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Original Article

Biophysical and spectroscopic characterization of the *S. mansoni* triosephosphate isomerase~NAD-binding domain of GAPDH protein-protein interaction: implications for anti-schistosomal therapeutics

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Abstract: Protein-protein interactions (PPIs) are essential to many biological processes. These PPIs are linked to various infectious diseases, and schistosomiasis is no exception. Objective: The *S. mansoni* Triosephosphate isomerase~NAD-binding (SmTPI~SmNBD) domain of Glyceraldehyde-3-Phosphate dehydrogenase protein complex (GAPDH), also known as the SmTPI~SmNBD of GAPDH, has been proposed to play an essential role in providing energy for the parasite to continue its life cycle and is also believed to be involved in other pathways driven by the formation of this. However, this interaction has been demonstrated only in silico; therefore, it is necessary to validate and characterize it *in vitro*. Methods: In this study, UV-visible (UV-Vis) spectroscopy, Raman spectroscopy, and Microscale Thermophoresis (MST) were used to investigate the interaction between the two schistosomal proteins. Results: Results support an interaction between TPI and the SmNBD of GAPDH. Raman spectra revealed that upon interaction, peaks at 1450 cm^{-1} and 3159 cm^{-1} increased in intensity. These peaks were not observed in the individual protein spectra, suggesting changes in their secondary structures upon interaction. The dissociation constant (K_D) obtained from the MST study was 2.35 μM, suggesting moderate binding between the two proteins. Conclusion: These findings provide insight into the interaction between TPI and the mof GAPDH, which is crucial for future research in anti-schistosomal drug discovery and development, particularly for targeting the SmTPI~SmNBD of the GAPDH protein complex with small-molecule inhibitors.

Keywords: Glyceraldehyde-3-phosphate dehydrogenase, microscale thermophoresis, protein-protein interactions, schistosomiasis, small-molecule inhibitors, triosephosphate isomerase

Introduction

Proteins are responsible for controlling most cellular processes and typically facilitate these cellular functions by interacting with other proteins [1, 2]. These protein-protein interactions (PPIs) take various forms, including binary interactions, multi-protein complexes, and long-range interactions [3]. These PPIs occur in proteins with associated functions, especially in the same pathways [4]. They play key roles in affecting the enzymatic activity by altering its kinetic properties, facilitating substrate channeling through a comprehensive mechanism, creating a new binding site for small effector molecules, subduing or deactivating a protein as needed, and lastly, adjusting the specificity of a protein for its substrate through interaction with various binding partners [5]. According to De Las Rivas and Fontanillo, these PPIs form a complex network in cells known as an "interactome", which is essential for physiological and pathological processes, such as the growth and reproduction of pathogens within the human host [3]. Since aberrant PPIs are implicated in disease evolution, they are linked to several human ailments, including infectious diseases. Therefore, proteins play a crucial role in disease

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progression and can serve as novel therapeutic targets or biomarkers [6].

In the case of schistosomiasis, a devastating parasitic disease affecting over 258 million people across the globe and claiming the lives of 300,000 sub-Saharan Africans annually [7, 8]. Studies have shown that Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) plays a pivotal role in perpetuating the disease [9]. This glycolytic protein converts D-glyceraldehyde 3-phosphate (G3P) to 1,3-bisphosphoglycerate, utilizing nicotinamide adenine dinucleotide (NAD^+) as a cofactor [10]. It generates nicotinamide adenine dinucleotide hydrogen (NADH) from NAD^+, which is used in the electron transport chain to produce adenosine triphosphate (ATP) [11]. This protein plays a crucial role in the survival of the Schistosoma parasite within its host [10]. It is known that glycolytic enzymes interact with cellular structures to form multienzyme complexes, which modify their functional properties [12]. A bioinformatic study conducted using the STRING protein-protein interaction network (https://string-db.org/) revealed that Triosephosphate isomerase (TPI) is a significant protein that interacts with Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) in schistosomes [13]. TPI catalyzes the co-enzyme-independent interconversion of dihydroxyacetone phosphate (DHAP) and Glyceraldehyde-3-phosphate (G3P) [14, 15], a substrate for GAPDH in glycolysis. The two proteins play this cooperative role in supplying energy and avoiding the host's immune system [15]. This PPI is essential for the survival of the schistosomal parasite in the human host; thus, blocking it may reduce schistosome survival by lowering their energy levels.

