Chapter-4
SUMMARY
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Chapter-1:
This chapter gives a brief overview of literature pertaining to archaeal molecular biology and biochemistry. A detailed account on archaeal translational apparatus, the ribosomal structure, organisation and assembly is presented. Salient features of bacterial ribosomes (E. coli) have also been described. The role of primary r-RNA binding proteins and the significance of homologous ribosomal proteins from different organisms has been documented. Objectives and scope of the present investigation have also been mentioned in this chapter.

Chapter-2:
In this chapter, the methodologies described are growth of organisms, isolation of ribosomes, ribosomal subunits, ribosomal proteins, fractionation of r-proteins by novobiocin, chromatographic procedure for the isolation of 50S core particles, thermal melting analysis of ribosomes, isolation of subunit core particles by salt extractions, electrophoresis in the presence of SDS, two-dimensional gel electrophoretic technique and immunoblotting procedure etc.

Chapter-3.1:
In this chapter, the ribosomes isolated by different methods from S. acidocaldarius were analysed for total r-proteins by SDS-poly acrylamide gel electrophoresis and two-dimensional gel electrophoresis. It was found that the ribosomes isolated under gentle lysis conditions do not contain high molecular weight non ribosomal proteins. A fractionation method involving precipitation of proteins with novobiocin has been developed to separate ribosomal proteins of
\textit{S. acidocaldarius}. Four groups of proteins were obtained corresponding to those precipitated with 0.2, 0.5, 1.0 mg/ml novobiocin and proteins soluble at 1.0 mg/ml novobiocin. The same procedure was also successful for the fractionation of ribosomal proteins from \textit{E. coli}. Two-dimensional gel electrophoresis showed that proteins present in each group were distinct with only a minor contamination of proteins from other groups. Our results indicate that proteins with similar charge and molecular weight can be separated by this method. This procedure may have applications for the fractionation of other complex protein mixtures.

\textbf{Chapter-3.2:}

In the present study an attempt is made to understand the thermal stability of \textit{S. acidocaldarius} ribosomes. \textit{S. acidocaldanus} ribosomes are more heat stable than the \textit{E. coli} ribosomes because of higher GC content in r-RNA and stronger r-RNA and r-protein interactions. Sepharose 4B chromatography of \textit{S. acidocaldarius} cell lysates and ribosomes yielded 50S core particle and split proteins. The proteins which are associated with r-RNA in 50S core particle strongly protect the r-RNA against thermal denaturation and are possibly the early assembly proteins. These proteins were identified as L2, L3, L4, L6, L7, L8, L11, L12, L14, L15, L18, L19, L20, L21, L23, L27, L30, L33, a, and b. The split proteins which were identified (S13, S15, S24, L1, L10, L13, L14, L15, L24, L25, L26, L27, and L28) could be the late assembly proteins. The chromatographically isolated 50S core particle could serve as a best source for the purification of primary r-RNA binding proteins. This method has no limitations with respect to the quantity of core particle isolated in one run.
Chapter-3.3:

*S. acidocaldarius* 30S particle obtained by hydrophobic chromatography contained most of the 30S subunit proteins. Core particles were isolated by treatment of 30S subunit with different concentrations of salt and urea. The proteins which are present in 4 M LiCl core particles were identified as S6, S10, S11, S16, S27 and a, which are primary r-RNA binding proteins as they are associated with r-RNA under highly stringent conditions. The primary r-RNA binding proteins protect 16S r-RNA strongly against thermal denaturation. The other set of proteins which are associated with 30S subunit after primary r-RNA binding proteins were identified as S3, S4, S7, S8, S12, S14, S15, S17, S18, S19, S20, b and c. These proteins were called secondary r-RNA binding proteins.

Chapter-3.4:

Ribosomes from *S. acidocaldarius* and *E. coli* were analysed for the presence of homologous proteins using immunoblotting technique. Antibodies raised against subunits (30S and 50S) of *E. coli* and *S. acidocaldarius* were used in the present study. *S. acidocaldarius* r-proteins S5, S6 and S11 and L8, LU and L16 cross-reacted with anti *E. coli* subunit antibodies. These proteins were members of the primary r-RNA binding proteins. *E. coli* r-proteins S5 and S18 and L1, L3, L6 and L9 cross-reacted with anti *S. acidocaldarius* subunit antibodies.