Expression and purification of *Mycobacterium tuberculosis* ß-ketoacyl-ACP reductase

Rosado, L.A., Silva, R.G., Santos, D.S., Basso, L.A.

Centro de Pesquisas em Biologia Molecular e Funcional, PUCRS

ß-ketoacyl-ACP reductase (MabA) is responsible for the second step of the fatty acid elongation system type II of bacteria, plants, and apicomplexan organisms, catalyzing the NADPH-dependent reduction of ß-ketoacyl-ACP to generate ß-hydroxyacyl-ACP and $NADP^+$. Here, the *mabA*-encoded ß-ketoacyl-ACP reductase from *Mycobacterium tuberculosis* has been expressed in soluble form and purified to homogeneity. The pET23a(+) vector harboring the MabA-encoding gene was introduced into *E. coli* BL21(DE3) host cells by electroporation and, after selection, the cells had been grown in LB medium until an OD_{600} = 0.5 was reached. IPTG (0.1 mM) was added to the culture, which was allowed to grown for additional 5 h and harvested by centrifugation. The pellet was re-suspended in 100 mM HEPES pH 7.0 (buffer A) and disrupted by sonication. The soluble sample was loaded on a High Trap Blue column and eluted with a linear gradient of 2M NaCl. The fractions containing MabA were pulled and applied to a Sephacryl S-200 column, which was washed with buffer A. The MabA-containing fractions were loaded on a Mono-S column and eluted with a linear gradient of 0.5M NaCl. SDS-PAGE analysis revealed no other protein was present in the eluted sample. The homogeneous MabA showed a specific activity of 12 U mg^{-1}. These results have paved the way for currently underway kinetic and thermodynamic studies on the Mab-A catalyzed reaction.