EXPRESSION AND PURIFICATION OF RECOMBINANT SMATPDASE2 PROTEIN (CD39-LIKE) FROM THE PARASITE SCHISTOSOMA MANSONI

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ATP-diphosphohydrolase 2 (ATPDase2) belongs to a family of enzymes that cleave ATP and ADP to AMP and Pi and are involved in inhibition of platelet aggregation. We have recently found it in the S. mansoni tegument by coimmunolocalization. The full-length sequence encodes a 564 aa protein, containing a single N-terminal end transmembrane region similar to other soluble ATPDase proteins such as human CD39L2 and CD39L4. Here we show expression of the cloned sequence that encodes the hydrophilic region (482 aa) fused with an hexa-histidine tag. E. coli BL21(DE3) and the pET21b vector were used, and inclusion bodies were obtained. Refolding and purification on column were performed but enzymatic activity was not detected. Subsequently, we coexpressed SmATPDase2 together with two chaperons (Cpn60 and Cpn10 from Oleispira antarctica) in E. coli at 12 °C, overnight, and obtained a soluble protein. Preliminary purifications using Ni-NTA and RR120 affinity columns showed in both elution fractions the presence of recombinant ATPDase2 with ~50kDa. Western blot using antibodies anti-his-tag and anti-ATPDase2 confirmed the identity of the protein. In parallel, expression of SmATPDase2 in P. pastoris strain GS115 is being performed using the expression vector pPIC9K. Further characterization of the soluble purified protein will include enzymatic activity and circular dichroism spectroscopy.

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