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Short communication

Structural and kinetic studies of *Schistosoma mansoni* adenylate kinases

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ABSTRACT

The human parasite *Schistosoma mansoni* is totally dependent on the purine salvage pathway in order to supply large quantities of purine precursors for its energy and DNA biosynthetic needs. Adenylate kinase (ADK) is responsible for the conversion of AMP (produced by the adenosine kinase reaction) into ADP, which is subsequently converted into ATP by nucleoside diphosphate kinase (NDPK). ADK and NDPK are the most active enzymes of the pathway, probably reflecting an evolutionary adaptation due to the intense use of the branch in which they participate. However, notwithstanding their importance very little information has been accumulated found regarding these enzymes. In this work two adenylate kinases from *S. mansoni* were cloned and heterologously expressed in *Escherichia coli*. The purified products were utilized in activity assays, and displayed kinetic parameters similar to the corresponding human orthologous proteins. The cytosolic *S. mansoni* ADK was crystallized and its structure solved allowing us to detect a difference in the nucleotide binding site when compared with the human ortholog.

Schistosoma mansoni is a human flatworm parasite, which is one of the agents responsible for the disease schistosomiasis. Unlike their human host, schistosomes and schistosomules are totally dependent on the salvage pathway to obtain purine nucleotides [1,2]. This pathway was elucidated in adult and schistosomules [1–3] and is composed of 14 enzymes in three sub-pathways, which form ATP and/or GTP from adenosine (Fig. S1). The sub-pathway composed of nucleoside and nucleotide kinases (adenosine kinase – AK, adenylate kinase – ADK and nucleoside diphosphate kinase – NDPK), which converts adenosine into ATP through AMP and ADP intermediates, accounts for approximately one-third of ATP production from adenosine as a precursor [1]. The activity for these kinases was first identified in 1973 and is higher when compared to various mammalian tissues, and also when compared to the other enzymes involved in purine salvage [4,5]. Another branch that produces ATP, and also employs the enzymes ADK and NDPK is the indirect pathway mediated by adenosine phosphorylase and adenine phosphoribosyltransferase, which is responsible for the remaining one-third of ATP production [5].

Schistosomes also convert the adenosine analogs 2-fluoroadenosine (2FA) and 2-fluoro-2′-deoxyadenosine into nucleotides via adenosine kinase (AK) reaction [6]. The catalysis of 2FA to 2FAMP was confirmed by using recombinant *S. mansoni* AK (to be published). Thus, SmAK converts 2-fluoro-AMP or 2-fluoro-2′-deoxy-AMP into 2-fluoro-ADP or 2-fluoro-2′-deoxy-ADP which are subsequently used to provide nucleoside triphosphate analogs via their nucleoside triphosphates. This is markedly different to the activity of ADK from human erythrocyte, in which 2-fluoro-2′-deoxyadenosine does not progress beyond the monophosphate nucleotide level, indicating that 2-fluoro-2′-deoxy-AMP is not a substrate for human ADK, demonstrating a different substrate specificity in comparison to *S. mansoni* ADK [6]. This sub-pathway is also responsible for the conversion of formycin A and tubercidin into their respective triphosphate analogs [6]. In this context, SmADK has a key function in the purine salvage pathway in *S. mansoni*.

Adenylate kinases (ADKs) are ubiquitous enzymes that catalyze the reversible reaction of phosphoryl exchange between nucleotides ATP + AMP ↔ ADP, which is critical for the cell's life cycle and is recognized as a sensitive reporter of the cellular energy state, translating small changes in the balance between ATP and ADP into relatively large changes in ATP concentration [7]. ADK provides a unique buffering role against rapid concentration changes of any component of this system [8]. Structurally, ADKs are composed of three domains: a core domain, containing a parallel β-sheet surrounded by α-helices; an NMP-binding domain (NMP domain), where the AMP binds, and a LID domain, that covers the nucleotides [9]. The core domain has a P-loop motif that interacts with the ATP phosphates. Usually, the LID and NMP domains undergo large conformational changes during the catalysis [10]. There are two canonical conformations for ADKs; the open conformation, for the apo enzyme, and the closed conformation, for the enzyme–ligand

Abbreviations: AK, adenosine kinase; ADK, adenylate kinase; NDPK, nucleoside diphosphate kinase; 2FA, 2-fluoroadenosine.

