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Cross-species activation of trypanosome S-adenosylmethionine decarboxylase by the regulatory subunit prozyme

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Abstract

The protozoan parasite *Trypanosoma cruzi* is the causative agent of Chagas disease (American trypanosomiasis), a neglected disease of Central and South America. Polyamines are small organic cations that are required for cell growth and their biosynthesis has been the target of drug discovery efforts in both *T. cruzi* and the related *T. brucei* parasites. Here we show that, as previously demonstrated for *T. brucei*, S-adenosylmethionine decarboxylase (AdoMetDC) from *T. cruzi* forms a heterodimer with prozyme, an inactive homolog that arose by gene duplication of the canonical enzyme uniquely in the trypanosomatids. The *T. cruzi* AdoMetDC/prozyme heterodimer is 110-fold more active than homodimeric AdoMetDC. Unlike for *T. brucei* AdoMetDC, the activity of the *T. cruzi* heterodimer is further stimulated by putrescine to generate an enzyme with similar catalytic efficiency to the fully activated *T. brucei* enzyme. The effects of prozyme on *T. cruzi* AdoMetDC are mediated by an increase in k_{cat}, while the predominant effect of putrescine is to lower the K_m. Finally we demonstrate that the cross-species heterodimers of *T. cruzi and T. brucei* AdoMetDC and prozyme pairs are functional, and that putrescine is required for prozyme to fully activate the mixed species heterodimers. These data demonstrate that prozyme mediated activation of AdoMetDC is a common mechanism required to regulate AdoMetDC activity in the trypanosomatids.

Keywords

Trypanosoma cruzi; Trypanosoma brucei; polyamines; putrescine; regulation

Introduction

The trypanosomatid parasites, *Trypanosoma cruzi* and *Trypanosoma brucei* cause significant human and veterinary disease worldwide [1-3]. In Africa 50 million people are at risk for contracting African sleeping sickness caused by *T. brucei*. The disease is fatal if untreated. In South America the causative agent of Chagas disease,*T. cruzi*, has an incidence of 200,000 cases each year and over 13 million people are chronically infected with the parasite. Chagas disease is a major cause of cardiomyopathy in the region. Current therapeutics for both Chagas

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disease and African sleeping sickness are limited, underscoring the need for the development of new and improved anti-trypanosomal drugs and drug targets.

Polyamines are small organic cations that are required for cell growth and proliferation [4]. Polyamines are synthesized from L-ornithine and decarboxylated S-adenosylmethionine (dcAdoMet) (Scheme 1), and the biosynthetic enzymes ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylases (AdoMetDC) have been studied as chemotherapeutic targets. α-Difluoromethylornithine (DFMO), a suicide inhibitor of ODC, is used clinically for the treatment of African sleeping sickness [5]. In contrast, *T. cruzi* does not encode an ODC gene, and therefore DFMO is not effective against this parasite (Scheme 1) [6,7]. Instead, the intracellular *T. cruzi* pathogen relies on putrescine uptake to obtain this polyamine precursor, which is facilitated by the abundance of putrescine found within host cells [8]. *T. cruzi* epimastigotes are also able to transport spermidine [9]. In contrast, *T. brucei*, an extracellular parasite, relies on *de novo* polyamine synthesis since there are limiting amounts of polyamines in the blood and lymph.

AdoMetDC catalyzes the decarboxylation of S-adenosylmethione to form dcAdoMet, which then serves as the amino propyl donor for the formation of spermidine from putrescine (Scheme 1). Both *T. cruzi* and *T. brucei* contain the gene for AdoMetDC, and inhibitors of this enzyme have been demonstrated to cure murine models of infection with either trypanosome species [10-12]. AdoMetDC is a pyruvoyl-dependent enzyme that derives the pyruvoyl cofactor from an auto processing reaction that cleaves the polypeptide chain into a larger α-chain, containing the pyruvate at the N-terminus, and a smaller β-chain [13]. The human AdoMetDC enzyme is a homodimer ($\alpha_2\beta_2$) that is allosterically activated by putrescine [14,15]. Previous work has shown that the recombinant AdoMetDC enzyme from *T. cruzi* forms a homodimer that is stimulated by putrescine, however it has a much lower catalytic efficiency than human or plant homologs [16-18]. We recently demonstrated that the *T. brucei* AdoMetDC is allosterically activated by a regulatory protein, prozyme [19]. Prozyme is an inactive paralog of AdoMetDC that arose by gene duplication and is found only in the trypanosomatids. *T. brucei* prozyme and AdoMetDC form a high affinity heterodimer that is the functional species of the enzyme in the parasite. Furthermore prozyme protein expression levels are regulated in *T. brucei*, providing a mechanism to control pathway flux that is unique to these parasites [20].

The functional role for prozyme as an activator has so far only been demonstrated for *T. brucei* AdoMetDC, though phylogenic analysis suggests that both *T. cruzi* and *Leishmania* AdoMetDC will also be activated by prozyme [19]. Here we show that *T. cruzi* AdoMetDC is also activated by prozyme. The AdoMetDC/prozyme heterodimer from *T. cruzi* forms with high affinity stimulating the activity of the enzyme; however, in contrast to the *T. brucei* enzyme, the activity of the *T. cruzi* heterodimer is putrescine-dependent. Finally we demonstrate that prozyme and AdoMetDC from *T. brucei* and *T. cruzi* are able to form functional cross-species heterodimers, demonstrating that the dimer interface and mechanism of activation are conserved between the species.