According to Moreira and co-workers, most PPIs feature specific amino acid residues on their interfaces that are essential for binding and free energy [16]. These locations are referred to as "hot spots" because when alanine is altered, there are noticeable increases in the binding free energy [16, 17]. Therefore, despite the immensity of the interface, PPI medications frequently target these hotspot amino acids [18], and small-molecule drugs have been shown to disrupt these important PPI hotspots successfully. These inhibitors can bind directly to the orthosteric hotspot in PPIs, but in their absence, PPIs can be modulated allosterically [18]. This suggests that modulating the SmTPI~SmNBD of GAPDH interaction using small-molecule inhibitors is a promising approach for the discovery and development of anti-schistosomal drugs. However, this PPI has not been demonstrated through wet-lab experiments; hence, this study characterized the SmTPI~SmNBD of GAPDH protein complex using biophysical and spectroscopic techniques to qualitatively and quantitatively determine this interaction. Therefore, this study offers an important preliminary experimental validation of the interaction between the SmTPI~SmNBD of GAPDH protein complex using complementary biophysical tools.

Experimental methods

Recombinant expression and purification of SmTPI and SmNBD of GAPDH

The *S. mansoni* TPI and NBD of GAPDH constructs were cloned into pET-11a and pGEX-6P-2 vectors, respectively, and successfully transformed into T7 Express *E. coli* cells using the heat-shock method. The transformed cells from both plasmids were plated on Luria-Bertani (LB) agar supplemented with 100 mg/mL Ampicillin and incubated overnight at 37°C. The following day, random colonies were selected to inoculate 10 mL starter cultures of LB broth containing 100 µg/mL Ampicillin, which were grown overnight at 37°C. These starter cultures were used to inoculate 2 L of LB broth supplemented with 100 µg/mL Ampicillin. Cells were then grown at 37°C until they reached an optical density (OD_{600}) of 0.4-0.6, a process that took approximately 4 hours. At this point, protein expression was induced using 0.5 mM isopropyl β-D-thiogalactoside (IPTG), and the cells were further grown overnight at 18°C with vigorous shaking. The following morning, the cells from the SmTPI cultures were harvested by centrifugation at 3,000 × g for 10 minutes at 4°C and pellets were re-suspended in lysis buffer (50 mM Tris, 40 mM Imidazole, 1 mM PMSF, and 1 mg/mL lysozyme, pH 7), sonicated (4 mins in total, with 30 seconds on and 30 seconds off cycle), and the resulting suspension was centrifuged at 15,000 × g for 20 minutes to obtain a clear total bacterial lysate. The clear bacterial lysate was then loaded onto a Nickel-NTA column, and 5 column volumes (CVs) of wash buffer (50 mM Tris and 40 mM imidazole) were used to wash the column, followed by 4 CVs of elution buffer (50 mM Tris and 250 mM

Imidazole) to elute the protein. The same process was carried out for harvesting and sonicating the SmNBD of GAPDH, except that the lysis buffer contained no Imidazole and purification was performed using a Glutathione-Sepharose column. The fractions containing both schistosomal proteins were then analyzed on a 15% SDS-PAGE gel, and the concentrations of purified SmTPI and SmNBD of GAPDH protein samples were determined using a NanoDrop ND2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Ultraviolet-visible (UV-vis) spectroscopy

UV-vis spectroscopy was used to assess the presence of any spectral changes consistent with potential interaction between the two schistosomal proteins. The purified SmTPI and SmNBD of GAPDH proteins were analyzed in a 96-well microtiter plate. Approximately 200 µL of the blank (protein elution buffer), 200 µL of each protein at a concentration of 0.5 mg/mL, as well as a mixture of both proteins in a 1:1 dilution, were pipetted into separate wells on the microtiter plate. The plate was scanned at 200-400 nm to obtain absorbance values, and the data were processed to construct a spectrum. It is important to note, however, that the UV-vis tool is not specifically designed to detect protein-protein interactions. Instead, the observations seen are interpreted as supportive evidence in conjunction with that obtained from Raman and MST data.

