

# Triosephosphate isomerase of *Taenia solium* (TTPI): phage display and antibodies as tools for finding target regions to inhibit catalytic activity

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**Abstract** Previous studies demonstrated that antibodies against triosephosphate isomerase of *Taenia solium* (TTPI) can alter its enzymatic catalysis. In the present study, we used antibodies produced against the NH<sub>2</sub>-terminal region of TTPI (1/3NH<sub>2</sub>TTPI) and the phage display technology to find target regions to inhibit TTPI activity. As a first step, we obtained polyclonal antibodies against non-conserved regions from the 1/3NH<sub>2</sub>TTPI, which had an inhibitory effect of about 74 % on catalytic activity. Afterward, they were used to screen a library of phage-displayed dodecapeptides; as a result, 41 phage mimotope clones were isolated and grouped according to their amino acid sequence, finding the consensus A1 (VPTXPI), A2 (VPTXXI), B (LTPGQ), and D (DPLPR). Antibodies against selected phage mimotope clones were obtained by rabbit's immunization; these ones clearly recognized TTPI by both Western blot and ELISA. However, only the mimotope PDTS16 (DSVTPTSVMVA) clone, which belongs to the VPTXXI consensus, raised antibodies capable of inhibiting the TTPI catalytic activity in 45 %. Anti-PDTS16 antibodies were confronted to several synthetic peptides that encompass the 1/3NH<sub>2</sub>TTPI, and they only recognized three, which share the motif FDTLQK belonging to the helix- $\alpha$ 1 in TTPI. This suggests that this motif is the main part of the epitope recognized by anti-PDTS16 antibodies and revealed its importance for TTPI catalysis.

**Keywords** Triosephosphate isomerase · Antibodies · Phage display · Mimotope · Inhibitors · Catalytic activity · *Taenia*

## Introduction

Humans, who carry the adult stage of *Taenia solium* (tapeworm) in their small intestine (taeniosis), can expel thousands of eggs through the feces and infect pigs and humans causing cysticercosis. Humans acquire taeniosis by pork consumption with viable cysticerci (larval stage). Cysticercosis is one of the most common public health problems that afflict more than 50 million people in a wide range of regions around the world, and its neurological form (neurocysticercosis) undermines the physical integrity of people and, in most cases, can be deadly (Schantz et al. 1993).

Triosephosphate isomerase (TPI, EC 5.3.1.1) is a ubiquitous and essential enzyme for living organisms and is one of the best studied during the last decades. It does not need any cofactor or metal ions to carry out its reaction, and it belongs to a structural family of enzymes that contain a highly conserved barrel structure ( $\alpha\beta$ )<sub>8</sub> (Wierenga 2001). TPI catalyzes the reversible interconversion of dihydroxyacetone phosphate (DHAP) and D-glyceraldehyde 3-phosphate (GAP); both triosephosphates are products of the preliminary reaction carried out by aldolase that constitutes an essential step in glycolysis. Only GAP follows to the next reaction of glycolysis; it is the substrate of glyceraldehyde phosphate dehydrogenase (GAPDH); therefore, DHAP must be converted into GAP to produce complete ATP and NADH in this pathway. In addition, TPI avoids the accumulation of DHAP, a highly toxic metabolite, which breaks down to methylglyoxal; both are potent glycation agents that modify the structure of nucleic acids and proteins (including TPI) (Li et al. 2008). Recently, it was demonstrated that the inhibition of TPI and GAPDH in cultures of neurons triggered an accumulation of DHAP followed by progressive cell death (Sheline and Choi 1998). Due to its features, TPI has been proposed as a promising target to develop novel drugs against pathogens (Oppendoes

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and Michels 2001). In this regard, the TPI of several parasites has been inhibited through different strategies: *Plasmodium falciparum* with synthetic peptides (Kuntz et al. 1992; Singh et al. 2001) and a sulphonated dye (Joubert et al. 2001); *Entamoeba histolytica* and *Trypanosoma cruzi* with chemical compounds methyl methanethiosulfonate (Rodríguez-Romero et al. 2002) and 3-(2-benzothiazolythio)-1-propanesulfonic acid (Tellez-Valencia et al. 2004), respectively; and *Schistosoma mansoni* and *Tr. cruzi* with its respective monoclonal antibody (Harn et al. 1992; Cortés-Figueroa et al. 2008).

Both polyclonal antibodies and a monoclonal antibody toward the whole TTPI and to an epitope contained within helix- $\alpha$ 6, respectively, inhibit the catalytic activity of TTPI (Jiménez et al. 2003; Sanabria-Ayala et al. 2013) in addition, vaccinated mice with TTPI were protected against cysticercosis caused by *Taenia crassiceps* (Jiménez et al. 2003). Moreover, TPI enzymes have three non-conserved regions at positions 17–63, 137–161, and 188–206 with remarkable differences (Jiménez et al. 2000).

Phage display technology uses filamentous phages of *Escherichia coli* (M13, fd, and f1) as vehicles to express proteins or peptides fused to surface proteins belonging to the phage (Smith and Petrenko 1997). It is a useful technique to investigate interactions between several ligands, such as protein-protein, carbohydrate-protein, and protein-nucleic acids, but is especially suitable for epitope mapping in the quest of new drug targets that offer solutions to treat infectious diseases (Mullen et al. 2006). Antibodies against specific epitopes from a protein can isolate peptides composed of different amino acids that mimic the original epitope; for this reason, they are referred to as mimotopes (Geysen et al. 1985, 1986). An active immunization using mimotopes induces antibodies to recognize the mimicked epitope. It has been successful in the development of diagnostic probes (González et al. 2010; Manhani et al. 2011; Gazarian et al. 2012a, b; Van Nieuwenhove et al. 2012) and has improved vaccines (Arnon et al. 2000; Guo et al. 2010; Gazarian et al. 2012a, b; Capelli-Peixoto et al. 2011).