Materials and Methods

Cloning of T. cruzi prozyme and AdoMetDC

The expression construct (TcAdoMetDC) for *T. cruzi* AdoMetDC has been described previously [16]. The gene for *T. cruzi* prozyme was found by blast search of geneDB (http://www.genedb.org/, ID: Tc00.1047053509167.110) using *T. brucei* prozyme as the search sequence. *T. cruzi* prozyme was cloned and amplified by PCR from genomic DNA, and ligated into the pET 15b vector generating an N-terminal His_6-tagged construct. A double expression vector (DEV) that allows for coexpression of His-tagged AdoMetDC and untagged prozyme in *E.coli* was created. First, the *T. cruzi* prozyme gene was introduced into the pET22b

with its stop codon to produce an untagged protein. Then, the ribosomal binding site (rbs), the T7 promoter and the His_6-AdoMetDC coding region was amplified by PCR from the TcAdoMetDC construct and ligated into this vector to create DEV. Primers used for cloning were as follows: Tc prozyme-Forward for pET 15b (5′-CGCCATATGTTGGAGAGCACCTGGGCAGCCG-3′, includes an Nde I site); Tc prozyme-Reverse for pET 15b (5′-CGCGGATCCTTATTCGGCGCGAATATAGCTGG -3′, includes a BamHI site); Tc DEV-Forward for pET 22b-DEV (5′-CGATAGTCGACTCGAAATTAATACGACTCACTATAGG-3′, includes a SalI site); Tc DEV-Reverse for pET 22b-DEV (5′-TATGCGGCCGCTTACTACTCTTCCACAGAATCTGTGG-3′, contains a NotI site).

Expression and purification of T. cruzi prozyme and AdoMetDC/prozyme complex

T. cruzi AdoMetDC or prozyme were expressed individually in *Escherichia coli* BL21/DE3 cells containing the respective construct, and purified in two steps by Ni^{2+}-agarose (Qiagen) and anion exchange column chromatography (Amersham Mono Q 5/50 GL column) as described [16], except that the buffer was exchanged after elution from Ni^{+2} agarose and Mono Q by a HiPrep 26/10 desalting column (Amersham) equilibrated in storage buffer (50 mM Hepes pH 8.0, 50 mM NaCl, 1 mM dithiothreitol). For co-purification, the *T. cruzi* DEV construct was expressed in *E. coli*, and the proteins were purified as above. Purified proteins were quantified using their respective extinction coefficients (at 280 nm): AdoMetDC, 61.3 $mM^{-1}\ cm^{-1}$; prozyme, 36.9 $mM^{-1}cm^{-1}$; AdoMetDC/prozyme complex, 98.2 $mM^{-1}\ cm^{-1}$. SDS-PAGE was used to assess purity. Cloning, expression and purification of His_6-tagged *T. brucei* AdoMetDC and prozyme has been described previously [19].

Sedimentation Equilibrium by Analytical Ultracentrifugation

The molecular weight of the *T. cruzi* AdoMetDC/prozyme complex was determined by equilibrium sedimentation analysis using a Beckman XLI analytical ultracentrifuge equipped with an AN60 Ti rotor. Samples in buffer (50 mM HEPES pH 8.0, 50 mM NaCl, and 1 mM β-mercaptoethanol) were loaded at 4, 6 and 8 μM (0.1-0.12 ml total volume) into a six-sector equilibrium centerpiece and equilibrated for data collection at 14,000 and 20,000 rpm. After equilibrium was reached (approximately 24 hours), absorption data were collected at 280 nm through sapphire windows using a radial step size of 0.001 cm. Base-line absorbance readings for each cell were acquired by over speed at 42,000 rpm.

Data sets were analyzed using equations describing a single ideal species model (eq. 1), or a monomer-heterodimer model (eq. 2), as previously described [21], or for the AdoMetDC/prozyme complex globally fitted to a single ideal species model using the Beckman XL-A/XL-I Data Analysis Software version 6.0. Both analyses gave similar results.

$$A=c^{*}\varepsilon^{*}d^{*}\exp\left[\left(M^{*}(1-v_{bar}{}^{*}\rho)^{*}\omega^{2*}\left(r^{2}-r_{o}^{2}\right)/2^{*}R^{*}T\right]+\delta \qquad \text{Eq. 1}$$

$$\begin{aligned}A=&c^{*}\varepsilon_{A}{}^{*}d^{*}\exp\left[\left(M_{A}{}^{*}(1-v_{barA}{}^{*}\rho)^{*}\omega^{2*}\left(r^{2}-r_{o}^{2}\right)/2^{*}R^{*}T\right]\\&+c^{*}\varepsilon_{B}{}^{*}d^{*}\exp\left[\left(M_{B}{}^{*}(1-v_{barB}{}^{*}\rho)^{*}\omega^{2*}\left(r^{2}-r_{o}^{2}\right)/2^{*}R^{*}T\right]\\&+c^{2*}(\varepsilon_{A}+\varepsilon_{B})^{*}d^{*}K_{a,AB}{}^{*}\exp\left[\left((M_{A}{}^{*}(1-v_{barA}{}^{*}\rho)+(M_{B}{}^{*}(1-v_{barB}{}^{*}\rho))^{*}\omega^{2*}\left(r^{2}-r_{o}^{2}\right)/2^{*}R^{*}T\right]+\delta\end{aligned}$$

Where: A = Absorbance at 280 nm at radial position r; c = concentration at the meniscus in abs units; ε= extinction coefficient (TcADoMetDC monomer 61310 $M^{-1}cm^{-1}$; Tcprozyme

monomer 36900 $M^{-1}cm^{-1}$; heterodimer 98210 $M^{-1}cm^{-1}$; caclulated by ExPASy ProtParam tool: http://www.expasy.ch/tools/protparam.html); d= pathlength (1.2 cm); M = Molar mass (g/mol) (M_a = TcAdoMetDC monomer 44,000 dal; M_b = Tcprozyme monomer 37,000 dal.); V_{bar} = partial specific volume (0.73 mL/g TcADoMetDC; 0.732 mL/g Tcprozyme; 0.731 mL/g Tc heterodimer); ρ = buffer density (1.005 g/ml); ω (angular velocity) = (2*π*rpm)/60; r = radius; r_o=reference radius; R = gas constant (8.314×10^7 $ergK^{-1}mol^{-1}$); T = temperature (277 ° Kelvin); δ = offset (experimentally determined from the over speed); K_a = association constant (M). V_{bar} and ρ were calculated using the program SEDNTERP [22].

