Abstract

An open reading frame, designated as *recX* is directly located downstream of *recA* in *E. coli* and it is found to be conserved in gram positive as well as gram negative bacteria. The *recX* gene of *E. coli* has been cloned and it has been found that this ORF encodes a protein of ~ 22 kDa. This protein has been overexpressed and purified to homogeneity. The molecular weight of the pure protein has been determined by MALDI-ToF. The structural characterization of RecX has been done using spectroscopic methods. Far UV-Circular dichroism study has been done for both native and denatured protein. The presence of a broad negative hump at 220 nm and the estimation of secondary structure content of RecX indicate the high propensity of α helical structure. Denaturation studies on the protein have been carried out using 8 M urea. In order to monitor the melting of the protein, the residue molar ellipticity at 220 nm for various concentrations of urea (0, 2, 4, 6, 8 M) has been recorded. The peak at 1650.795 cm^{-1} in the amide 1 transition from FTIR spectroscopy indicates that the protein is completely helical. As the protein contains three Trp residues, the normalized fluorescence spectra of the native and denatured protein have been studied in the excitation wavelength at 280 nm. The spectrum corresponding to the native protein (λ_{max} = 339 nm) is blue-shifted relative to that of the unfolded protein (λ_{max} = 355 nm) and suggests that the tryptophan molecules are located within a less polar environment in the native protein than in the unfolded state. To further investigate the microenvironments of the tryptophan molecules both the native and unfolded proteins have been subjected to acrylamide quenching. The upward curvature of the Stern-Volmer plots suggested that all the tryptophanyl residues are equally accessible to the quencher molecules. This is also indicated by the large values of static quenching constant. As there no structure homologous to RecX has been found in the PDB, theoretical modeling of RecX 3D structure has been done using fold prediction techniques. The *In silico* study of RecX reveals that the protein is completely helical which has been validated by all spectroscopic studies. The model reveals that the three Trp residues are equally accessible to the solvent.

The RecA mediated ATP hydrolysis indicates that with rise in concentration of RecX, the ATPase activity gradually decreases. This suggests that RecX downregulates RecA function by interacting with the protein. To study the mechanism of interaction, RecA-RecX interaction has been studied by docking the proteins using GRAMM

software. An algorithm has been developed to find out the interacting zone between the proteins. It predicts that, besides other interacting residues, Gln 194 in RecA protein present near to L1 and L2 (disordered loop regions, reported as DNA binding region) is proposed to interact with the phosphate group of DNA. However, in presence of RecX, the negatively charged residues (Glu 107 and Asp 26) of RecX binds to the DNA binding region of RecA replacing the DNA. A part of the RecX molecule sits on the RecA filament at its DNA binding site thus inhibiting the entry of the substrate and reducing its ATPase activity. The different structural parameters of the RecA-RecX complex has been compared with known protein-protein complex structures.

Key words

RecX, RecA, Purification, Western Blot, Circular dichroism, Fluorescence, FTIR, Modeling, protein-protein interaction, MALDI-ToF, GRAMM, threading, energy minimization, ATPase assay.