In silico analysis of binding capability of nucleosides in PKA regulatory subunit of *Trypanosoma cruzi*

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Cyclic AMP (cAMP) signalling has shown to be essential in *Trypanosoma cruzi* biology. However, little is known about downstream effectors in the cAMP-dependent pathways in the parasite. Previous reports showed the presence of several ORFs in *T. cruzi* genome that possess putative Cyclic Nucleotide Binding domains (CBDs), such as cAMP Responsive Protein 1 (CARP1) and Protein Kinase A (PKA). However, while it has been demonstrated that CARP1 binds cAMP, unlike its mammalian counterpart, PKA from *T. brucei* and *T. equiperdum* are unable to bind the cyclic nucleotide. In this regard, sequence alignment with canonical cAMP effectors have shown differences in some key aminoacids in the Phosphate Binding Cassette (PBC) of the CNB present in PKA, a crucial region for cAMP-protein interaction. In order to increase the knowledge about cAMP and other nucleosides mediated pathways of *T. cruzi*, we modelled two proteins homologues to the regulatory subunit of mammalian PKA, TcCLB.506227.150 and TcCLB.510879.50, which have differences in some residues that could elicit changes in nucleotide and nucleoside binding.

Metodology

A BLAST search was performed in order to find homologues with both PKA Regulatory subunits (PKAR) of *T. brucei* (UniProt ID: Q385V6) and *T. equiperdum* (UniProt ID: A0A068EPY7), using *T. cruzi* CL Brener strain as target organism. Two protein sequences with the lowest E-values were retrieved from TritrypDB in their FASTA format: **TcCLB.506227.150 (TcPKAR)** and **TcCLB.510879.50 (TcPKAR_like)**. A multiple alignment was implemented with canonical CBD-containing proteins and these two candidates, using ClustalW algorithm. Protein sequence of each candidate was submitted to SWISS-MODEL web service for the elaboration of tridimensional protein structures, using the best predicted templates for homology modelling; PDB ID: 6FLO for TcPKAR and PDB ID: 6H4G for TcPKAR_like (both templates corresponding to TbPKAR, co-crystallized with inosine). Energy minimization of each PDB file was performed with *FoldX* software. Rigid docking simulations with nucleosides (inosine, adenosine and guanosine) were built using *Smina* (*Vinardo* algorithm) for each minimized model, previously converted into PDBQT files with AutodockTools software (ADT), with default conversion parameters. Another 3 deazanucleosides analogues (3-deazaguansine; 9-deazainosine and 7-iodo-7-deazaadenosine), with anti-trypanosomal activity reported, were also included in docking simulations. All generated structures were visualized and analyzed with PyMOL.

Trypanosomatid PKARs lacks an arginine on both PBCs, a crucial residue for cAMP-protein interaction

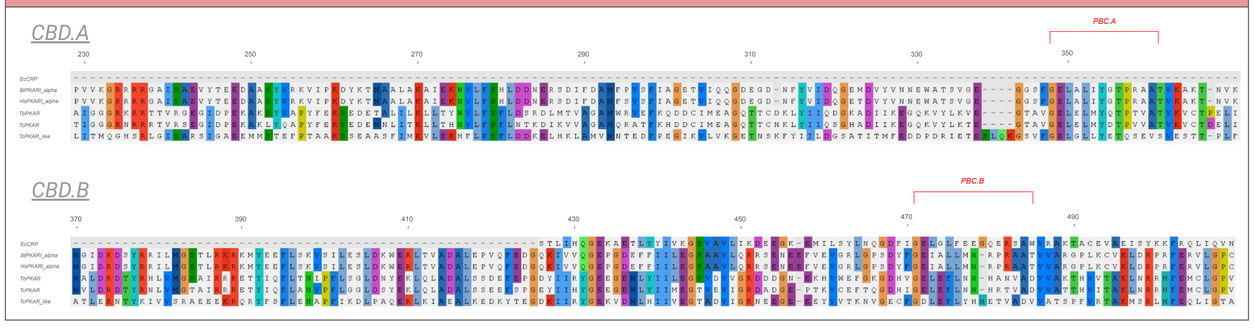

Figure 1. Multiple sequence alignment of canonical cAMP-bound proteins and Trypanosomatid PKARs. Both CBDs of TcPKAR and TcPKAR_like were aligned with known cAMP effectors from different species: *E. coli* Catabolite Response Protein (Uniprot ID: A0A0A6V8W6), *B. taurus* and *H. sapiens* PKA Regulatory subunits (P00514 and P10644, respectively), using ClustalW algorithm. PKA regulatory subunit of *T. brucei* (Q385V6) was also included in the alignment. The Phosphate Binding Cassette (PBC) of each domain, a highly conserved region within all CBDs and critical for cAMP binding, is marked with red lines above each alignment. Aminoacid positions are marked relative to TcPKAR_like.