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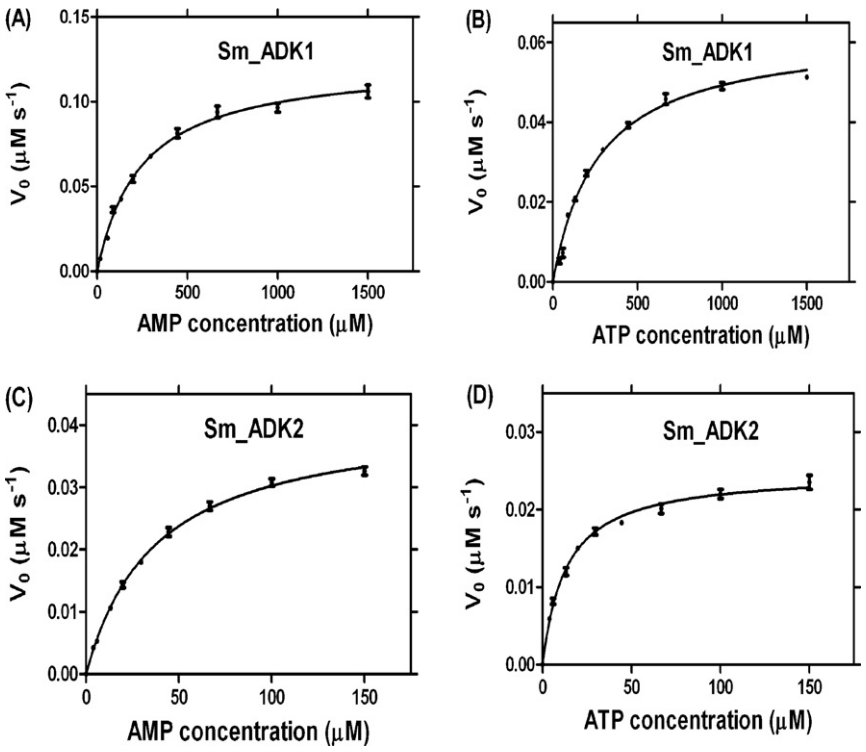

Fig. 1. Michaelis–Menten plot for AMP and ATP as substrate for SmADK1 (A and B) and SmADK2 (C and D). The reaction buffer used was 50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 5 mM $MgCl_2$, 1 mM phosphoenolpyruvate, 5 U of lactate dehydrogenase and pyruvate kinase. The SmADK1 assay used 0.4 mM β-NADH and 1.5 mM ATP, whilst that for SmADK2 used 0.2 mM β-NADH and 250 μM ATP. The SmADK concentration used in these assays was 5 nM. Each point was measured in quadruplicate. (A) Assay for SmADK1 with AMP as variable substrate. The assay was performed in 12 points starting with AMP concentration of 1500 μM using a serial dilution of 1:1.5. (B) Assay for Sm_ADK1 with ATP as substrate. Ten data points were measured starting with an ATP concentration of 1500 μM using a serial dilution of 1:1.5. In the SmADK2 assay 10 data points were measured using an initial concentration of ATP or AMP of 150 μM in 1:1.5 serial dilution. Assay for SmADK2 with AMP (C) and ATP (D) as variable substrate.

complex, as have been observed in ADK structures from *Escherichia coli* [11]. Based on the length of the LID domain, the ADKs are classified as either short or long. In the long isoforms the LID domain has 25–40 residues and has typically four β-strands. As for the short isoforms, the LID domain is just a small loop.

