Summary
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Chapter 1 (Introduction):

A review of the cell and molecular biology of the archaea, their classification and characteristics followed by a review of the histone and histone like proteins present in the prokaryotes is covered in this chapter. This is followed by objectives and scope of the present investigation.

Chapter 2 (Materials and methods):

This chapter lists the materials used in the study and a brief description of the methods used during the course of the investigation. Purification protocol for nucleoid, S-layer and HSNP A are presented. The nucleoid was investigated and its components were determined and analyzed by micrococcal nuclease digestion, RNase digestion and formaldehyde fixation. The changes in the nucleoid composition during the growth phases were determined. Effect of temperature on the nucleoid components was monitored.

The solution state of HSNP A was studied by formaldehyde and dimethylsuberimidate cross linking. The nucleic acid binding properties of HSNP A were studied by flourimetry, gel mobility shift assay and thermal denaturation experiments. Effect of chemical modification of lysine and tryptophan residues of HSNP A on its DNA binding properties was also studied.

Chapter 3 (Results):

This chapter deals with the results that were obtained in the present investigation and contain the following sections.
3.1: Biochemical characterization of thermooacidophilic archaenal nucleoid of *Sulfolobus acidocaldarius*.

Sephacryl S-200 chromatography of the micrococcal nuclease digested nucleoid shows HSNP C and HSNP A coeluting in the later fractions. Of the two peaks resolved during the Sephacryl S-1000 column chromatography of the nucleoid peak I was found to have a higher protein to DNA ratio whereas the protein, DNA and RNA content was higher in peak II. The two peaks are different forms of the nucleoid. RNA was found to be a major component of the nucleoid and play an important role in maintaining the structural integrity of the nucleoid.

3.2: Studies on growth phase dependent variations in nucleoid

The elution profile of sephacryl S-1000 was found to vary during exponential and stationary growth phases. Peak I from nucleoid of stationary phase cells shows a profile similar to that of RNase digested exponential phase nucleoid. DBNP B protein is observed to be relatively more abundant whereas HSNP C levels are relatively reduced. Digestion of nucleoid with RNase does not have any effect on the peak I profile and only slightly shifts the peak II elution volume.

3.3: Effect of temperature on nucleoid fractions

The high molecular aggregate form of peak I was found to be maintained at the optimum growth temperature conditions of *Sulfolobus acidocaldarius* i.e., 70°C. The aggregate form is lost upon incubation at 37°C and regained on incubation at 70°C. There is no temperature induced change in the peak II profile.

3.4: Purification and characterization of S-layer proteins

The S-layer protein was purified as described by Grogan et al. The purified S-layer consists of two proteins, a 130 kDa protein and a 65 kDa protein. The 65 kDa protein bound to ds DNA cellulose column and eluted out at 300 mM. Nucleoid preparations and both peak I and peak II show a prominent band at ~65 kDa which could be this
protein which binds to the genomic DNA and is collected along with it during nucleoid purification.

3.5: Purification of HSNP A and characterization of its DNA binding properties
Helix stabilizing nucleoid protein A (HSNP A) is a nucleoid associated protein which protects DNA against thermal denaturation. The protein was purified and characterized for its biochemical and nucleic acid binding properties. It is a 10 kDa protein which was observed to occur as a tetramer or octamer in solution. Spectroscopic properties of the protein were also studied. The protein binds both ss and ds DNA, with greater affinity to ds DNA. The nucleic acid properties were studied by fluorescence titration, gel mobility shift assays and electron microscopy.

3.6: Studies on DNA binding properties of chemically modified HSNP A
HSNP A was treated with modification agents to modify the tryptophan and lysine residues. There was no inactivation of the DNA binding ability on modification. The modified protein showed an increased ability to protect the DNA against thermal denaturation.

Chapter 4: (Discussion):
This chapter deals with the conclusions derived from and the implications of the results observed during the course of the investigation.

Micrococcal nuclease digestion studies along with the electron microscopy studies of HSNP C’ implicates HSNP C to act like a scaffold protein. The co elution of HSNP A along with HSNP C’ during DNA cellulose chromatography and also during the chromatography of micrococcal nuclease digestion products shows a close interaction between the two proteins. The above observations and the occurrence of relatively high levels of HSNP A, DBNP B and HSNP C’ during the regeneration of the Sulfolobus culture shows the need for further investigation into the roles of these proteins.

The two peaks resolved by the Sephacryl S-1000 chromatography of the nucleoid are both confirmed to be nucleoid components. The peak II has most of the nucleic acid
and the associated proteins of the nucleoid. Peak I has a higher protein to DNA ratio and is rich in S-layer proteins. The strong affinity to ds DNA observed in case of the small 65 kDa subunit of the S-layer protein along with the protein profile of peak I indicates peak I to comprise S-layer bound fraction of genomic DNA formed either during the purification of the nucleoid or comprising the genomic DNA and RNA involved in the coupled transcription and translation of the S-layer proteins.

RNA was observed to form a major component of the nucleoid. RNase digestion studies and the analysis of its effect on Sephacryl S-1000 column profile indicate a major role to RNA in maintaining the structural integrity of the nucleoid.

Temperature dependent variations are observed in peak I protein profile. Further investigation into the component(s) responsible for these changes is required.