Raman spectroscopy

The WITec Alpha300R Raman spectrometer (Oxford Instruments, Abingdon, United Kingdom) was used to record the Raman spectra. Raman spectra provide information on a protein's chemical structure and molecular interactions. It is also used to study the structural changes that occur during an interaction between two proteins. The Raman spectrometer is coupled with a confocal microscope, while the dispersive system consists of a single grating with 600 grooves/mm, providing a resolution of 1 cm^{-1}. The Raman signal was collected by a liquid-nitrogen-cooled charge-coupled device (CCD). Only 2 µL of the *S. mansoni* protein samples was needed for the measurement, which was placed on a glass slide and subjected to Raman scattering. The spectral data were recorded across a 50-3800 cm^{-1} range using a 532.150 nm laser for excitation at 2 mW, combined with a 50X objective and a 100 µm optical fibre to focus and collect the Raman-scattered light for spectrum generation.

Microscale thermophoresis

The Monolith NT.115 instrument (NanoTemper Technologies, Munich, Germany) was used to assess SmTPI binding to the SmNBD of GAPDH. The SmTPI was fluorescently labeled using the Monolith protein labeling kit RED-NHS 2nd generation and diluted to 30 mM in assay buffer (1X PBS and 0.05% Tween 20). This protein was selected for labeling instead of SmNBD due to its stability and compatibility with the NHS dye. This was followed by preparing a 541.18 mM solution of the SmNBD of GAPDH in assay buffer and mixing it with a 30 mM solution of SmTPI in a PCR tube. Subsequently, the labeled SmTPI was diluted with assay buffer at a 1:1 ratio in another PCR tube. Both samples were incubated at room temperature for 5 minutes. The samples were loaded into four different capillaries via capillary action and then loaded into the Monolith NT.115 instrument to conduct the binding check analysis at room temperature, using 90% excitation and 40% MST power.

Using a previously published procedure with some minor adjustments [19], a binding affinity experiment was additionally performed to measure the binding of SmTPI to the SmNBD of GAPDH. Different concentration ranges of the SmNBD of GAPDH were prepared by mixing 10 µL of the diluted fluorescent-labeled SmTPI with 10 µL of serially diluted (16 dilutions) SmNBD of GAPDH. Thereafter, the SmTPI~SmNBD of GAPDH protein complex was incubated for 5 minutes at room temperature before being loaded into capillaries for measurement using the Monolith NT.115 instrument at 90% excitation and 40% MST power. The experiment was run in triplicate, and the results were analyzed using MO-Affinity analysis software v2.1.3 (NanoTemper Technologies, Munich, Germany) to calculate the dissociation constant (K_D).

Results

Bacterial expression and purification

A large-scale expression (2 L) of the SmTPI protein in T7 *E. coli* cells and purification using a

