_m$ of *E. coli* DNA alone was 61 °C). With the other three proteins the $T_m$ of *E. coli* DNA was increased by 9–22 °C. With poly(dA).poly(dT) an increase in $T_m$ of 21 °C was observed by the

**Discussion**

*S. acidocaldarius*, an extremely acidothermophilic archaebacterium, was chosen for analyzing its chromatin structure and composition because phylogenetic data indicated that this archaebacterium is more closely related to eukaryotes evolving as a separate line of descent from the progenote (Woese, 1981; Fewson, 1986). Nucleoid has not been isolated and studied from any archaebacterium. Hence, we isolated the nucleoid from *S. acidocaldarius* and purified the histone-like proteins for their characterization. *S. acidocaldarius* cells can be easily lysed by dilute nonionic detergents. This is probably due to the absence of a rigid cell wall structure (Brock *et al.*, 1972). We have not purified further by washing the nucleoid with high salt because of the possibility of removing some of the proteins which are genuinely associated with DNA and whose interaction with DNA is salt-sensitive.
proteins have been studied. Antibodies to purified proteins with the four proteins. Immunodiffusion results indicated that the four proteins are antigenically distinct. However, cross-scale purification of these proteins is also achieved by developing techniques, and quenching of the intrinsic fluorescence of the proteins. The helix stabilizing effect of HSNP-A, -C, and -C' reported here. Further work is necessary to determine the identity among the histone-like proteins isolated from different archaeabacteria.

The nucleoid histone-like proteins may have some common domain which may be involved in DNA binding.

CD spectra of the proteins suggest the presence of ordered secondary structural elements in all the four proteins. The spectra have the unusual property of intersecting the base line around 205–210 nm (usually 200 nm). HSNP-A showed a CD spectrum characteristic of an α-helical protein, but estimation of α-helical content gave low values. This may be because of the low signal intensity observed for this protein and also because of the uncertainty associated with structure predictions based on CD measurements. The CD spectra of some of the low molecular weight DNA binding proteins isolated from *S. acidocaldarius* are available in the literature (Dijk and Reinhardt, 1986). By comparison it appears that the proteins 7e, 10b, and 8a described by Dijk and Reinhardt (1986) may correspond to HSNP-C', DBNP-B, and HSNP-C, respectively.

The amino acid compositions of the nucleoid proteins show certain unique features such as lack of methionine and very low content or absence of proline. The basic amino acid content of these proteins is similar to that of eukaryotic histones; however, the content of lysine is relatively higher than most of the eukaryotic histones (H2A, H2B, H3, and H4) and is more like that of H1. Overall, the amino acid compositions of the histone-like proteins of this archaeabacterium show similarities with eukaryotic histones as well as with those of the histone-like proteins of the eubacteria, although there are distinct differences in the composition of certain amino acids. Among the archaeabacterial histone-like DNA binding proteins isolated, the amino acid composition of a 7-kDa protein from *S. solfataricus* (Kimura et al., 1984) shows strong similarities with the composition of HSNP-C'. A methylated DNA binding protein of 7 kDa from *S. solfataricus* whose amino acid sequence has been determined (Choli et al., 1988a) also shows strong similarities in its amino acid composition and DNA binding to HSNP-C'. The 7-kDa protein isolated and sequenced from *S. acidocaldarius* (Choli et al., 1988b) shows similarities in its amino acid composition to HSNP-C' reported here. Further work is necessary to determine the identity among the histone-like proteins isolated from different archaeabacteria.

The nucleoid proteins HSNP-A, -C, and -C', and DBNP-B bind nucleic acids, and the first three strongly protect DNA against thermal denaturation. The purified proteins bind both native and denatured DNAs with varying degrees of affinity as measured by affinity chromatography, filtration techniques, and quenching of the intrinsic fluorescence of the proteins. The helix stabilizing effect of HSNP-A, -C, and -C' was observed at different ratios of protein to DNA and at different salt concentrations with maximum protection being reached at a ratio of 1–2. The helix stabilizing effect of these proteins is specific to DNA, and double stranded RNA is not at all stabilized (Reddy and Suryanarayana, 1988). The amount of these proteins, which are the only acid-soluble proteins, is 90% by weight of the DNA in the nucleoid.
Therefore, the observed protection by these proteins is physiologically significant. The results of protection of DNA when mixtures of proteins were added indicates cooperative and synergistic effect of these proteins in protecting DNA against thermal denaturation. These results clearly point out the strong and specific helix stabilizing effect of the nucleoid proteins. Although eubacterial histone-like protein (protein HU or protein NS) condenses duplex DNA into nucleosomal structure (Rouvière-Yaniv and Kjeldgaard, 1979; Broyles and Pettijohn, 1985), it could not protect DNA against thermal denaturation to any appreciable extent (Miano et al., 1982 and our own results). Among the various DNA binding proteins isolated until now from eubacteria and archaeabacteria, the proteins HSNP-A, HSNP-C and HSNP-C' are special in their helix stabilizing property which is physiologically very important. Thermal denaturation studies performed at high salt (0.1 M) also showed strong protection by these proteins with \( T_m \) increases of 10 to 15 °C over and above the increasing rate due to increased salt concentration (not shown). The protection of DNA against thermal denaturation by these proteins may be through both electrostatic and hydrophobic interactions.

Comparison of the histone-like proteins of the archaeabacteria with those of eubacteria and eukaryotic histones will help in understanding the evolutionary relatedness of organisms as well as the evolution of the histone-like proteins. Another interesting aspect is the interaction of histone-like proteins with nucleic acids which can be used as a model system for studying the general nature of the protein-nucleic acid interaction. Work is progressing in our laboratory on these lines.

REFERENCES