In this work, we propose a method to identify regions involved in enzymatic catalysis. In this case, we use antibodies against the NH<sub>2</sub>-terminal region of TTPI (1/3NH<sub>2</sub>TTPI) and phage display technology, which allowed us to isolate one clone that carried the peptide (PDTS16) that mimics an epitope on helix- $\alpha$ 1 of TTPI. Anti-PDTS16 antibodies were able to inhibit TTPI activity and were useful to identify the epitope region on TTPI.

## Materials and methods

### Preparation of antigen (1/3NH<sub>2</sub>TTPI)

In a first instance, a recombinant TTPI (rTTPI) fused to six histidines was produced using the pRSET vector in *Es. coli*

(strain TG1); the procedure was carried out as described before by Jiménez et al. (2000). To prepare the 1/3NH<sub>2</sub>TTPI fragment, a computational analysis was carried out using the program PC/GENE 6.8 (IntelliGenetics, USA) to choose the most suitable protease and site of cleavage on TTPI; then, we selected the V8 protease from *Staphylococcus aureus* (Sigma-Aldrich, USA). Therefore, 1 mg of purified TTPI was digested with V8 protease in ammonium acetate buffer (0.1 M, pH 7.8) at 37 °C. Samples from the digestion were taken at several times (2–32 h). The product of the digestion was passed through metal affinity chromatography, and the binding fractions were eluted with a linear gradient from 10 to 250 mM of imidazole in phosphate-buffered saline (PBS), to obtain the 1/3NH<sub>2</sub>TTPI. Samples from V8 digestion and fractions of the chromatography were visualized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

### Immunization of animals

New Zealand rabbits were immunized with 50  $\mu$ g of 1/3NH<sub>2</sub>TTPI or TPIs or TTPI synthetic peptides mixed with 10  $\mu$ g of saponin, or with 10<sup>13</sup> pfu of selected phage mimotope clones or with wild-type phage M13KE in 200  $\mu$ L of sterile PBS, applying a boost at days 15 and 30. One week after the last immunization, sera were obtained. Titers of sera against TPIs, TTPI peptides, and mimotopes were determined by ELISA. IgG fractions (antibodies) of all sera were purified by Protein A-Sepharose (Sigma, USA). All experiments done with rabbits comply with the current institutional ethical regulations and the Official Mexican Norms: NOM-062-ZOO-1999 for the use, care, and killing of laboratory animals.

### Western blot assays

TPIs (2  $\mu$ g) were separated through SDS-PAGE at 12 % and transferred onto nitrocellulose membranes (Millipore, USA). The membranes were blocked with PBS containing 5 % dry milk and then incubated with the antibodies against each TPIs from preimmune and immunized rabbits (1:1,000) for 1 h at 37 °C. Peroxidase-conjugated goat anti-rabbit IgG (Zymed) was used as secondary antibody. Bound antibodies were developed with 0.5 mg of 3-3'-diaminobenzidine and 2  $\mu$ L of 1 % H<sub>2</sub>O<sub>2</sub> in PBS for 15 min at temperature (RT).

### Screening of phage display library

Four successive rounds of biopanning with anti-1/3NH<sub>2</sub>TTPI antibodies were carried out with a random dodecapeptide phage display library, to determine which peptides are displayed in fusion to the pIII protein of the filamentous phage M13KE (PhD12, New England Labs). In brief, one EIA/RIA 8-well strip (Corning, Corning, NY, USA) was coated with

5 µg/mL of anti-1/3NH<sub>2</sub>TTPI antibodies in carbonate buffer (15 mM Na<sub>2</sub>CO<sub>3</sub>, 35 mM NaHCO<sub>3</sub>, 0.2 g/L NaN<sub>3</sub>, pH 9.6) overnight at 4 °C. Plates were blocked with 0.25 % bovine serum albumin (BSA) in Tris-buffered saline (TBS) buffer (50 mM Tris-HCl, 150 mM NaCl, pH 7.5) for 2 h, washed with TBS three times, and incubated overnight at 4 °C with 10<sup>11</sup> pfu in 100 µL of TBS per well. Wells in the plates were washed three times with TBS with 0.05 % Tween-20, and phage display mimotopes were released by incubating for 10 min with 1.0 mL of 100 mM triethylamine (pH 12) and immediately neutralized with 0.5 mL of 1 M Tris-HCl (pH 7.4). Eluted phages were added to 1 mL TG1 bacterial cultures. Cultures were added to 100 mL of 2XYT medium and incubated overnight at 37 °C. Cultures were centrifuged and supernatants were transferred to a fresh tube, and phages were precipitated at 4 °C adding 1/6 volume of PEG/NaCl (20 % polyethylene glycol 8,000/2.5 M NaCl) and, afterward, centrifuged at 1,950×g, and the pellet was resuspended in TBS. Titration of amplified phages was carried out by the agar overlay technique. For the second round, we coated wells with 4 µg/mL anti-1/3NH<sub>2</sub>TTPI antibodies, and TBS with 0.1 % Tween-20 was used for washings; for the third round, 2 µg/mL of anti-1/3NH<sub>2</sub>TTPI antibodies, and TBS with 0.3 % Tween-20 for washings; and for the fourth round, 1 µg/mL of anti-1/3NH<sub>2</sub>TTPI antibodies, and TBS with 0.5 % Tween-20 for washings. We alternated BSA and skimmed milk for blocking buffer preparation to avoid unwanted selection elicited by these proteins. Last round of panning was plated on LB/IPTG (isopropyl-β-D-1-thiogalactopyranoside)/Xgal (5-bromo-4-chloro-3-indoyl-β-D-galactoside) plates, and several blue plaques were picked randomly for sequencing.

