

Rv0805: Cyclic Nucleotide Phosphodiesterase from *M. tuberculosis*

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Cyclic nucleotides play a crucial role in signaling pathways as second messengers and are generated by nucleotide cyclases. The intracellular level of cyclic nucleotides is controlled by the presence of cyclic nucleotide phosphodiesterases. In our efforts to understand the role of cyclic nucleotides in bacterial systems, our attempt is to systematically characterize the relevant proteins from mycobacteria. Bioinformatic analyses revealed the presence of a protein Rv0805 from *M. tuberculosis* related to a recently characterized phosphodiesterase from *E. coli* (Class III). We developed a large-scale expression and purification procedure for Rv0805, which was shown to be a potent cAMP-phosphodiesterase. Rv0805 appears to be expressed mainly as a dimer in several expression strains of *E. coli*. The protein is partially nicked, which however doesn't seem to disturb the dimer formation. The biological role of the cleavage is not known yet. The position of the proteolytic cleavage was determined by the N-terminal sequencing and mass spectrometry of the nicked protein. Rv0805 is a metallo-enzyme. Highly concentrated solutions of the enzyme (several 10 mg/ml) are colored brown. The presence of iron and manganese in the active site was shown by the Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Crystallization of the phosphodiesterase Rv0805 is in progress.

Keywords: cyclic nucleotides, phosphodiesterase, metallo enzymes