ABSTRACT

Rifampicin is a major chemotherapeutic agent used against mycobacterial and nocardial infections. High level resistance is primarily due to mutational alterations in the \textit{rpoB} gene encoding the \(\beta\) subunit of RNA polymerase. When challenged, these bacteria may inactivate rifampicin by one of four mechanisms: decomposition, ADP-ribosylation, glucosylation and phosphorylation. ADP-ribosylation occurs in many mycobacterial pathogens but nothing is known about the properties of the enzyme responsible. Consequently mutational analysis may be used to explore structure-function relationships in this protein.

Three mutants with changes in the open reading frame were selected and studied. The altered \textit{arr} gene in pMG1 was obtained by \textit{in vivo} selection whilst in pMG2 and pMG4 by \textit{in vitro} mutagenesis. The mutated \textit{arr} gene in pMG1 and pMG2 conferred resistance to 50 \(\mu\)g/ml of rifampicin while in pMG4 to 200 \(\mu\)g/ml. This suggested that alterations near the N-terminus resulted in lowered activity because of closer proximity to the active site. This is the first successful report of induced \textit{arr} gene expression. This over-expression of the Arr ADP-ribosyltransferase and its mutants assisted in their later purification by metal affinity chromatography.