Chapter 1 : Introduction

This chapter begins with an introduction to Archaea - the third biological kingdom, followed by the molecular biological aspects of the archaeal kingdom. In addition to this it also provides a review of the proteins that are histone like from bacterial and archaeal organisms. A brief account of eukaryotic scaffolding proteins namely the HMG group of DNA binding proteins is also given. The first chapter was followed by scope and objectives of the present investigation.

Chapter 2 : Materials and Methods

This chapter provides a detailed description of the methodologies used to carry out the present work. In addition, information about the chemicals and the materials used for the methods used is given. The present investigation was carried out with the help of various techniques such as mobility shift assays, fluorescence titrations, affinity chromatography on nucleic acid matrices, DNA aggregation and renaturation assays.

Chapter 3 : Results

The three abundant isoforms of HSNP-C were purified by CM cellulose chromatography (HSNP-C\(a\), HSNP-C and HSNP-C\(b\)). The amino terminal sequencing of HSNP-C (the most abundant of the three isoforms) showed that it is 100% identical to Sac 7d protein. Crosslinking studies with formaldehyde showed that the physiological state of HSNP-C is an oligomeric structure. Gel filtration experiment and affinity chromatography experiments on HSNP-C coupled epoxy sepharose showed the association of HSNP-C with other proteins. The nucleic acid binding properties of HSNP-C and its isoforms were studied by gel mobility shift assays, fluorescence titrations, affinity chromatography on nucleic acid matrices, nuclease protection assay. The protein binds strongly to ds DNA and reasonably strongly to ss DNA. Of the three isoforms, HSNP-C\(b\) showed strongest affinity to ds DNA as seen by both affinity
chromatography and fluorescence titrations. The binding constant K for the three isoforms were calculated. Apart from nucleic acid binding, all the three isoforms showed binding to GTP. HSNP-C aggregated both double stranded and single stranded DNA. The aggregates formed remained stable with increase in the concentration of the protein (HSNP-C). Complementary single stranded DNA was renatured by HSNP-C into high molecular weight network DNA. Renaturation promoted by HSNP-C did not require Mg\(^{2+}\) or GTP and showed an optimum pH of 5.0. Interestingly, HSNP-C and its isoforms showed ribonuclease activity with tRNA and ribosomal RNA as substrates inspite of strong DNA binding properties. All the three isoforms showed weak binding to RNA. Immunological homology of HSNP-C was observed with eukaryotic DNA binding proteins namely the HMG 1/2 group of proteins.

Chapter 4: Discussion

Crosslinking of HSNP-C with formaldehyde has shown that this protein exists as multimeric aggregates physiologically. Fluorescence titrations suggest strong binding of HSNP-C to double stranded DNA and weak binding to single stranded DNA. Gel mobility shift assays indicate strong and cooperative binding of the protein to double stranded DNA with greater affinity for the supercoiled form. Of the three isoforms, HSNP-C\(^b\) showed strongest affinity for double stranded DNA. Chromatography of the three isoforms on DNA cellulose columns revealed an interesting heterogeneity in the isoforms. The salt required to elute each isoform differed depending on the extent of methylation of the forms. Apart from nucleic acid binding, HSNP-C and its isoforms showed nucleotide binding i.e to GTP with a mild GTPase activity. This GTPase activity could be of importance in some other processes in the cell. HSNP-C aggregated both double stranded and single stranded DNA which suggests its role in condensation of DNA in the organism. Aggregation of DNA increases the effective concentration of DNA and facilitates many important reactions like renaturation of complementary single strands. Renaturation and aggregation promoted by HSNP-C was more non specific like that shown by histones (Cox and Lehman, 1981). The associated ribonuclease activity of HSNP-C and its isoforms support the earlier studies of p2 and p3 endoribonucleases.
(Fusi et al., 1993). The ribonuclease activity and nucleotide binding reflect the multifunctions of the protein HSNP-C apart from DNA binding. Strong immunological homology of HSNP-C was seen with the eukaryotic scaffolding proteins namely the HMG 1/2 group.