Cloning, Expression and Characterization of a Protein Phosphatase 2C from *Mycoplasma synoviae*

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Protein phosphorylation is a critical mechanism regulating a number of cellular processes in eukaryotes and in a variety of pathogenic bacteria. *Mycoplasma synoviae* is a major pathogen of poultry throughout the world, being the causative agent of respiratory diseases, synovitis and systemic diseases. The analysis of *M. synoviae* genome reveals the presence of a gene (*prpC*) corresponding to a putative protein phosphatase 2C (PP2C). This work presents the cloning, expression and preliminary characterization of this serine/threonine protein phosphatase from *M. synoviae*. The gene *prpC* was amplified by PCR from genomic DNA of *M. synoviae* 53 and cloned into the expression vector pET14b. The recombinant protein (PrpC) was purified by metal affinity chromatography. Using p-nitrophenyl phosphate as substrate we determined the optimal PrpC activity at pH 9.5 with preference for Mn$^{2+}$ over Mg$^{2+}$. Through size-exclusion chromatography we discovered that PrpC is a monomeric enzyme with a molecular mass of 30.0 kDa, and its identity was confirmed by mass spectrometry. Circular dichroism (CD) analysis showed that PrpC exhibits an α-helical secondary structure profile. We also evaluated the effect of Mn$^{2+}$ and Mg$^{2+}$ on the overall structure of PrpC by CD and fluorescence. The presence of these metals does not cause major changes in the overall structure. However, when we analyzed the PrpC denaturation profile by CD in the presence and absence of these metals, we observed that in the absence of metals the protein is more susceptible to thermal denaturation. Altogether, these results demonstrate that PrpC is an active protein phosphatase 2C.

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