RecA Protein from the Multi-drug Resistance Bacterium *Mycobacterium Smegmatis*: Expression, Purification, Crystallization and Characterization

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Abstract—*The RecA protein is essential for the drug resistance of Mycobacterium smegmatis and other Mycobacterium species. Drugs for tuberculosis lead to DNA damage and the bacterium relies on RecA to fix this damage. RecA protein is a key component in DNA repair and homologous genetic recombination process. Previous study found that it plays an important role in recombination, repair and maintenance of DNA in Escherichia coli. However, for RecA protein to function in these processes, it must assemble on ssDNA to form a nucleoprotein filament known as the presynaptic complex. For full understanding of both these processes and ensuing application in Mycobacterium smegmatis, structural knowledge of RecA protein is vital. In this work, the RecA protein of Mycobacterium smegmatis was overexpressed into E.coli BL21 (DE3) and purified to high quality by using several techniques such as centrifugation, Anion exchange chromatography, Ni-NTA affinity chromatography and size exclusion chromatography. Polymine was used to remove nucleic acid properties. Our purpose was to crystallize protein without DNA. However, it is assumed that DNA molecules can enhance the crystal growth. Crystallization of protein was done by sitting drop method using in-house Mosquito Crystal (TTP Lab-Tech) automated liquid dispensing system. Initial screening was with JSB, Hampton, PEG suit 1 and PEG suit 2 Research Index HT screen. PEG suit 1 screen resulted in two crystals of the RecA appearing within hours of setup. Secondary structure for RecA protein was predicted using Circular dichroism. K2D2 predicted 1.86 % of α helix and 49.03% of β helix. Once the structure of RecA protein is resolved further we can find out likelihood of inhibitors for RecA protein. The inhibitors of RecA may serve to delay or prevent the appearance of bacterial drug resistance. In this context, structural and functional insights into the crystal structures could be useful for design of inhibitors against RecA from M. tuberculosis. This may be of significance in the process of improving the tuberculosis management through better drugs and vaccines.*