Chapter-5
SUMMARY & CONCLUSIONS
Summary and Conclusions

Chapter 1: Introduction

This chapter deals with the introductory aspects of the biological kingdom in general, but with special emphasis on archaea (previously referred to as the archaebacteria). These organisms are grouped into a third novel kingdom comprising of organisms that are specially adapted to living in the extremeties of temperature, pH and salinities. In addition to these, the various aspects of DNA binding proteins such as histone-like proteins from the three kingdoms are also presented in this chapter.

Chapter 2: Materials and Methods:

This chapter provides information about the chemicals, materials and methodologies used during the course of the thesis. Apart from these, it provides sufficient details regarding the procedures and protocols made use of in the present study along with minor modifications if any. The present task was accomplished with the help of various techniques such as fluorescence titrations, mobility shift assays, studies on binding affinities to immobilized nucleic acid matrices, Tm studies, DNA aggregation, electron microscopy and chemical modification studies.

Chapter 3: Results

HSNP-C', an 8 kDa DNA binding protein is associated with the ribosome free domains of genomic DNA in the cell. The abundant protein was purified from S. acidocaldarius to homogeneity as analysed by SDS-PAGE followed by silver staining. HSNP-C exists in solution as a multimeric aggregate as analysed by cross-linking studies as well as immunoblotting studies. The nucleic acid binding
properties of the protein were studied by fluorescence titrations, affinity chromatography on nucleic acid matrices, electron microscopy, DNA aggregation and protection of DNA against thermal denaturation. The protein binds to dsDNA with a site size of 4 and a binding constant "K" of $4 \times 10^6 \text{M}^{-1}$ at 20 mM NaCl. It has weak affinity to single stranded DNA and no affinity to RNA. HSNP-C also binds to mononucleotides through a site which is probably distinct from the dsDNA binding site. The protein exists in atleast two distinct forms which differ in the extent of methylation of lysine residues. Electron microscopic studies suggest that the protein forms compact structures with folded dsDNA domains.

Chemical modification studies suggest importance of aromatic amino acids in the binding of protein to dsDNA and that the electrostatic interactions stabilise the binding of the protein. Chemical modification studies and proteolytic digestion also suggest very compact structure of the protein which is resistant to proteolytic enzymes and modifying reagents.

**Chapter 4: Discussion**

Cross-linking studies using both bifunctional cross-linkers (DMS and DFDNB) as well as zero level cross-linkers revealed the tendency of the protein, HSNP-C to exist as multimeric aggregates in solution. The protein has a very compact structure and binds strongly and co-operatively to dsDNA. Electron microscopic studies also strengthen the view that HSNP-C forms compact structures with DNA suggesting the condensation of intracellular DNA of the organism. HSNP-C was found to aggregate DNA and protect DNA against thermal denaturation. The protection offered by HSNP-C against thermal denaturation in the presence as well as absence of polyvalent cations suggests the involvement of hydrophobic/hydrogen bonding interactions in the stabilisation of
DNA in the organism

Chemical modification studies yielded information suggesting the involvement of tyr and trp in the nucleic acid binding domain of the protein. Our study gives evidence for the model proposed for Sso 7d by Bauinann et al. (1995). This is the first detailed study on the nucleic acid binding properties of the protein which has the capacity to condense DNA into compact structures.