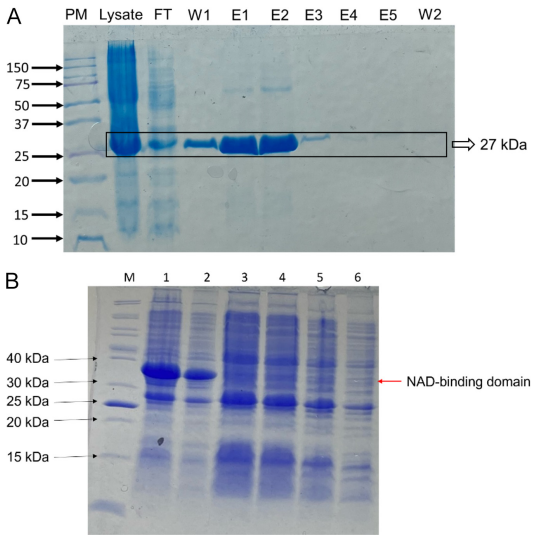


Figure 1. A. A 15% SDS-PAGE gel of the purification of recombinant *S. mansoni* TPI (SmTPI) pET-11a construct expressed in T7 *E. coli* cells at ~27 kDa. PM is the molecular mass marker, lane 1 (Lysate) represents the soluble fraction obtained after cell lysis of a 2 L SmTPI culture. Lane 2 (FT) represents the flow-through from the Nickel-NTA column, and lanes 3 & 9 (W1 & W2) are washes with 50 mM Tris containing 40 mM imidazole. Lanes 4 to 8 (E1 to E5) are from 50 mM Tris containing 250 mM imidazole elutions from the Nickel-NTA column, each 6 mL. B. A 15% SDS-PAGE gel showing large-scale production of the *S. mansoni* NBD of GAPDH pGEX-6P-2~SmNBD of GAPDH (SmGST-SmNBD of GAPDH) expressed in T7 bacterial cells at ~37 kDa. M is the molecular mass marker, while lanes 1 & 2 are the soluble total bacteria cell lysate of the protein from a 2 L culture; loaded 20 and 10 µL, respectively. Lanes 3 to 6 represent the insoluble fractions with no protein band corresponding to the SmNBD of GAPDH obtained after centrifugation; loaded 20, 10, 15, and 5 µL, respectively. The SmNBD of GAPDH protein was subsequently purified from the soluble fractions shown in lanes 1 & 2.

Nickel-NTA column were conducted. The 15% SDS-PAGE analysis in **Figure 1A** demonstrates the successful expression and purification of the protein, with the majority of SmTPI being expressed in the soluble fraction (Lysate Lane) at the expected size of ~27 kDa. Furthermore, the protein was successfully purified using the Nickel-NTA column, as shown in lanes E1 and E2, where a relatively pure protein was eluted with 250 mM imidazole. For the SmNBD of GAPDH, the large-scale expression conducted in a 2 L culture was also successful. The SDS-PAGE analysis reveals conspicuous bands in lanes 1 and 2, corresponding to 20 µL and 10 µL of the total bacterial cell lysate, respectively (**Figure 1B**). These bands are not observed in lanes 3-6, which contain 20, 15, 10, and 5 µL of the insoluble fraction obtained after centrifugation. The results indicate that the protein was overexpressed at ~37 kDa, since GST is ~27 kDa. Consequently, the soluble protein fraction was purified and recovered for downstream biophysical and spectroscopic analyses. In summary, both the SmTPI and SmNBD of GAPDH constructs were successfully expressed in T7 *E. coli* cells at their expected molecular weights and purified using the Nickel-NTA column and the Glutathione-Sepharose column, respectively. The purified *S. mansoni* protein samples were concentrated from 9.20 mg/mL (SmTPI) and 3.82 mg/mL (SmNBD of GAPDH) to 1 mg/mL for subsequent characterization studies.

Supporting evidence of the interaction between SmTPI and the SmNBD of GAPDH

The protein characteristics were analyzed using UV-vis spectroscopy. **Figure 2** shows three significant peaks between 280 and 300 nm, attributed to aromatic side chains, tyrosine, and tryptophan [20]. The SmTPI (orange) peak shows a lower absorbance than the SmNBD of GAPDH (blue). This difference can be attributed to differences in the quantity of these aromatic side chains. The SmTPI~SmNBD of GAPDH protein complex (green) exhibits a peak absorbance that is the average of the absorbance of the two individual proteins. It is proposed that this change in the absorbance of the aromatic side chains is due to a protein-protein interaction [21].