Steady-state kinetic analysis

Steady state kinetic analysis was performed by trapping liberated $^{14}CO_2$ on a filter paper soaked in saturated barium hydroxide as described [16,18,19]. Reactions were performed over a range of enzyme (1 - 4 μM homodimer or 25-400 nM heterodimer) and substrate (1-[^{14}C]-AdoMet (25 μM) plus unlabeled AdoMet (0 - 975 μM)) concentrations with or without saturating putrescine (5 mM) at 37 °C in buffer (100 mM HEPES pH 8.0, 50 mM NaCl, and 1 mM DTT). Reactions were allowed to proceed for various times (2.5 - 20 min) before quenching with 6 M HCl. Substrate titration data were fitted to the Michaelis-Menten equation (Eq. 3) to determine the steady-state kinetic parameters using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com). Samples were assayed in triplicate.

$$v_o = V_{max}[S] / (K_m + [S]) \quad \text{Eq. 3}$$

To analyze the activation of AdoMetDC by prozyme from the other trypanosome species, the proteins were incubated together with an excess of prozyme (1:10 AdoMetDC: prozyme) during the AdoMetDC assay. Substrate titration was performed as described above, and the data was analyzed by Michaelis-Menten kinetics (Graph pad Prism).

Results

T. cruzi AdoMetDC and prozyme form a tight heterodimer

We have previously shown that *T. brucei AdoMetDC* and prozyme form a heterodimer, and here we examined the recombinant *T. cruzi* proteins to determine if they also formed a complex (Figure 1 A). Recombinant *T. cruzi* AdoMetDC undergoes auto-processing to generate the larger α (32 kdal) and smaller β (10 kdal) subunits (Figure 1A, lane A), while the *T. cruzi* prozyme was not processed and purified as a single polypeptide chain (Figure 1A, lane P) that had no catalytic activity. Recombinant His_6-tagged AdoMetDC and untagged prozyme were co-expressed from a single plasmid, and then co-purified. The subunits remained associated over three chromatography steps: Ni^{+2}-agarose, anion exchange and size exclusion (Figure 1A, lane C), similar to the *T. brucei* proteins.

To further analyze the AdoMetDC/prozyme complex, we used sedimentation equilibrium on the Analytical Ultracentrifuge to determine the molecular weight and dissociation constant of the complex (Figure 1B). The data were fitted to a single ideal species model (Equation 1), which predicted a species mass of 71.8 ± 4 kDa (Figure 1B, left panel). Since the predicted size of the heterodimer is 80.6 kDa, this indicates that the sample composition contains a mixture of dimers and monomers (predicted sizes of 44 kDa for AdoMetDC or 36.6 kDa for prozyme). To determine the affinity of prozyme and AdoMetDC in the heterodimer, the data were also fitted to a heterologous association model (Equation 2), and the K_d for heterodimerization was found to be < 0.5 μM (Figure 1B, right panel). The *T. cruzi* AdoMetDC homodimer was previously found to have a dissociation constant of 15 - 30 μM [17], thus the

heterodimer forms with an affinity that is at least 25 - 50 fold higher than the homodimer, indicating that the heterodimer will be the physiologically relevant enzyme form.

Catalytic efficiency of the T. cruzi AdoMetDC is stimulated by prozyme

To characterize the catalytic efficiency of the *T. cruzi* AdoMetDC homodimer in comparison to the AdoMetDC/prozyme heterodimer, we measured the activity of the recombinant proteins over a range of substrate concentrations (Figure 2 A and B). As previously reported [16], the *T. cruzi* AdoMetDC enzyme was stimulated by putrescine and we observed a 17-fold increase in catalytic efficiency. However, in comparison to recombinant human AdoMetDC (k_{cat} = 2.6 s^{-1} and k_{cat}/K_m= 44,000 $M^{-1}s^{-1}$ in the presence of 5 mM putrescine [16]), the putrescine activated enzyme has a slower catalytic rate (k_{cat} = 0.02 s^{-1}) and lower catalytic efficiency (k_{cat}/K_m= 150 $M^{-1}s^{-1}$). In contrast, the catalytic efficiency of the *T. cruzi* AdoMetDC/prozyme heterodimer is 110-fold greater than that of the basal *T. cruzi* AdoMetDC, and the addition of putrescine to the assay further stimulated the heterodimer by 5-fold (Figure 2b and Table 1). The effect of putrescine addition on the heterodimer is seen as a decrease in K_m (~ 4 fold decrease), where the stimulation by prozyme is observed as an increase in k_{cat} (40-120 fold). In the presence of putrescine the *T. cruzi* AdoMetDC/prozyme heterodimer has a catalytic efficiency that is 550-fold greater than the unstimulated AdoMetDC homodimer, and has a similar activity to that observed for the *T. brucei* heterodimer. We have previously reported that *T. brucei* AdoMetDC was stimulated by prozyme by 1200-fold on k_{cat} [19]. Putrescine stimulates the catalytic efficiency of *T. brucei* AdoMetDC by 10-fold, however in contrast to the result for the *T. cruzi* heterodimer, the *T. brucei* heterodimer is fully active in the absence of added putrescine [19].

