Cloning, expression, crystallization and structure resolution of the enzyme Adenosine Kinase from *Schistosoma mansoni*

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Schistosoma mansoni, does not posses the "the novo" purine pathway and depends on it's host for purire requirements. The enzyme Adenosine Kinase (AK) (E.C 2.7.1.20) is one key component of this pathway. AK catalyzes reaction adenine + ATP ⇔ AMP +ADP. The cDNA of the AK was amplified from a total mRNA, using specific reverse primer by PCR. The AK gene was inserted in pET28a expression vector. The AK protein was produced after IPTG induction. The protein was purified using two affinity steps (nickel and AMP-agarose) yielding 4mg/L. The purified protein was concentrated until ~4mg/mL and was submitted to robotic crystallization trials. The AK was crystallized in 100mM Bis-tris pH 6.5, 25% PEG 3350 and 200mM $LiSO_4$ in two different crystal forms. Two datasets were collected in the LNLS MX2 beamline up to 2.3Å in complex with AMP and up to 2.8Å in the presence of ATP. The AK-AMP complex belong to the space group $P2_12_12$ with cell dimensions of a=59.9Å, b=180.3Å and c=78.3Å, the second crystal in a complex with ATP belongs to the space group $P3_2$ with cell dimensions of a=b=76.4Å and c= 392.3Å. The AK-AMP complex was solved by molecular replacement (MR) using Phaser employing human AK as a search model (1BX4), the AK-ATP complex was also solved by MR using partially refined AK-AMP structure as a search model. These structures are currently under refinement. We expected that Schistosoma AK structures could help the development of new compounds against schistosomiasis.

Supported by Fapesp and CNPq.