Native and conformational changes observed due to the protein complex formation

Raman spectroscopy was used to analyze the secondary structures and hydrophobic residues present in the SmTPI protein and the SmNBD of GAPDH. Additionally, the conformational changes induced by the interaction between the two proteins were identified. **Figure 3A** (SmNBD of GAPDH) and **Figure 3B** (SmTPI) indicate the presence of seven and six noticeable peaks, respectively. Peak 1, corresponding to 855 cm^{-1}, signifies tyrosine residues, while peak 3, at about 1060 cm^{-1}, is attributed to lysine residues. Peak 2, corresponding to approximately 932 cm^{-1}, is attributed to C-C stretching vibrations, which are indicative of α-helices and are one of the most

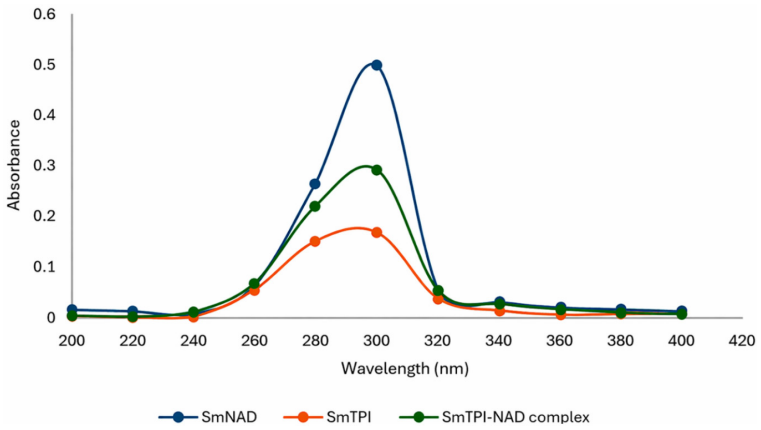


Figure 2. The UV-vis spectrum of the SmTPI, SmNBD of GAPDH, and the SmTPI~NBD of GAPDH protein complex. The absorbance of the protein samples was measured on a UV-Vis spectrophotometer over 200-400 nm.

significant elements in both the secondary structures of the SmTPI and SmNBD of GAPDH proteins [22]. The peak between 1270 cm^{-1} and 1300 cm^{-1} represents the Amide III band, which has significant contributions from N-H bending vibrations and C-N stretching vibrations, with minor contributions from C=O bending and C-C stretching vibrations [23]. This band indicates the presence of β-sheets, which are the major secondary structure component in both proteins. The sixth and seventh peaks, located at approximately 1340 cm^{-1} and 1550 cm^{-1}, respectively, correspond to the vibrations of the indole ring of tryptophan and indole [22]. The last peak, between 1665 cm^{-1} and 1680 cm^{-1}, observed only in the SmNBD of GAPDH spectrum, corresponds to the amide I band. This peak arises from the C=O stretching vibrations. Hence, it is used to analyze the secondary structure of proteins [24]. This band reveals the presence of α-helices, a structural element in the SmNBD of GAPDH. The observed peak shifts and the relative increase in intensity of the 1450 cm^{-1} band indicate changes in secondary structure and side-chain packing, which are compatible with the formation of protein complexes.

The SmTPI~NBD of GAPDH protein complex, as observed in **Figure 3C** and **3D**, shows the conformational changes undergone by the two proteins during interaction. Three prominent differences are observed; the first is a shift in peak positions (band shifts). The second difference is observed approximately at 1450 cm^{-1}, corresponding to the formation of a peak produced by the CH_2 deformation band [25]. This band is connected to changes in the bond angle. Lastly, the peak around 3159 cm^{-1} for the SmTPI~NBD of GAPDH protein complex is of low intensity (low CCD counts) compared to the spectra of the individual proteins.