Prozyme activates AdoMetDC homolog from the other trypanosome species

The prozyme proteins from *T. cruzi* and *T. brucei* share 48% sequence homology while the AdoMetDC proteins are 68% homologous. This high degree of conservation prompted us to examine the ability of prozyme from one species to activate the AdoMetDC from the other species. Each recombinant protein was expressed with a His_6-tag, and the proteins were assayed in a ratio of 1:10 AdoMetDC to prozyme. The specific activity of the *T. brucei* heterodimer was found to be lower when the two subunits are mixed rather than co-purified, probably due to the aggregation of prozyme when expressed alone [19]. The activity of the *T. cruzi* heterodimer generated by mixing was likewise slightly lower than for the co-purified heterodimer (Tables 1 and 2). Both sets of cross-species heterodimers (TbAdoMetDC/ Tcprozyme and TcAdoMetDC/Tbprozyme) were evaluated by subunit mixing experiments. In the absence of putrescine, *T. cruzi* prozyme stimulated *T. brucei* AdoMetDC by 60-fold, while the *T. brucei* prozyme was able to increase the activity of *T. cruzi* AdoMetDC by 70-fold (Figure 3A, C and Table 2). This level of activation was significantly less than observed for the same species heterodimers. However when putrescine was included, both cross-species heterodimers were further activated yielding mixed species heterodimers with similar catalytic efficiency to the same species heterodimers (Figure 3B, C and Table 2). Thus, the orthologous prozyme can function with the AdoMetDC from another trypanosome species, but only in the presence of putrescine.

Discussion

The requirement for AdoMetDC to be activated by heterodimer formation with the inactive homolog, prozyme, is unique to the trypanosomatid species. Here we have demonstrated that the *T. cruzi* prozyme can form a high affinity heterodimer with AdoMetDC and increase the catalytic efficiency, similar to what we have previously reported for the *T. brucei* heterodimer [19]. We have shown that the *T. cruzi* AdoMetDC/prozyme heterodimer is further activated by putrescine, and have demonstrated that the prozyme from one species can increase the

catalytic efficiency of AdoMetDC from another species in the presence of putrescine. This confirms that prozyme activation of trypanosomatid AdoMetDC is a general mechanism in these parasites.

As previously described for *T. brucei* AdoMetDC [19], *T. cruzi* AdoMetDC forms a high activity heterodimer with *T. cruzi* prozyme. In the absence of putrescine, the *T. cruzi* AdoMetDC/prozyme heterodimer is less efficient than the *T. brucei* heterodimer. However, in the presence of putrecine, the efficiency of the *T. cruzi* heterodimer increases to a similar level as observed for the *T. brucei* heterodimer. Thus, the prozyme is required for activation of both trypanosomal AdoMetDCs, while putrescine is important only for the *T. cruzi* heterodimer. The activity of the *T. cruzi* heterodimer is increased 5-fold in the presence of putrescine. Both k_{cat} and K_m of *T. cruzi* AdoMetDC homodimer are stimulated by putrescine [18]; in contrast the effect of putrescine on the heterodimer is confined to a reduction in K_m, while prozyme acts by increasing k_{ca}. Structural studies of the human enzyme show that the dimer interface and putrescine binding sites are distant from the active site [14,23]. This indicates that both prozyme and putrescine will use allosteric mechanisms of activation; however the different molecules regulate activity by causing changes in different catalytic parameters.

The requirement for both prozyme and putrescine to fully activate the *T. cruzi* AdoMetDC/prozyme heterodimer shows that *T. cruzi* is able to regulate decarboxylation of AdoMet on two levels. This additional level of control by putrescine may possibly allow the parasite to monitor the outside environment. *T. cruzi* is an intracellular parasite, whereas the related *T. brucei* is an extracellular pathogen. While most cell types have abundant polyamine pools, the blood, where the *T. brucei* parasites live, is a poor source of polyamines. *T. cruzi* relies on putrescine uptake for the synthesis of spermidine and can also import spermidine much more efficiently than *T. brucei* [24]. The species difference in putrescine regulation of AdoMetDC/prozyme may be related to the differences in polyamine biosynthesis and environmental niche between these trypanosomes.

The structural basis for the trypanosome species differences in AdoMetDC putrescine activation are unclear as no X-ray structure of the trypanosome AdoMetDC's has been reported. Site-directed mutagenesis of the *T. cruzi* AdoMetDC residues predicted to be within the putrescine binding-site based on the human AdoMetDC structure, demonstrated that the putrescine binding-site is not well conserved between the parasite and human enzymes [16, 17]. This finding makes it difficult to predict where the putrescine binding-site will be located in the parasite AdoMetDCs. Despite this several residues have been shown to have a possible role in putrescine binding to *T. cruzi* AdoMetDC (D174, S111 and F285) and all are conserved in *T. brucei* AdoMetDC. Thus if trypanosome species differences in the putrescine binding-site are present they have not yet been identified.

The ability of the prozyme to form functional cross-species heterodimers with AdoMetDC from the other trypanosome species indicates that the amino acid residues important for the energetics of dimerization and for the activation mechanism are conserved between the two species. Interestingly putrescine is required for full activation of the cross-species heterodimers, suggesting that the *T. cruzi* component of these heterodimers may drive this requirement. Functional cross-species dimers have also been reported to form between *T. brucei* and mammalian ODC [25]. *T. brucei* and mouse ODC share ~60% sequence identity, slightly higher than the 50% sequence identity shared between *T. brucei* and *T. cruzi* prozyme.