#### TPI activity assay

Enzymatic activity was determined in a coupled assay by the formation of DHAP from G3P as previously reported (Jiménez et al. 2003). For the inhibition assay, antibodies were preincubated at different amounts (50 and 100 µg) with 10 µg of TTPI in 1 mL TED buffer (10 mM triethanolamine, 1 mM EDTA, 1 mM dithiothreitol) at RT for 30 min. The activity assay was initiated by adding 6 ng of preincubated TTPI in a cuvette that contained 1 mL of reaction mixture (100 mM triethanolamine, 10 mM EDTA, 1 mM DTT, 1 mM glyceraldehyde 3-phosphate, 0.2 mM β-NADH, 2.5 U of α-glycerophosphate dehydrogenase), and kinetics was monitored by absorbance decay at 340 nm in spectrophotometer Ultrospect 3100 pro (Amersham Biosciences, Sweden).

#### ELISA techniques

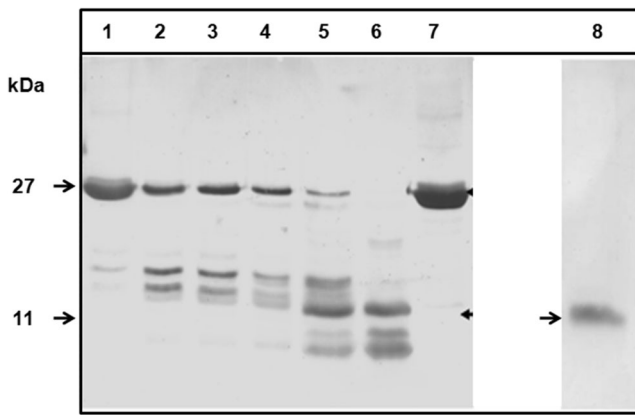
ELISA plates were coated with a solution of 5 µg/mL of each TTPI synthetic peptides and TTPI in carbonate buffer (15 mM Na<sub>2</sub>CO<sub>3</sub>, 35 mM NaHCO<sub>3</sub>, 0.2 g/L NaN<sub>3</sub>, pH 9.6) during 48 h

at 4 °C. Following the removal of the binding solution, the plates were rinsed five times with PBS containing 0.3 % Tween-20, 0.3 % BSA, and 0.3 % skimmed milk (blocking buffer) to saturate protein binding sites and, then, incubated for 1 h at RT. The solution was shaken out, and the plates were rinsed in PBS containing 0.3 % Tween-20, and drained. At this time, the anti-mimotope or anti-peptide antibodies were diluted 1:1,000 and added to the wells, and the plates were incubated at RT for 2 h. The plates were then washed three times with PBS containing 0.3 % Tween-20, and 50 µL of goat anti-rabbit IgG conjugated to peroxidase (Zymed, San Francisco, CA) was diluted 1:2,000 with blocking buffer, and 50 µL of this antibody was added to each well. After incubation for 1 h at RT, the plates were washed with PBS containing 0.3 % Tween-20. Finally, the wells were incubated with 100 µL/well of a solution containing orthophenyldiamine (0.4 mg/mL) and 1 % H<sub>2</sub>O<sub>2</sub> in citrate buffer pH 5.5. The plates were incubated at RT and the reaction stopped with 20 µL of 2 N sulfuric acid per well and measured in a spectrophotometer at 492 nm (Multiskan Ascent, Thermo Scientific, USA). For binding confirmation of phage clones, ELISA plates were coated with anti-TTPI-1, 2, and 3 antibodies at a concentration of 5 µg/mL in carbonate buffer at 4 °C overnight. Phages were applied in duplicates at 10<sup>10</sup> pfu/mL in TBS per each selected clone for 3 h at RT. As negative control, we used a phage clone with no peptide fusion displayed, and as a positive control a solution of TTPI at 1 µg/mL in PBS. The bound phages were detected with anti-M13 antibody conjugated with HRP (GE Healthcare). The signal was visualized as previously mentioned.

