SITE-DIRECTED MUTAGENESIS OF THE AROE-ENCODED SHIKIMATE DEHYDROGENASE FROM Mycobacterium tuberculosis H37RV AND EXPRESSION OF THE MUTANT PROTEINS

Rodrigues-Junior,V.S.^[1,2]; Basso,L.A.^[1]; Santos,D.S.^[1]

^[1]Centro de Pesquisas em Biologia Molecular e Funcional - PUCRS; ^[2]Programa de Pós-Graduação em Biologia Celular e Molecular - UFRGS

Among all infectious diseases that afflict humans, tuberculosis remains the deadliest. The emergence of multi-drug-resistant strains of Mycobacterium tuberculosis and the unbearable side effects of the available drugs have increased the need for new effective antitubercular drugs. The shikimate pathway is an attractive target for the development of antitubercular agents because it is essential in Mycobacterium tuberculosis but absent in human host. Mycobacterium tuberculosis aroE-encoded shikimate dehydrogenase (MtbSD) catalyzes the NADPH-dependent reduction of 3-dehydroshikimate to shikimate. Three-dimensional structure studies involving other shikimate dehydrogenases have suggested that Lys69 and Asp105 are likely to play an important role in catalysis and/or substrate-binding. Recent studies have proposed both kinetic and chemical mechanisms of MtbSD, and have suggested Lys69 and Asp105 to be involved in catalysis and/or substrate-binding. The objective of this study is to investigate the kinetic properties of the following mutant enzymes: K69A, K69H, K69I, K69Q, D105A, D105N. Mutagenesis was performed using a PCR-amplification technique. The sequences of the mutated aroE genes have been verified and the expression of the mutant proteins was observed in Escherichia coli C41(DE3) grown with IPTG induction. Protein purification will provide enzymes for kinetic studies. A better understanding about the enzyme catalytic and chemical mechanisms may help in the rational design of MtbSD inhibitors.