We have recently discovered that prozyme is a key factor in the regulation of polyamine and trypanothione biosynthesis in *T. brucei* [20]. In unchallenged cells, prozyme is limiting, and not all of the cellular AdoMetDC is activated. Genetic or chemical depletion of AdoMetDC activity results in increased levels of prozyme protein. These data suggest that *T. cruzi* parasites

may also use prozyme to regulate polyamine synthesis and pathway flux. *T. cruzi* has a requirement for putrescine uptake suggesting prozyme levels could be regulated in response to the available extracellular putrescine in order to maintain balance in the pathway. In support, a 10-fold increase in putrescine was observed after genetic knockdown of AdoMetDC in *T. brucei*, and this effect remains a possible trigger for the increased prozyme expression observed under these conditions [20].

In conclusion, we have shown that the prozyme from *T. cruzi* and *T. brucei* act similarly to activate AdoMetDC. Further functional cross-species heterodimers between prozyme from one species can be formed with AdoMetDC from the other species. Together, these data demonstrate that prozyme evolved as a general mechanism to regulate AdoMetDC activity in the trypanosomatids. The *T. cruzi* heterodimer can be further stimulated by putrescine, which may be a consequence of the lack of ODC and subsequent need for putrescine import from the invaded host cell environment of these parasites. The trypanosomatid AdoMetDC/prozyme heterodimer interaction is unique to the trypanosomatids and offers a species-selective mechanism to target AdoMetDC in the treatment of trypanosomiasis.

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Abbreviations

AdoMetDC, S-adenosylmethionine decarboxylase; AdoMetDC/prozyme, the purified complex between AdoMetDC/prozyme; ODC, ornithine decarboxylase; DFMO, α-Difluromethylornithine; AdoMet, S-adenosylmethionine; dcAdoMet, decarboxylated AdoMet.

References

1. Barrett MP, Boykin DW, Brun R, Tidwell RR. Human African trypanosomiasis: pharmacological re-engagement with a neglected disease. Br J Pharmacol 2007;152:1155–1171. [PubMed: 17618313]
2. Stuart K, Brun R, Croft S, Fairlamb A, Gurtler RE, McKerrow J, Reed S, Tarleton R. Kinetoplastids: related protozoan pathogens, different diseases. J Clin Invest 2008;118:1301–1310. [PubMed: 18382742]
3. Tarleton RL, Reithinger R, Urbina JA, Kitron U, Gurtler RE. The challenges of Chagas Disease-- grim outlook or glimmer of hope. PLoS Med 2007;4:e332. [PubMed: 18162039]
4. Casero RA Jr. Marton LJ. Targeting polyamine metabolism and function in cancer and other hyperproliferative diseases. Nature reviews 2007;6:373–390.
5. Barrett MP, Boykin DW, Brun R, Tidwell RR. Human African trypanosomiasis: pharmacological re-engagement with a neglected disease. Br J Pharmacol 2007;152:1155–1171. [PubMed: 17618313]
6. Carrillo C, Cejas S, Gonzalez N, Algranati I. Trypanosoma cruzi epimastigotes lack ornithine decarboxylase but can express a foreign gene encoding this enzyme. FEBS letters 1999;454:192–196. [PubMed: 10431805]
7. Ariyanayagam MR, Fairlamb AH. Diamine auxotrophy may be a universal feature of Tryapnsoma cruzi epimastigotes. Mol. and Biochem. Parasitol 1997;84:111–121. [PubMed: 9041526]
8. Le Quesne SA, Fairlamb AH. Regulation of a high-affinity diamine transport system in Trypanosoma cruzi epimastigotes. Biochem. J 1996;316:481–486. [PubMed: 8687391]
9. Carrillo C, Canepa GE, Algranati ID, Pereira CA. Molecular and functional characterization of a spermidine transporter (TcPAT12) from Trypanosoma cruzi. Biochem Biophys Res Commun 2006;344:936–940. [PubMed: 16631600]

10. Bacchi CJ, Nathan HC, Yarlett N, Goldberg B, McCann PP, Bitonti AJ, Sjoerdsma A. Cure of murine Trypanosoma brucei rhodesiense infections with an S-adenosylmethionine decarboxylase inhibitor. Antimicrobial agents and chemotherapy 1992;36:2736–2740. [PubMed: 1482141]

11. Yakubu M, Majumder S, Kierszenbaum F. Inhibition of S-adenosyl-L-methionine (adomet) decarboxylase by the decarboxylated adomet analog 5′{[(Z)-4-amino-2-butenyl]methylamino}-5′-deoxyadenosine (MDL73811) decreases the capacities of Trypanosoma cruzi to infect and multiply within a mammalian host cell. J. Parasitol 1993;79:525–532. [PubMed: 8331473]

12. Barker RH Jr. Liu H, Hirth B, Celatka CA, Fitzpatrick R, Xiang Y, Willert EK, Phillips MA, Kaiser M, Bacchi CJ, Rodriguez A, Yarlett N, Klinger JD, Sybertz E. Novel S-adenosylmethionine decarboxylase inhibitors for the treatment of human African trypanosomiasis. Antimicrobial agents and chemotherapy 2009;53:2052–2058. [PubMed: 19289530]

13. Pajunen A, Crozat A, Janne O, Ihalainen R, Laitinen P, Stanley B, Madhubala R, Pegg A. Structure and Regulation of Mammalian S-Adenosylmethionine Decarboxylase. J. Biol. Chem 1988;263:17040–17049. [PubMed: 2460457]

14. Ekstrom J, Mathews I, Stanley B, Pegg A, Ealick S. The crystal structure of human S-adenosylmethionine decarboxylase at 2.25 Å resolution reveals a novel fold. Structure 1999;7:583–595. [PubMed: 10378277]