## Results

#### Obtaining of 1/3NH<sub>2</sub>TTPI

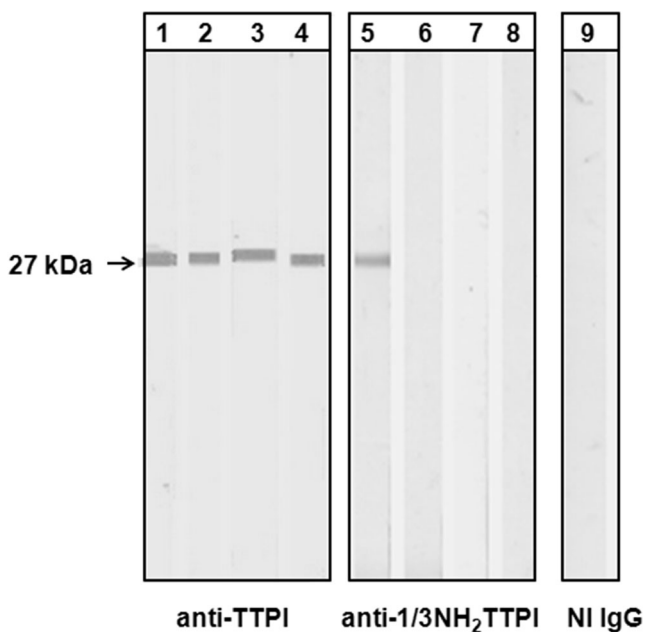
We chose protease V8 from *St. aureus* to digest TTPI because it is an endopeptidase that cuts specifically after glutamic acid (E) from carboxyl to amino terminal, under specific conditions of use (Houmard and Drapeau 1972; Fontana et al. 2004); in turn, we were able to identify several cleavage sites throughout TTPI. The optimal time of digestion was determined; we collected samples at different times and analyzed them through SDS-PAGE. In Fig. 1, a gradual progression of digestion of TTPI can be observed; lanes 1–4 (2, 4, 8, and 16 h) show vanishing of the 27-kDa band and an enrichment of smaller fragments; in lane 5 (24 h), the presence of an ~11-kDa fragment is notorious, but TTPI is still present; however, in lane 6 (32 h), TTPI disappeared and the band of ~11 kDa is fully perceptible. This His-tagged fragment was purified through nickel affinity chromatography (lane 8). Lane 7 shows the TTPI with no protease. We started the digestion with 1,000 µg of TTPI and 150 µg of protease V8, and after purification, we recovered 400 µg of the amino terminal fragment.



**Fig. 1** SDS-PAGE of the purification of the first third of the NH<sub>2</sub>-terminal region of TTPI (1/3NH<sub>2</sub>TTPI). *Left panel* shows TTPI digestion with protease V8 from *St. aureus* at different times: 2 h (*lane 1*), 4 h (*lane 2*), 8 h (*lane 3*), 16 h (*lane 4*), 24 h (*lane 5*), and 32 h (*lane 6*). The gel strip on the right (*lane 8*) shows the purified fragment of ~11 kDa (1/3NH<sub>2</sub>TTPI) obtained with 32-h digestion and after affinity chromatography on metal chelating column. As control, TTPI without protease V8 was included (*lane 7*)

### Specificity of antibodies against 1/3NH<sub>2</sub>TTPI

All anti-1/3NH<sub>2</sub>TTPI, TPI, and TTPI peptide sera of immunized rabbits had a titer of 1:10,000 determined by ELISA. Figure 2 shows the specificity of anti-1/3NH<sub>2</sub>TTPI antibodies tested by Western blot with the used TPIs. Anti-TTPI antibodies recognized whole TTPI (strip 1) and anti-TPIs antibodies



**Fig. 2** Western blot shows the specificity of antibody anti-1/3NH<sub>2</sub>TTPI. Strips with TPI of *Taenia solium* (1, 5, and 9), *Sus scrofa* (2 and 6), *Schistosoma mansoni* (3 and 7), and *Entamoeba histolytica* (4 and 8). Strips 1–4 were tested with the respective homologous anti-TPI antibodies. Strips 5–8 were tested with anti-1/3NH<sub>2</sub>TTPI antibodies. Strip 9 was tested with non-immune IgG from rabbit, as negative control (NI IgG)

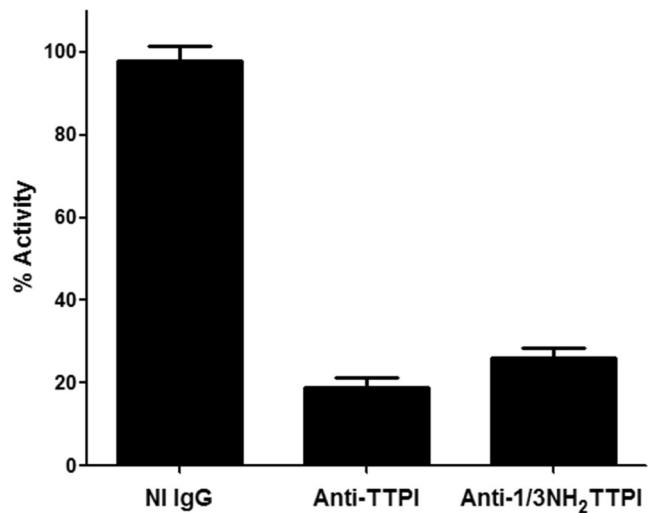
raised against *Sus scrofa*, *Sc. mansoni*, and *En. histolytica* recognized their respective TPI (strips 2–4); in contrast, anti-1/3NH<sub>2</sub>TTPI antibodies only recognized TTPI (strip 5). As negative control, IgG from non-immune rabbit serum was included (strip 9) and did not recognize any TPIs tested.

### Inhibition assay of TTPI

The inhibition assay performed with anti-1/3NH<sub>2</sub>TTPI and anti-TTPI antibodies demonstrated that both were able to inhibit TTPI activity (Fig. 3). The graph shows the reduction in activity of TTPI; after being incubated with 100 μg of anti-TTPI or anti-1/3NH<sub>2</sub>TTPI antibodies, the inhibition was 81 and 74 %, respectively. As negative control, IgG from non-immune rabbit serum was used, and as expected, it did not alter the catalysis.

