

ABSTRACT

Rifampicin is a major chemotherapeutic agent used against mycobacterial and nocardial infections. High level resistance is primarily due to mutational alterations in the *rpoB* gene encoding the β 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 *arr* gene in pMG1 was obtained by *in vivo* selection whilst in pMG2 and pMG4 by *in vitro* mutagenesis. The mutated *arr* gene in pMG1 and pMG2 conferred resistance to 50 $\mu\text{g/ml}$ of rifampicin while in pMG4 to 200 $\mu\text{g/ml}$. 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 *arr* gene expression. This over-expression of the Arr ADP-ribosyltransferase and its mutants assisted in their later purification by metal affinity chromatography.