PKAR and PKAR_like 3D models generated were highly conserved and structurally favoured

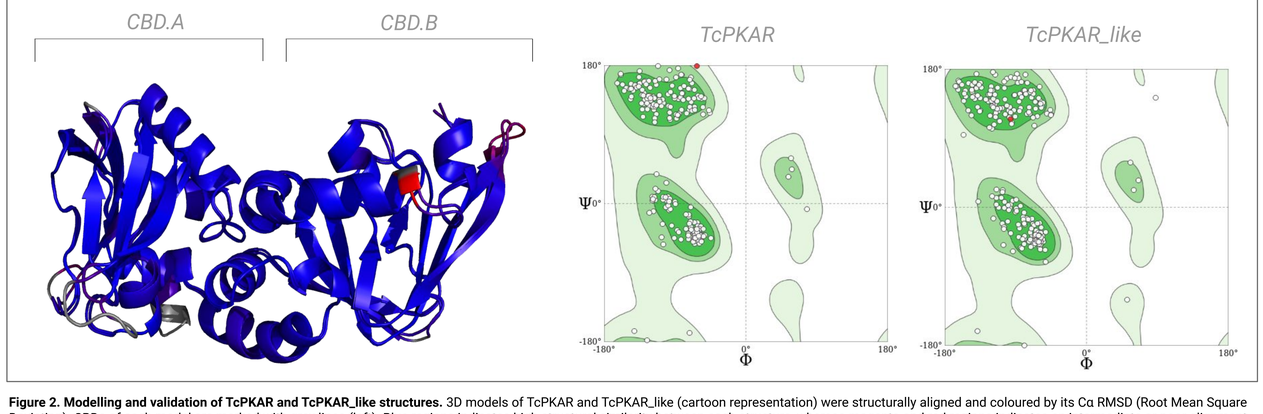

Figure 2. Modelling and validation of TcPKAR and TcPKAR_like structures. 3D models of TcPKAR and TcPKAR_like (cartoon representation) were structurally aligned and coloured by its Cα RMSD (Root Mean Square Deviation). CBDs of each model are marked with grey lines (left). Blue regions indicates high structural similarity between each structure, whereas magenta and red regions indicates an intermediate or poor alignment, respectively. Grey regions correspond to unaligned residues. Average RMSD obtained was 0.87 Å. Ramachandran plots were also generated in order to validate each model by its phi/psi (φ/ψ) torsion angles on each residue (white and red dots; right). There were no unfavoured torsion angles for TcPKAR model, while only 1 residue was sterically forbidden (ARG 333) for TcPKAR_like. Red dots on each plot indicates bad clash scores for atoms on that residue (*i.e* non-donor–acceptor atoms overlapping by more than 0.40 Å). These outliers were not given critical importance since they are not ubicated on hypothetical binding site of nucleosides. Energy minimization and rotamer outliers were corrected using FoldX service, before proceeding to docking simulations.

Docking simulations shows favourable ligand poses for most nucleoside and deazanucleosides in both CBDs of TcPKAR, but not for TcPKAR_like