### Obtaining of mimotopes and assessment of mimicry with TTPI

To map the epitopes on 1/3NH<sub>2</sub>TTPI, a PhD12 library was screened decreasing the anti-1/3NH<sub>2</sub>TTPI antibodies and increasing Tween-20 along the four rounds of biopanning; this allowed us to select those clones with high affinity. Between the first and last rounds, there was an enrichment of  $6.2 \times 10^4$  (Table 1). Thus, from the last round, 41 clones were randomly picked and DNA-sequenced. With the analysis of deduced amino acid sequences of the peptides, we sorted them in groups: consensus A, the largest one, was subdivided into two groups, according to the presence of a second proline, they are A1 (VPTXPI) and A2 (VPTXXI); B with DPLPR;



**Fig. 3** Inhibition of *Taenia solium* TPI (TTPI) activity with antibodies. TTPI was incubated with 100 μg of IgG from non-immunized rabbit (NI-IgG, negative control), anti-TTPI (positive control), and anti-1/3NH<sub>2</sub>TTPI. The values represent the percentages of the residual activities in regard to the enzyme activity assayed in the absence of any antibody (data not shown)

**Table 1** Enrichment of phage clones interacting with anti-1/3NH<sub>2</sub>TTPI antibodies

Round	TBST wash	Inputted phages (pfu)	Eluted phages (pfu)	Recovery rate <sup>a</sup>
1	0.05 %	8×10 <sup>11</sup>	8.5×10 <sup>3</sup>	1.06×10 <sup>-8</sup>
2	0.1 %	8×10 <sup>11</sup>	6.2×10 <sup>6</sup>	7.75×10 <sup>-6</sup>
3	0.3 %	8×10 <sup>11</sup>	1.7×10 <sup>8</sup>	2.12×10 <sup>-4</sup>
4	0.5 %	8×10 <sup>11</sup>	5.3×10 <sup>8</sup>	6.62×10 <sup>-4</sup>

pfu plaque forming unit  
<sup>a</sup> Phages eluted/phages inputted

and C with LTPGQ. An interesting fact here was that none of the peptides obtained with anti-1/3NH<sub>2</sub>TTPI antibodies showed any remarkable sequence homology with it (Fig. 4). In order to demonstrate mimicry between the selected random peptides and the 1/3NH<sub>2</sub>TTPI, six rabbits were immunized with one or more phage clones that represent each consensus (see Table 2). Likewise, we immunized a rabbit with the wild-type phage M13KE (with no peptide insert) to be used as negative control. Western blot from Fig. 5 shows that all anti-mimotope antibodies cross-reacted with TTPI, contrary to anti-M13KE antibodies and IgG from non-immune rabbit serum that did not recognize it. As positive control, anti-TTPI antibodies were used, and as expected, they recognized TTPI (strip 1).

**Inhibition assay with anti-mimotope antibodies**

The inhibition assay was carried out with the antibodies raised toward M1-M6; in addition, we produced antibodies toward peptides TTPI-1, TTPI-2, and TTPI-3, which are contained within the 1/3NH<sub>2</sub>TTPI fragment. Figure 6 depicts the nomenclature of all peptides used in the following assays, as well as their sequences and positions.

The graph on Fig. 7 shows the inhibition assay of TTPI activity. In the case of antibodies against M1-M3, which belong to consensus A1 and A2, slight inhibition of the

**Table 2** Phage clones used as immunogens

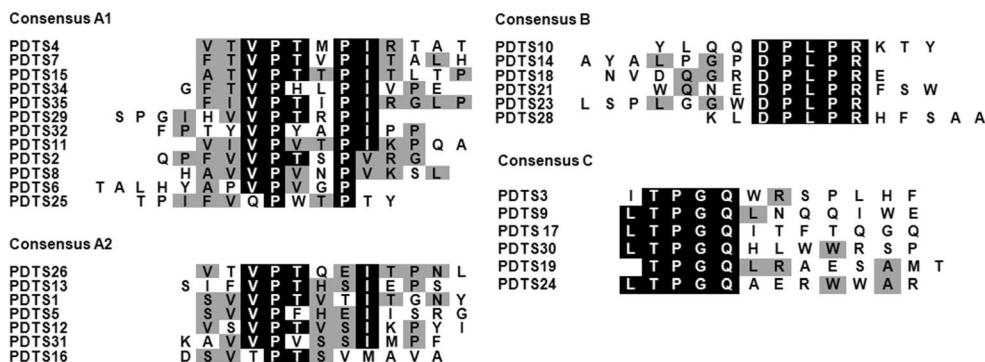
Immunogen	Phage clones	No. times appeared
M1	PDTS4 (VTVP <b>TMPI</b> RTAT)	3
M2	PDTS11 (VIVPV <b>TPIK</b> PQA) PDTS 8 (HAVVPV <b>NPVK</b> SL)	2
M3	PDTS 5 (SVVP <b>FHEI</b> SRG) PDTS13 (SIV <b>PTHSI</b> EPS) PDTS31 (KAVVPV <b>SSIM</b> PF)	1
M4	PDTS16 (DSVT <b>PTSVM</b> AVA)	6
M5	PDTS 9 (L <b>TPGQ</b> LNQQIWE) PDTS30 (L <b>TPGQ</b> HLWWRSP)	1
M6	PDTS10 (YL <b>QQDPL</b> PRKTY) PDTS18 (NVD <b>QGRDPL</b> PRE)	1

Letters in bold represent the conserved aminoacids in displayed peptides of the isolated clones

activity of around 10 % was observed, whereas anti-mimotope 4 antibodies (consensus A2) caused an inhibition of 45 %. The latter was noteworthy, because it appeared in the selection more times than the others and was used as single immunogen (PDTS16, DSVTPTSVMAVA). Anti-mimotope 5 and 6 antibodies, which were raised against clones from consensus B and C, respectively, did not show any degree of inhibition. We used anti-1/3NH<sub>2</sub>TTPI antibodies as positive control—it inhibited 74 %—and non-immune IgG and anti-M13KE antibodies as negative controls. On the other hand, anti-TTPI-1 and anti-TTPI-2 antibodies inhibited catalytic activity of TTPI in 40 and 30 %, respectively. In contrast, anti-TTPI-3 antibodies did not inhibit it.