Quantification of the binding of SmTPI to SmNBD of GAPDH

Microscale thermophoresis is a biophysical technique that is used to characterize biomolecular interactions. This method enables the study of ligand-target interactions by measuring the movement of a fluorescently labeled molecule along a microscopic temperature gradient. When the fluorescing molecule interacts with the non-fluorescent ligand, it changes its hydration shell, charge, and size. This alteration will impact the molecule's thermophoresis, providing insight into the interaction [26]. As shown in the binding check analysis of SmTPI to SmNBD of GAPDH (**Figure 4A**), there is a significant binding between the two proteins based on the critical shift in the normalized fluorescence (mean: 858.5) of the bound and unbound state (mean: 848.76) of SmTPI (P = 0.0029). The binding between the two proteins was quantified using a binding affinity experiment (**Figure 4B**), which showed a gradual increase in SmTPI fluorescence with increasing GAPDH SmNBD concentrations. The signal-to-noise ratio was 8.3951691, which is above 5; this indicates that the sigmodal curve obtained has more signal than noise and can therefore be used to determine the K_D value, which is the value used to assess the binding strength between a ligand and a target molecule [27]. These results provide evidence of binding between the two proteins. The obtained binding affinity was a K_D of 2.35 μM, which indicates moderate interaction between the two proteins [28]. The K_D confidence was ± 1.1482E-06 (**Table 1**), meaning that the obtained K_D value is acceptable. Lastly, the stoichiometry (n) was found to be 2, indicating that the SmNBD of GAPDH protein must be present at twice the concentration of the SmTPI for maximal binding to occur. Because a shortened protein construct was used, this conclusion is interpreted

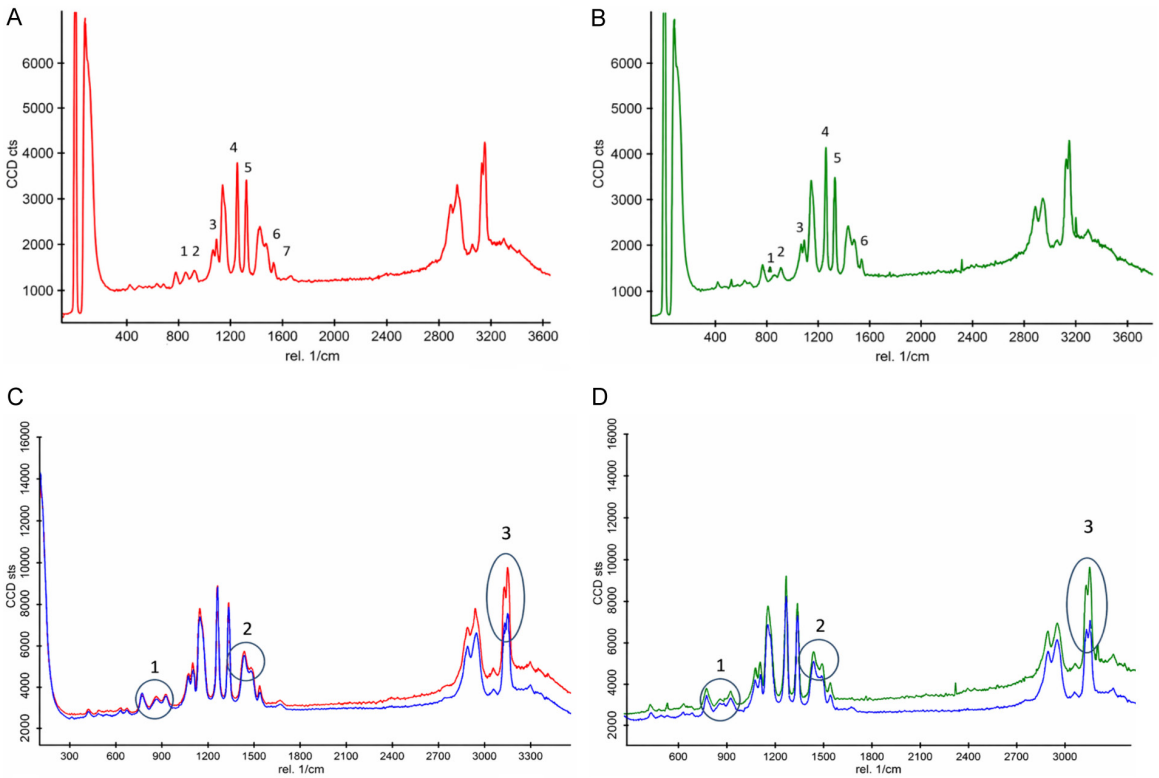


Figure 3. The Raman spectra of (A) SmNBD of GAPDH, (B) SmTPI, (C) SmTPI~NBD of GAPDH overlayed on the SmNBD of GAPDH spectrum, and (D) SmTPI~NBD of GAPDH overlayed on the SmTPI spectrum. All spectra revealed characteristic secondary structure elements and amino acid residues.