15. Pegg A, Xiong H, Feith D, Shantz L. S-adenosylmethionine decarboxylase: structure, function and regulation by polyamines. Biochem. Soc. Trans 1998;26:580–586. [PubMed: 10047786]

16. Beswick T, Willert E, Phillips M. Mechanisms of allosteric regulation of Trypanosoma cruzi S-adenosylmethionine decarboxylase. Biochemistry 2006;45:7797–7807. [PubMed: 16784231]

17. Clyne T, Kinch L, Phillips M. Putrescine activation of Trypanosoma cruzi S-adenosylmethionine decarboxylase. Biochemistry 2002;41:13207–13261. [PubMed: 12403622]

18. Kinch LN, Scott J, Ullman B, Phillips MA. S-adenosylmethionine from *Trypanosoma cruzi*: cloning, expression and kinetic characterization of the recombinant enzyme. Mol. and Biochem. Parasitol 1999;101:1–11. [PubMed: 10413038]

19. Willert EK, Fitzpatrick R, Phillips MA. Allosteric regulation of an essential trypanosome polyamine biosynthetic enzyme by a catalytically dead homolog. Pro. Nat. Acad. Sci. USA 2007;104:8275–8280.

20. Willert EK, Phillips MA. Regulated expression of an essential allosteric activator of polyamine biosynthesis in African trypanosomes. PLoS Pathog 2008;4:e1000183. [PubMed: 18949025]

21. Lebowitz J, Lewis M, Schuck P. Modern analytical ultracentrifugation in protein science: a tutorial review. Protein Sci 2002;11:2067–2079. [PubMed: 12192063]

22. Laue, T.; Shah, B.; Ridgeway, T.; Pelletier, S. Computer-aided interpretation of analytical sedimentation data for proteins. In: Harding, S.; Rowe, A.; Horton, J., editors. Analytical Ultracentrifugation in Biochemistry and Polymer Science. Royal Society of Chemistry; Cambridge: 1992. p. 90-125.

23. Ekstrom J, Tolbert W, Xiong H, Pegg A, Ealick S. Structure of a human S-adenosylmethionine decarboxylase self-processing ester intermediate and mechanism of putrescine stimulation of processing as revealed by the H243A mutant. Biochemistry 2001;40:9495–9504. [PubMed: 11583148]

24. Taylor MC, Kaur H, Blessington B, Kelly JM, Wilkinson SR. Validation of spermidine synthase as a drug target in African trypanosomes. Biochem. J 2008;409:563–569. [PubMed: 17916066]

25. Osterman AL, Grishin NV, Kinch LN, Phillips MA. Formation of functional cross-species heterodimers of ornithine decarboxylase. Biochemistry 1994;33:13662–13667. [PubMed: 7947774]

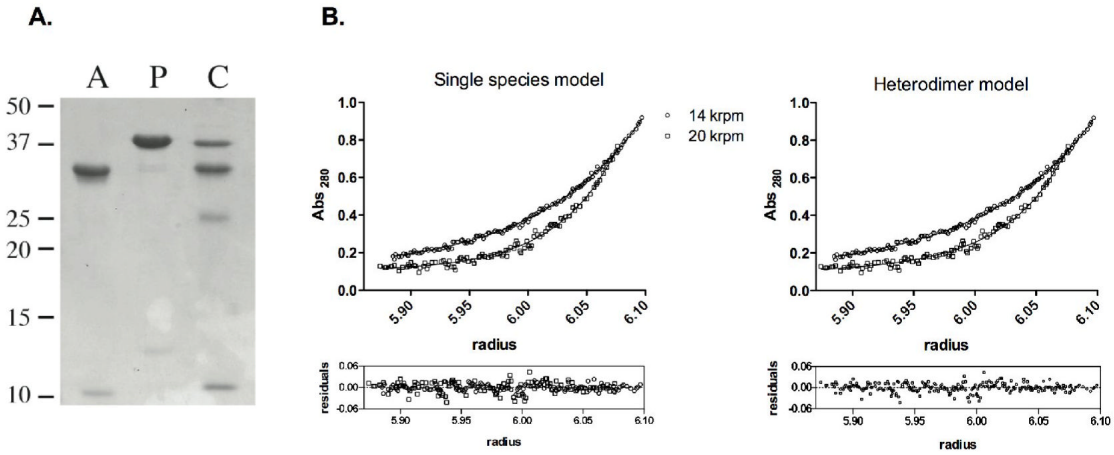


Figure 1. *T. cruzi* AdoMetDC forms a tight heterodimer with prozyme

A. Purification of recombinant proteins from *T. cruzi*: His_6-tagged AdoMetDC (A), His_6-tagged prozyme (P) and the co-purified complex of His_6-tagged AdoMetDC and untagged prozyme (C). Proteins were purified by Nickel, anion exchange and size exclusion chromatography. **B.** Sedimentation equilibrium analysis of analytical ultracentrifugation of *T. cruzi* AdoMetDC/prozyme complex (8μM). Data were fitted to a single species model (left) and a heterologous association model (right).