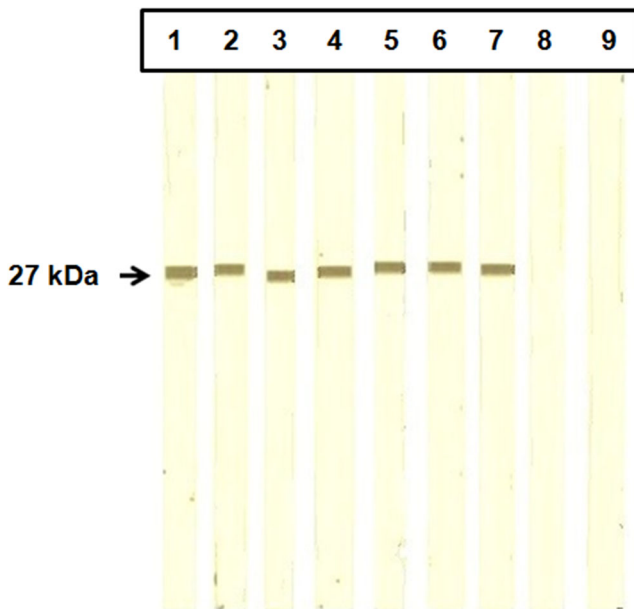
**Determination of binding site of anti-PDTS16 antibodies**

Figure 8a shows that anti-TTPI-1 and anti-TTPI-2 antibodies recognized TTPI and the peptides TTPI-1, 2, 12, N, and TS16, whereas anti-TTPI-3 antibodies only recognized TTPI, TTPI-N, and TTPI-3. Figure 8b shows that anti-TTPI antibodies



**Fig. 4** Alignment of deduced amino acid sequences of phage-displayed peptides mimotopes. After four panning rounds of a random peptide phage display library (PhD12) using anti-1/3NH<sub>2</sub>TTPI antibodies

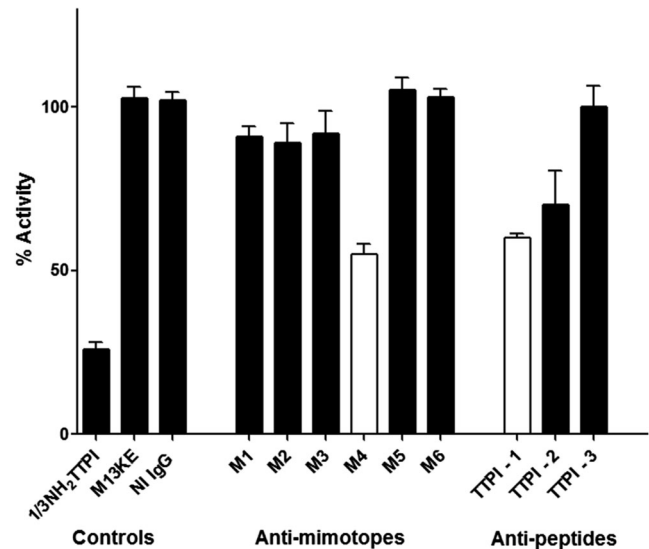
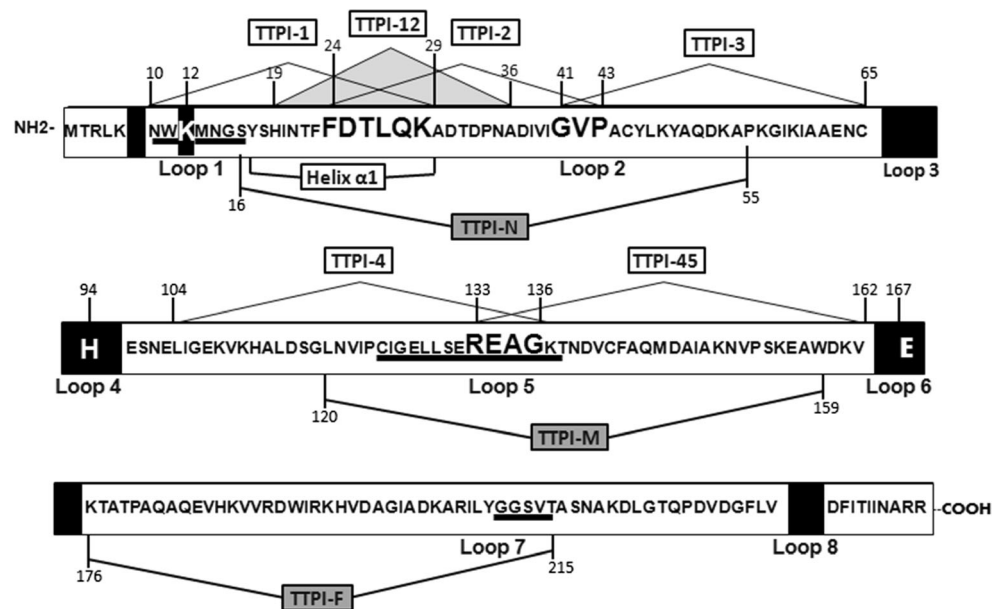
revealed four consensus: A1(VPTXPI), A2(VTPXXI), B(DPLPR), and C(LTPGQ); they are indicated in *black*. Amino acids with shared positions among the peptides are indicated in *gray*



