Title:	Molecular Characterization of Oxidative Stress-Inducible LipD of Mycobacterium tuberculosis H37Rv
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Abstract:	The Mycobacterium tuberculosis has developed intricate strategies to evade the killing of microorganism and support its survival in phagocytes. The genome sequence of bacterium revealed the presence of several genes for lypolytic enzymes. Rv1923 gene, a member of Lip family in M. tuberculosis demonstrated the least sequence similarity with its counterpart in non-pathogenic strain M. smegmatis. The expression of Rv1923 gene (LipD) was not observed in in vitro growing cultures of M. tuberculosis H37Ra while an upregulation of transcription of Rv1923 gene was noticed in oxidative conditions. For detailed characterization of LipD enzyme the Rv1923 gene was cloned in pQE30-UA vector and expressed in E. coli M15 cells. LipD was purified from inclusion bodies and refolded with nearly 40 % protein yield. The specific activity of enzyme was calculated to be 16 U/mg with pNP-palmitate as a preferred substrate. Kinetic analysis showed K m 0.645 mM and V max 24.75 U/ml with pNP-palmitate. Ser-102, Asp-342, and His-369, predicted as the members of the catalytic triad, were confirmed by mutagenesis. Mutagenesis studies revealed that catalytic serine residues located in β-lactamase motifs (S-X-X-K) were responsible for lipolytic activity. Secondary structure analysis by CD spectroscopy demonstrated the presence of α helices and β sheets in the canonical structure of LipD. The enzyme was stable up to 50 C and was active even at pH 6.0. The expression of enzyme under stress conditions and its activity and stability at high temperature and low pH suggested the possible role of LipD in the survival of mycobacterium in macrophage compartment.
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