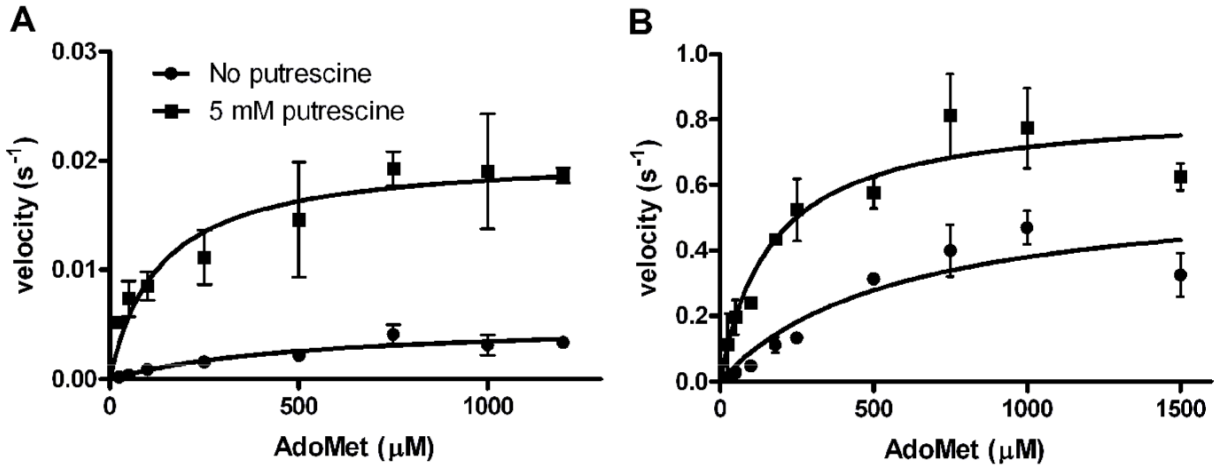


Figure 2. Steady-state kinetic analysis of recombinant *T. cruzi* AdoMetDC species

A. Steady-state kinetics of *T. cruzi* AdoMetDC homodimer without (circles) and with (squares) 5 mM putrescine. **B.** Steady-state kinetics of *T. cruzi* AdoMetDC/prozyme heterodimer without (circles) and with (squares) 5 mM putrescine. Error bars represent n=3 replicates for each experimental point.

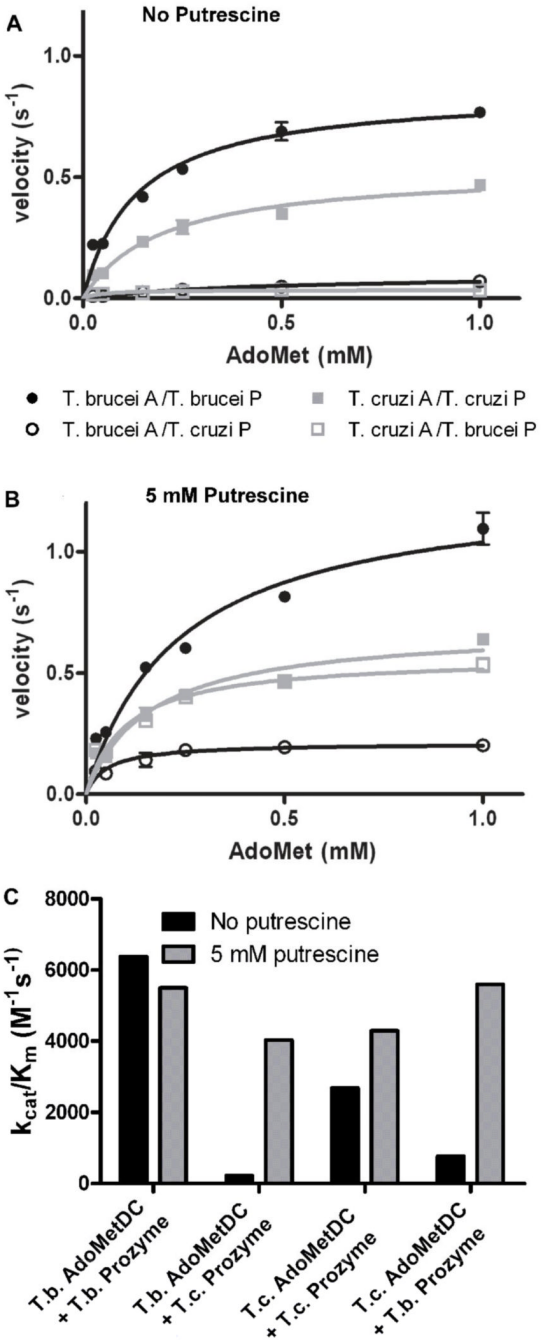
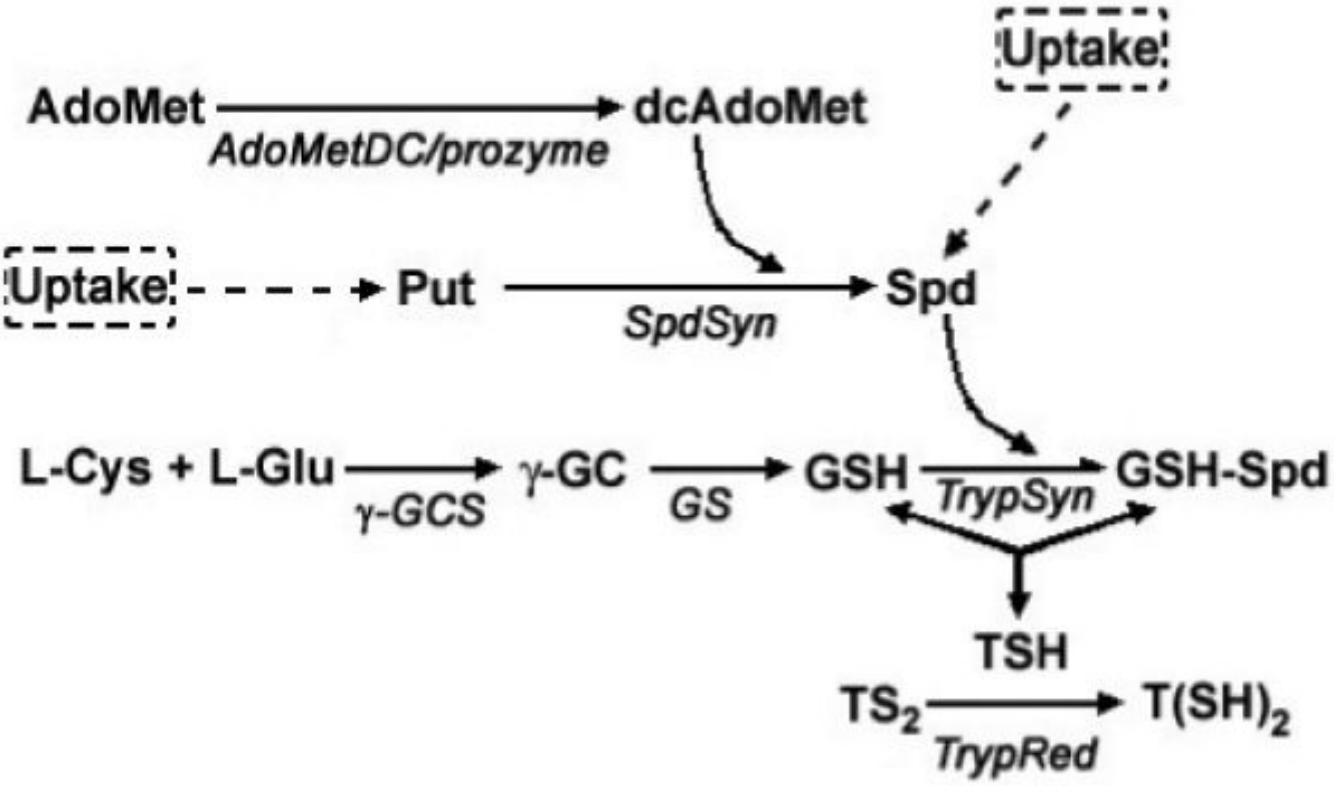


