Chapter 1 (Introduction):

A brief review of archaea and their molecular biological aspects is given in this chapter. It was followed by a review of histone like DNA binding proteins from eubacteria and archaea. A brief survey on proteins which promote renaturation and aggregation from eubacteria and eukarya is also presented. This was followed by objectives and scope of the present investigation.

Chapter 2 (Materials and methods):

This chapter lists the materials used in the study and gives a brief description of the methods that were followed in the investigation. A purification procedure for nucleoid from *S. acidocaldarius* was presented. A purification protocol for DBNP-B is presented. The protein chemical properties were studied by using various cleaving agents like V8 protease, trypsin, chymotrypsin and cyanogen bromide. The solution state of DBNP-B was studied by employing formaldehyde and dimethyl suberimidate crosslinking. The nucleic acid binding properties of the 11 kDa DBNP-B were studied by employing a variety of techniques like fluorimetry, gel mobility shift assays, renaturation assays, nicking assays and aggregation assays. The functional properties of DBNP-B were also studied. The effect of DBNP-B on various enzymes like topoisomerase I, topoisomerase II, DNA polymerase and RNA polymerase are presented.

Chapter 3 (Results):

This chapter deals with the results that were obtained in the present investigation and contain the following sections.

3.1: Characterisation of *S. acidocaldarius* nucleoid:

Nucleoid from *S. acidocaldarius* was purified and passed through a sephacryl S-1000 column for further purification which resulted in the separation
of nucleoid into two peaks, peak-1 and peak-11 which differ in quality. Peak-1 nucleoid shows high molecular weight proteins in abundance along with low amounts of low molecular weight histone like proteins. The DNA looks like a high molecular weight sharp band. Peak-11 nucleoid shows low molecular weight histone like proteins in abundance along with high molecular weight proteins. The DNA is resolved as a broad smear on an agarose gel. This separation of nucleoid into two peaks is observed in freshly harvested early logarithmic phase cells and midlogarithmic phase cells and is thus not growth phase dependent. The nucleoid proteins in both the peaks which bind to DNA were characterised by passing through a DNA cellulose column. The tight binding proteins in the nucleoid were also characterised. Micrococcal nuclease digestion of nucleoid resulted in ~ 600 bp to 1500 bp fragments.

3.2: Purification and properties of DBNP-B:

DBNP-B was purified from cell extracts by a single CM-cellulose chromatographic step. The solution state of DBNP-B was studied by formaldehyde crosslinking at three different temperatures 37°C, 65°C and 80°C. Crosslinking results indicate that DBNP-B predominantly exists in a dimeric state in pH 5.0 containing buffer at 37°C and 65°C and it probably exists as oligomeric forms in the presence of MgCl₂ at 80°C. DBNP-B when passed through a gel filtration column, sephadex G-50 eluted as aggregates corresponding to dimer predominantly along with some tetrameric protein at room temperature. DBNP-B was cleaved chemically with cyanogen bromide and proteolytically with V8 protease, trypsin and chymotrypsin. DBNP-B seems resistant to trypsin and chymotrypsin.

3.3: Nucleic acid binding properties of DBNP-B:

The interaction between DBNP-B and DNA as well as RNA was studied using mobility shift assays, fluorescence assays, DNA renaturation and other assays. The binding interaction between DBNP-B and poly (U) or poly (A) was characterised and the site size of DBNP-B was calculated from the fluorescence titrations with poly (U) and poly (A). DBNP-B aggregated double stranded DNA. The aggregation was dependent on protein concentration and increases with increase in DBNP-B concentration. Complementary ss DNA was renatured into
high molecular weight network by DBNP-B. This DNA renaturation requires Mg^{++} and showed an optimum of pH 5.0. Renaturation was dependent on protein concentration and high concentrations of protein reduced renaturation. pUC19 supercoiled DNA showed increased sensitivity to DNase I at intermediate concentrations of DBNP-B and at high concentrations, a protection from DNase I digestion was observed. A non specific nicking activity of DNA by DBNP-B was observed which is Mg^{++} dependent and protein concentration dependent.

DBNP-B neither inhibits nor enhances the activity of DNA topoisomerase I and DNA topoisomerase II. DBNP-B enhances the activity of DNA polymerase I and RNA polymerase at low concentrations and inhibits their activity at high concentrations. Chemical modification of tyrosine residues in the protein by tetranitromethane resulted in the decreased affinity of DBNP-B to DNA-cellulose.

Chapter 4 (Discussion):

This chapter deals with the implications of the results obtained in the present investigation.

*S. acidocaldarius* nucleoid can be isolated as a rapidly sedimenting nucleoprotein complex. In order to ascertain which of the proteins are truly components of the nucleoid, the nucleoid was purified by gel filtration chromatography and DEAE-cellulose chromatography. Nucleoid isolated from cells grown upto early and midlogarithmic phases showed similar characteristics on gel filtration chromatography. Both peak-I and peak-II nucleoid fraction have essentially similar DNA binding proteins including the low molecular weight proteins. The purified nucleoid contained apart from the four DNA binding acid soluble proteins (HSNP-A, DBNP-B, HSNP-C and HSNP-C') a 15 kDa protein and atleast two to three 25-27 kDa proteins. Micrococcal nuclease digestion results of nucleoid suggest that the overall condensation of DNA and its organisation by the nucleoid proteins in archaea may be different from the organisation of eukaryotic DNA.

DBNP-B seems to exist in a very high molecular weight aggregated state. The largest multimeric state that is clearly identifiable is an octameric aggregate.
However under most of the crosslinking conditions, dimeric, tetrameric and hexameric species are dominant indicating that stable aggregated form of DBNP-B could be a tetramer or a hexamer. The protein is remarkably stable to digestion by proteolytic enzymes suggesting highly compact 3 dimensional structure. Results on nucleic acid binding, aggregation, renaturation etc which occur optimally in the presence of Mg$^{2+}$ and the observed fluorescence enhancement suggest a functional involvement of aromatic amino acids in the interaction of DBNP-B. Nucleic acid binding of DBNP-B by mobility shift assays suggest that the protein binds all types of DNA ss or ds or circular or linear with varying affinities. The coaggregation complexes formed by DNA:DBNP-B show that the aggregation of DNA by DBNP-B is stimulated by Mg$^{2+}$ ions with an optimum concentration of 10 mM.

DBNP-B promotes renaturation of complementary ss DNA not only to network like structures but also to form linear ds molecules. Renaturation promoted by DBNP-B could be an independent function of this protein or it could be also a generalised effect of DBNP-B such that the topology or structure of DNA in the DNA:DBNP-B complexes at a particular protein concentration may be suitable for the action of other enzymes and proteins acting on DNA.

Results presented on the effect of DBNP-B on enzymes acting on DNA show that DNA in DNA:DBNP-B complexes is not only available for action by these enzymes but is in a more favourable conformation. The novel and interesting activity detected during the course of this study is the nicking activity of DBNP-B which is optimal in a broad pH range of 7 to 8.5 and is absolutely dependent on Mg$^{2+}$ ions. The preferential specific nicking activity of circular DNA of size 4-5 kb may suggest formation of specific DNA:protein complex that is amenable to cleavage by the protein which is not possible with linear DNA or short circular duplexes. DBNP-B may have a multifunctional role in *S. acidocaldarius* facilitating several reactions connected with DNA metabolism such as DNA replication, transcription and recombination.