

**Analysis of Prokaryotic Fatty Acid Double Bond Hydratases,  
Members of Myosin Cross-Reactive Antigen Family**

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## Abstract

The myosin cross-reactive antigen (MCRA) protein family is highly conserved among different bacterial species ranging from Gram-positive to Gram-negative bacteria. Beside their ubiquitous occurrence, knowledge on the biochemical and physiological function of MCRA proteins is scarce. The present work shows that MCRA protein from *Streptococcus pyogenes* M49 (SPH) and *Lactobacillus acidophilus* NCFM (LAH) are flavin adenine dinucleotide (FAD) enzymes which act as hydratases on (9Z) and (12Z) double bonds of C-16, C-18 non-esterified fatty acids. Products are 10-hydroxy and 10,13-dihydroxy fatty acids. Kinetic analysis suggests that FAD rather stabilizes the active conformation of the enzymes and is not directly involved in catalysis. Analysis of *S. pyogenes* M49 grown in presence of either oleic or linoleic acid showed that 10-hydroxy and 10,13-dihydroxy derivatives were the only products. No further metabolism of these hydroxy fatty acids was detected. Deletion of the hydratase gene caused twofold decrease in minimum inhibitory concentration (MIC) against oleic acid, but increased survival of the mutant strain in whole blood. Adherence and internalization properties to human keratinocytes were reduced in comparison to the wild type. These results indicate that the previously identified MCRA protein can be classified as a FAD containing double bond hydratase, within the carbon-oxygen lyase family, that plays a role in virulence of at least *S. pyogenes* M49. *De novo* crystal structure of selenomethionine substituted apo-LAH was solved by X-ray diffraction with MAD phasing. LAH has the highest structural homology to the family of flavin containing amine oxidoreductases. The structural homology assignment resulted in identification of four distinct homologous domains (hDs), where hD1 mediates FAD binding and hD2–hD4 should be responsible for a completion of the active site. Particularly, flexible hD4 domain may function as a lid of LAH active site. Despite high level of overall structure similarity between LAH and the members of flavin containing amine oxidoreductase family no active site residues and almost none of FAD binding residues are conserved.

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