Figure 3. Steady-state kinetic analysis of cross-species prozyme activation of AdoMetDC orthologs

A. Steady-state kinetics of AdoMetDC and prozyme combinations (1:10 AdoMetDC to prozyme) in the absence of putrescine. **B.** Steady-state kinetics of AdoMetDC and prozyme combinations in the presence of 5 mM putrescine. *T. brucei* AdoMetDC (black circles) was mixed with *T. brucei* prozyme (closed circles) and with *T. cruzi* prozyme (open circles). *T. cruzi* AdoMetDC (grey squares) was mixed with *T. cruzi* prozyme (closed squares) and with *T. brucei* prozyme (open squares). **C.** Comparison of the catalytic efficiency of AdoMetDC and prozyme mixtures in the absence (black) and presence (grey) of 5 mM putrescine. Error bars represent n=2 replicates for each experimental point.



Scheme 1. The polyamine biosynthesis pathway in *T. cruzi*

AdoMet (S-adenosylmethionine); dcAdoMet (decarboxylated AdoMet)Put (putrescine); Spd (spermidine); SpdSyn (Spd synthetase); γ-GC (gamma-glutamylcysteine); γ-GCS (γ-GC synthetase); GSH (glutathione); GS (GSH synthetase); GSH-Spd (glutathionyl-spermidine); TSH (trypanothione); TrypSyn (TSH synthetase); TS_2 (oxidized TSH); $T(SH)_2$ (reduced TSH); TrypRed (TSH reductase).

Table 1

Kinetic comparison of *T. cruzi* AdoMetDC and AdoMetDC/prozyme heterodimer. AdoMetDC and prozyme proteins were coexpressed in *E. coli* followed by copurification as described in Materials and Methods. Error is the standard error of the fit

	No putrescine			5 mM putrescine			Fold change by putrescine		
	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($M^{-1}s^{-1}$)	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($M^{-1}s^{-1}$)	k_{cat}	K_m	k_{cat}/K_m
A. *T. cruzi* AdoMetDC									
TcAdoMetDC	0.005 ± 0.001	540 ± 350	9.3 ± 6.6	0.02 ± 0.003	130 ± 63	150 ± 76	4.0	0.24	17
TcAdoMetDC/Tcprozyme	0.60 ± 0.09	580 ± 200	1030 ± 400	0.84 ± 0.06	170 ± 42	5100±1900	1.4	0.29	4.9
Fold change by prozyme	120	1.1	110	41	1.3	32			
B. *T. brucei* AdoMetDC*									
TbAdoMetDC	0.0013	0.38	3.4	0.0082	0.24	35	6.3	0.63	10
TbAdoMetDC/Tbprozyme	1.4	0.11	12,000	1.7	0.17	11,000	1.2	1.5	0.92
Fold change by prozyme	1100	0.3	3500	210	0.71	310			

*Data taken from [19]

Table 2

Kinetic comparison of *T. cruzi* and *T. brucei* AdoMetDC/prozyme heterodimers with cross-species heterodimers. AdoMetDC and prozyme proteins were expressed and purified individually prior to mixing for the kinetic analysis. Error is the standard error of the fit

	No putrescine			5 mM putrescine		
	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($M^{-1}s^{-1}$)	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($M^{-1}s^{-1}$)
Tb AdoMetDC/Tb Prozyme	0.86 ± 0.04	130 ± 20	6600 ± 1100	1.3 ± 0.09	230 ± 46	5600 ± 1200
Tb AdoMetDC/Tc Prozyme	0.09 ± 0.01	450 ± 130	200 ± 62	0.21 ± 0.02	52 ± 17	4000 ± 1400
Tc AdoMetDC/Tc Prozyme	0.53 ± 0.04	200 ± 38	2700 ± 540	0.69 ± 0.05	160 ± 35	4300 ± 990
Tc AdoMetDC/Tb Prozyme	0.03 ± 0.003	440 ± 14	680 ± 210	0.57 ± 0.03	100 ± 20	5700 ± 1200