In this work we present the kinetic characterization of two adenylate kinases from *S. mansoni*, designated SmADK1 (Smp_071390; XM_002578280.1) and SmADK2 (Smp_061940.3; XM_002577421.1) and the crystallographic structure of SmADK1. SmADK1 and SmADK2 code for proteins of 197 and 239 residues and predicted molecular weights of 22.3 and 27.2 kDa, respectively. These ADKs share only 28% sequence identity. When compared to their human counterparts, the SmADK1 shares 54% with HuADK1 and SmADK2 shares 51% compared to HuADK2. SmADK1 is a short type adenylate kinase and SmADK2 is a long one. Previous work has shown that SmADK1 is produced during in vitro miracidium-to-sporocyst transformation of *S. mansoni* [12], indicating substantial energy requirements during this transformation.

The two target sequences of adenylate kinases were identified by searching the *S. mansoni* genome (http://www.genedb.org/Homepage/Smansoni). The genes were amplified from an enriched transcript library using the following primer pairs: SmADK1_F-NheI: 5′-ACT GTA TCA TAT GAT GAC TGA TCA GAA GTT AGC CAA AGC-3′; SmADK1_R-XhoI: 5′-TAC AGT CTC GAG TTA TTT CAC ACC GAA TTT TTG AAG-3′; SmADK2_F-NheI: 5′-CTG CAT ATG TTG TAT GAT TTC GCT AGT TGG TAT AG-3′; SmADK2_R-XhoI: 5′-CAG TCT CGA GCT ATT TTT CCT CTG CTA ATT TCG-3′ and cloned into the expression vector pET28a(+) in NheI and XhoI restriction sites. The recombinant vectors were used to transform *E. coli* BL21 (DE3) cells. Expression was induced with 0.1 mM IPTG for 4 h at 37 °C (SmADK1) and overnight at 18 °C (SmADK2). The yields were 20 mg/L and 8 mg/L for SmADK1 and SmADK2 respectively. The purification of the enzymes was performed by nickel affinity chromatography followed by gel filtration chromatography using a Superdex-200 column after overnight cleavage with thrombin. Both adenylate kinases presented a monomeric gel filtration profile. The final buffer for the enzymes was 20 mM Tris–HCl (pH 7.4), 150 mM NaCl and 5 mM β-mecarptoethanol.

The activity assays were performed on a Spectramax plus 384 in 96 well UV microplates by continuous monitoring at 340 nm of the consumption of β-NADH in a coupled assay [13]. The kinetic parameters for SmADK1 and SmADK2 for both AMP and ATP substrates were determined. The SmADK concentration used in these assays was 5 nM. Fig. 1A and B shows the plot of the initial velocity for SmADK1 and Fig. 1C and D for SmADK2, for both AMP and ATP. The data were fitted using the non-linear Michaelis–Menten equation using the GraphPad prism software. The values of K_{Mapp} for SmADK1 for ATP and AMP were 272 ± 16 and 244 ± 14 μM, respectively. These values are in the same range as those for human ADK1: 230 μM and 180 μM, also for ATP and AMP respectively [14]. The K_{Mapp} values of SmADK2 for ATP and AMP were 13 ± 0.8 and 37 ± 2 respectively, as observed in Table 1. These values are also in agreement with those for human liver mitochondrial ADK2 which presents a K_{Mapp} of 12 μM for ATP and 56 μM for AMP [8]. These kinetic data indicate that the selective pressure is not for a decrease in K_M values within a species, but at the level of enzyme expression, as demonstrate by the early work of Senft and coworkers [5], who showed high levels activity of both ADK and NDPK in *S. mansoni* extracts.

Crystallization assays were conducted on the Honeybee 963 robot using several crystallization kits from Qiagen and Hampton Research and an ADK concentration of 10 mg/mL in Greiner CrystalQuick 96 plates. Several attempts were conducted in the