with caution, even though the observed stoichiometry (n = 2) may indicate numerous binding sites or cooperative binding. The reported KD (~2.35 μM) falls within the range commonly associated with transient interactions between proteins, especially between metabolic enzymes.

Discussion

The over-reliance on Praziquantel (PZQ) to treat schistosomiasis has fallen short in recent years due to the emergence of resistant strains. This highlights the importance of discovering and developing novel drugs that effectively target the schistosome parasite. According to Mabonga and colleagues, proteins that interact with others are more likely to be involved in disease-related processes, unlike non-interacting proteins, which makes PPIs therapeutically sensitive for the development of new drugs [29]. Therefore, the use of small-molecule inhibitors has become a promising approach to combat drug resistance in schistosomes. Such inhibitors can be used to modulate the binding between disease-causing PPIs, such as the SmTPI~SmNBD of GAPDH protein complex. These moonlighting proteins are not only crucial to glycolysis but are also expressed in other pathways that increase schistosome survival in the human host by over 40 years [30]. This study aimed to validate the putative interaction between SmTPI and SmNBD of GAPDH through biophysical and spectroscopic techniques.

The assessment of SmTPI binding to SmNBD of GAPDH using UV-vis spectroscopy suggests modifications to the environment surrounding aromatic residues that are compatible with (although this is not entirely conclusive) hydrophobic interactions linked to protein binding. This was depicted by the change in the absorbance of the aromatic residues in the protein complex as compared to that of the individual proteins, thus suggesting that there is a change in the environment of these residues, as these hydrophobic aromatic residues became more buried and less exposed to the aqueous environment and interacted by forming hydrophobic interactions, which then led to the conformational changes in the active sites of the proteins [21, 31].

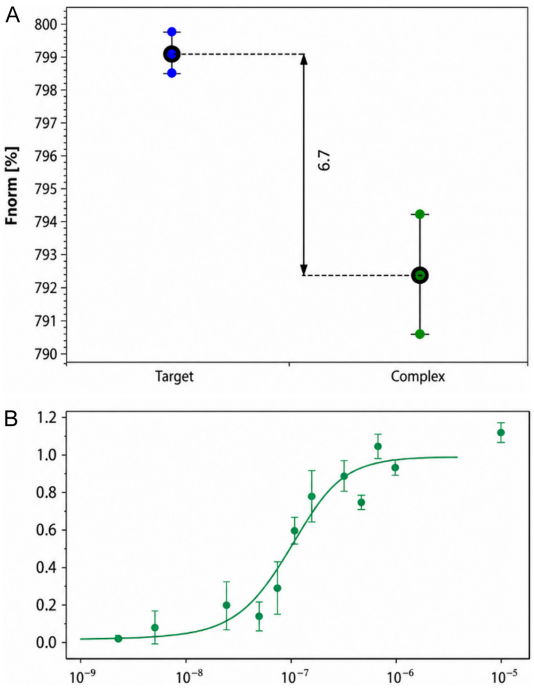


Figure 4. A. Binding check analysis showing the difference in SmTPI normalized fluorescence in an unbound state and bound state with SmNBD of GAPDH. The SmNBD of GAPDH enhanced SmTPI fluorescence, indicating an interaction between the two proteins. B. Quantification of the binding affinity of SmTPI to SmNBD of GAPDH to obtain the K_D value and ascertain the strength of binding. The observed KD (~2.35 μM) falls within the range typically associated with transient protein-protein interactions, particularly among metabolic enzymes.

