Activity *in vitro* and Identification of Targets Phosphorylation of Ser/Thr Phosphatase Sit4 from *Saccharomyces cerevisiae*

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The gene *SIT4* from the yeast *Saccharomyces cerevisiae* encodes a serine / threonine phosphatase that regulates processes such as control of cell cycle, glycogen metabolism and cell wall integrity. Sit4p participates in a conserved pathway TOR (*target of rapamycin*) that is linked to the availability of nutrients for cell growth and is activated in cancer cells. In this work we have cloned and expressed the protein Sit4p determined a protocol to purify and characterize its activity. The optimal activity for ?NPP hydrolysis was of 37°C in buffer 100mM Tris-HCl pH 7.5, 5mM ß-mercaptoethanol, 12.5 µM $MnCl_2$, 4.5 mM ?NPP and 300mM NaCl. The maximum velocity in these conditions is 12.61 nmoles.mg/min and Km of 2.13 mM for ?NPP. Using 1mM of cantharidin its activity was inhibited by 80% in a non-competitive manner. Molecular modeling experiments showed that cantharidin binds to Mn^{2+}, an essential co-factor for the activity of Sit4p. Differential proteomic analysis of phosphorylated proteins gave us two targets of Sit4p involved in the metabolism of glucose: fructose bi-phosphate aldolase and pyruvate decarboxylase 1. To confirm this data we measured pyruvate decarboxilase activity in extracts and show that deletion of *SIT4* when compared to wild-type show reduction in 42% the activity of pyruvate decarboxylase.