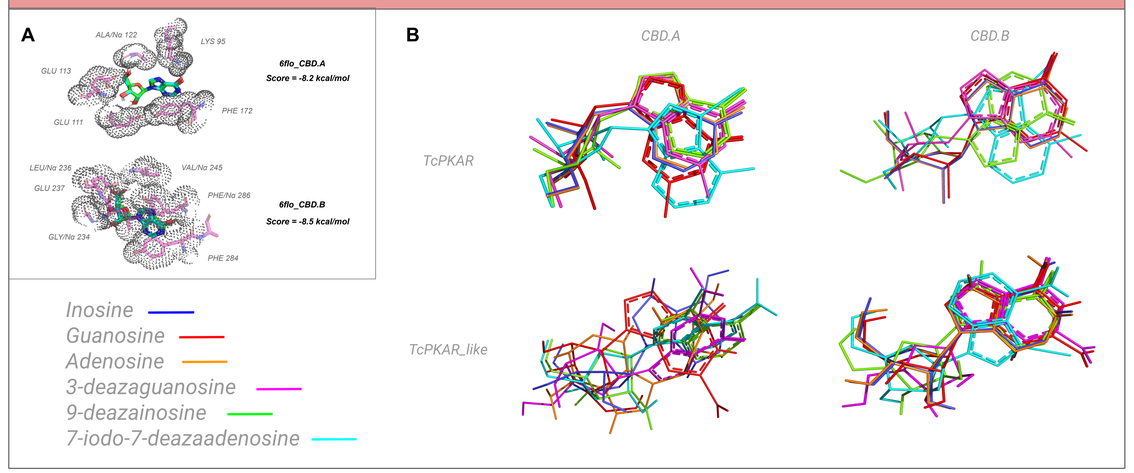

Figure 3. Rigid docking simulations of 6 different nucleosides in PKA regulatory subunit models of *T. cruzi* (A) In order to validate the docking algorithm used in this work, Inosine co-crystallized with TbPKAR (PDB ID: 6flo) was re-docked, showing an almost identical ligand superposition (carbons in cyan representing crystallized inosine; carbons in green showing best ligand pose predicted by the algorithm). Nearby residues within a maximum distance of 3.5 Å are shown with dots and transparent sticks representation (carbons in magenta). **(B)** 6 proposed ligands for TcPKARs were superimposed according to best predicted pose on each domain. Each color in lines representation represent a different ligand, as indicated at the bottom left of the figure.

		Vinardo Docking Score (kcal/mol)					
		Inosine	Guanosine	Adenosine	3-deazaguanosine	9-deazainosine	7-iodo-7-deazaadenosine
TcPKAR	CBD.A	-8.6	-5.5	-8.6	-8.3	-8.8	-8.6
	CBD.B	-5.5	-5.2	-5.4	-3.1	-4.0	1.3
TcPKAR_like	CBD.A	-3.3	-3.3	*n.a*	-4.4	-4.7	-4.2
	CBD.B	-5.2	-4.6	-5.2	-4.1	-4.0	-2.4

Table 1. Docking scores obtained from TcPKAR and TcPKAR_like. Best docking score values obtained between each receptor (TcPKAR and TcPKAR_like) and ligands (nucleosides and deazanucleosides), as indicated in the table. There was no any favoured ligand pose predicted for adenosine in CBD.A of TcPKAR_like, so no docking score was assigned in this case.

Highlights

- Despite conservation of some important residues that are reported to confer stability to both CBD structures , multiple sequence alignment shows differences on each PBC between canonical cAMP effectors and trypanosomatid PKAs. These different residues (particularly in ARG482, important for cAMP binding by its phosphate group) could elicit differences in ligand specificity.
- Despite low sequence identity between TcPKAR and TcPKAR_like (36.01%), each 3D model of both CBDs shows high structural similarity between them and, maybe, showing similar affinity for same ligands.
- TcPKAR appeared to be a stronger candidate to bind nucleosides and its analogs, compared to TcPKAR_like. CBD.A of TcPKAR presents similar affinities and favourable ligand poses for proposed ligands (except for guanosine) to that compared of TbPKAR with inosine, used as control. CBD.B predicted minor affinities, but with favourable ligand poses. CBD.A of TcPKAR_like presented low affinities for each ligand, with also unfavoured ligand positions on the PBC. CBD_B showed a pattern similar to CBD_B of TcPKAR.

References: **i.** Berman et al., Proc Natl Acad Sci U S A. 2005. doi:10.1073/pnas.0408579102; **ii.** Jäger et al., Mol Biochem Parasitol. 2015. doi.org/10.1016/j.molbiopara; **iii.** Calabokis et al., J Immunoass Immunochem. 2016. dx.doi.org/10.1080/15321819.2016.1162799; **iv.** Gould et al., Antimicrob Agents Chemother. 2013. dx.doi.org/10.1128