The structural changes that occurred during the interaction between the two proteins were analyzed using Raman spectroscopy. Three notable differences were observed in the SmTPI~SmNBD of GAPDH protein complex sample: the appearance of the CH_2 deformation peak at 1450 cm^{-1}, a shift in peak positions, and an increase in the intensity of the peak at 3159 cm^{-1}. These differences strongly suggest an interaction between these two glycolytic proteins. Changes in the protein complex spectrum, compared with the individual protein spectra, indicate that during interaction, these proteins undergo conformational changes to facilitate binding [25]. It is also important to note that the Amide I band observed in the SmNBD of GAPDH spectrum is also present in the protein complex spectrum, suggesting that the α-helix of the SmNBD of GAPDH plays a significant role in interacting with the SmTPI protein.

Table 1. Dataset overview of the MST results for the interaction between SmTPI and SmNBD of GAPDH

Name	TPI & NAD
Graph colour	Green
Target name	TPI
Target concentration	15 nM
Ligand name	NAD
Ligand concentration	135 μM to 0.00826 μM
n	2
Comments	-
Excitation Power	90%
MST Power	40%
Temperature	22.8°C
Kd	2.35E-06
Kd Confidence	± 1.1482E-06
Response Amplitude	9.7378151
Target Conc	1.5E-08 [Fixed]
Unbound	858.5
Bound	848.76
Std. Error of Regression	1.3152379
Reduced χ^2	10.483245
Signal to Noise	8.3951691

The binding check and binding affinity of the SmTPI~SmNBD of GAPDH protein complex validated the interaction between the two proteins. The MST study showed moderate binding between SmTPI and SmNBD of GAPDH, with a K_D value of 2.35 μM. A K_D value in the nanomolar range or lower indicates strong binding, while a higher K_D value greater than the millimolar range indicates weak binding [28]. Based on the K_D value obtained in this study, it can be concluded that there is moderate binding between the SmTPI and SmNBD of GAPDH [32], characterized by non-covalent interactions, including hydrogen bonds, ionic bonds, van der Waals forces, and hydrophobic interactions [33]. This implies that these two proteins associate to perform a cooperative biological function for a specific period, then dissociate once the role is completed [34]. In addition, the observed K_D falls within the range typically observed for transient protein-protein interactions, especially among metabolic enzymes. These moderate affinities indicate the physiological significance of this interaction in vivo by facilitating the dynamic association-dissociation cycles required for effective substrate channeling and metabolic control. Therefore, it

is essential to discover small-molecule inhibitors that can modulate the synergistic role of the SmTPI~SmNBD of GAPDH protein complex. In schistosomiasis, altering this relationship would represent a significant advance toward developing novel approaches to address anti-schistosomal drug resistance.

Conclusion

This study highlighted the vital interaction between the SmTPI and SmNBD of GAPDH, which plays a significant role throughout the life cycle of *Schistosoma mansoni*, the causative agent of schistosomiasis. Strong evidence of this interaction was demonstrated using biophysical and spectroscopic methods, which show that both hydrophobic and conformational modifications are necessary for binding. The MST study revealed moderate affinity, suggesting a relationship stabilized by multiple non-covalent interactions that allow these proteins to work cooperatively. This PPI can be further validated by additional investigations into its molecular and structural mechanism of action as a possible anti-schistosomal druggable target. Although there are various limitations associated with this study such as the use of the NAD-binding domain of GAPDH rather than a full-length protein which may have had a bearing on the affinity and stoichiometry results as well as the study being conducted *in vitro* which may not fully encapsulate the intracellular environment of the parasite, the study clearly presents preliminary data showing interaction that can be manipulated for drug design. Considering the concerning increase in drug resistance in schistosomiasis, targeting this PPI with novel small-molecule inhibitors offers a promising approach for therapeutic development. Therefore, further exploration and validation of this interaction may lead to substantial advancements in combating schistosomiasis and overcoming the challenges posed by resistant strains, thus paving the way for more effective strategies against the disease.

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Disclosure of conflict of interest

None.

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