**Fig. 5** Western blot of anti-TTPI mimotope antibodies against TTPI. Strips 2–7 were tested with rabbit anti-mimotopes antibodies raised against M1 and M2 (VPTXPI), M3 and M4 (VPTXXI), M5 (DPLPR), and M6 (LTPGQ). As positive control, we used rabbit anti-TTPI antibodies (*strip 1*), and as negative controls (*strip 8*) rabbit anti-M13KE (wild-type phage) antibodies and non-immune IgG from rabbits (*strip 9*)

(positive control) recognized TTPI and all peptides used, whereas the anti-1/3NH<sub>2</sub>TTPI antibodies recognized TTPI and the peptides TTPI-N, 1, 2, 3, and 12, but they did not recognize peptides located out of 1/3NH<sub>2</sub>TTPI (TTPI-4 and 45). The anti-PDTS16 antibodies show a similar recognition to anti-1/3NH<sub>2</sub>TTPI antibodies; however, they did not recognize the TTPI-3 peptide.

**Fig. 6** Schematics of the whole TTPI (250 amino acids). Each one of the three segments represents around 83 residues. Composition and length of synthetic peptides are indicated as TTPI-1, TTPI-2, TTPI-12, TTPI-3, TTPI-4 and TTPI-45, TTPI-N, TTPI-M, and TTPI-F. *Largest letters* inside indicate overlapping sequences among peptides; *white letters* indicate amino acids of the active site; *underlined sequences* and *black segments* indicate conserved motifs. Positions of loops and helix- $\alpha$ 1 are indicated under each respective sequence

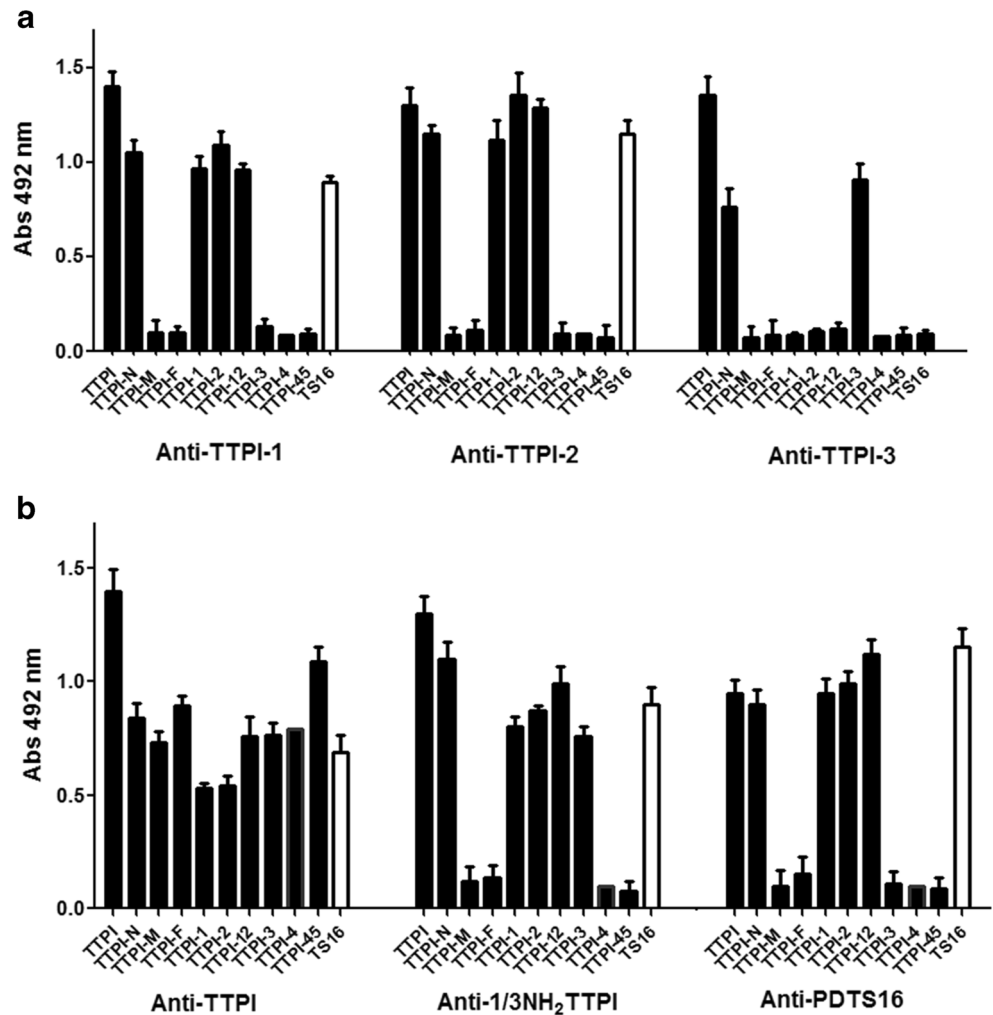


**Fig. 7** Inhibition of *Taenia solium* TPI (TTPI) activity by anti-mimotopes antibodies. TTPI was incubated with anti-mimotopes antibodies M1 and M2 (consensus VPTXPI), M3 and M4 (consensus VPTXXI), M5 (consensus DPLPR), and M6 (consensus LTPGQ). Only anti-mimotope 4 antibodies caused a marked inhibition. Additionally, antibodies toward peptide TTPI 1–3 were tested, and only anti-TTPI-1 antibodies show a level of inhibition similar to anti-mimotope 4 antibodies. As positive control, anti-1/3NH<sub>2</sub>TTPI antibodies were included, and as negative controls, anti-M13KE and non-immune IgG (NI IgG) antibodies. All antibodies used in this assay were raised in rabbits and 100  $\mu$ g was used

#### Localization of the mimotopes of 1/3NH<sub>2</sub>TTPI

To determine the localization of the mimotopes along the 1/3NH<sub>2</sub>TTPI, we confronted, by ELISA, anti-TTPI peptide (1, 2, 3, and 12) antibodies against mimotope

**Fig. 8** ELISA that confronts all synthetic peptides contained in TTPI and mimotope synthetic peptide TS16 with antibodies raised against **a** TTPI-1, TTPI-2, and TTPI-3 synthetic peptides. **b** TTPI, 1/3NH<sub>2</sub>TTPI, and mimotope synthetic peptide TS16. As positive control, TTPI was used in this assay, and peptides TTPI-M, 4, 45, and F, located out of the target 1/3NH<sub>2</sub>TTPI, were taken as negative controls



phage clones 1–6. We found that anti-TTPI-1 and 2 recognized mimotope clone consensus from VPTXPI, VPTXXI, and TTPI used as positive control; in contrast, they did not recognize the phage M13KE (negative control). In addition, anti-TTPI antibodies recognized mimotopes from the consensus DPLPR, and anti-TTPI-3 antibodies recognized mimotopes from the consensus LTPGQ (not shown). Based on these data, we made an alignment of the sequence of TTPI, which is encompassed by peptides TTPI-1 and TTPI-2, with the mimotopes of the consensus A1 (VPTXPI), A2 (VPTXXI), and B (DPLPR), and another alignment of TTPI-3 with mimotopes of consensus C (LTPGQ) was performed. Alignment analysis showed that the mimotopes of the consensus A1 and A2 presented partial homology with the region YSHINTFFDLQK of TTPI. In turn, consensus DPLPR had partial homology with the region DTLQKADTPNAD of TPI, which is predominantly part of TTPI-2. Finally, consensus C had partial homology with LKYAQDKAPKGI, region of TTPI encompassed by peptide TTPI-3 (Fig. 9).

## Discussion

This work was aimed at finding epitopes involved in the catalysis of TTPI by producing inhibitory antibodies against non-conserved epitopes present in 1/3NH<sub>2</sub>TTPI. Therefore, our first step consisted in isolating the 1/3NH<sub>2</sub>TTPI using the V8 protease from *St. aureus* (Houmard and Drapeau 1972; Fontana et al. 2004). In addition, we took advantage of knowing the position of the glutamic residues on the amino acid sequence of TTPI, and of having a plasmid construction that produces an amino terminal histidine-tagged TTPI. The purification and digestion processes yielded a fragment of ~11 kDa (1/3NH<sub>2</sub>TTPI) that coincides with the expected cleavage at glutamic-88 in TTPI. The second step consisted in producing specific anti-1/3NH<sub>2</sub>TTPI antibodies that have no cross-reaction with other TPis tested by Western blot, especially with *Su. scrofa* (intermediary host). The fact that the antibodies reacted with the TTPI in western blot may be an indication that linear epitopes are involved in the inhibition; nevertheless, it is possible that some epitopes recognized by the antibodies have a complex conformation.





is suggested that residue  ${}_{24}\text{FDTLQK}_{29}$  is part of the epitope and is located on the helix- $\alpha 1$ , which is exposed and near to the interface between the monomers. These findings reveal the important interaction between this helix with the helix- $\alpha 3$  of the adjacent subunit providing stability of the dimer.

Nowadays, there are many works that report the use of mimotopes as tools for studying mechanisms of molecular recognition, mimetic molecules for receptors, or as basis for designing compounds with potential use in therapy or diagnosis. The most important feature lies in developing vaccines and efforts to obtain effective therapies for pathogens, such as *Plasmodium vivax* (Demangel et al. 1998), *Sc. mansoni* (Arnon et al. 2000), *Cryptococcus neoformans* (Fleuridor et al. 2001), *P. falciparum* (Casey et al. 2004; Sabo et al. 2007), *Fasciola hepatica* (Villa-Mancera et al. 2008, 2011), *Streptococcus pneumonia* (Smith et al. 2009), *Toxoplasma gondii* (Cunha-Júnior et al. 2010), and *Helicobacter pylori* (Li et al. 2010).

The present work demonstrated that antigenic mimicry of the mimotopes can help to mapping epitopes recognized by antibodies, which allows for the localization of the binding site (Petit et al. 2003; Pacios et al. 2008; Donker et al. 2011). In this case, we identified by antibodies and phage display technique one peptide that mimics a linear epitope on the region  ${}_{17}\text{YSHINTFFDTLQK}_{29}$ , located on the helix- $\alpha 1$  of TTPI, which is important for TTPI catalytic activity. Our results also indicated the potential of using mimotopes as an alternative to develop therapeutic and preventive strategies toward neglected diseases, such as the taeniosis/cysticercosis binomial.

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**Conflict of interest** The authors declare that they have no conflict of interests.

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