Published in final edited form as:

Biochem Biophys Res Commun. 2009 August 21; 386(2): 333–337. doi:10.1016/j.bbrc.2009.06.028.

Translationally controlled tumor protein is a novel heat shock protein with chaperone-like activity

Munirathinam Gnanasekar, Gajalakshmi Dakshinamoorthy, and Kalyanasundaram Ramaswamy

Department of Biomedical Sciences, College of Medicine, University of Illinois, Rockford, IL 61107

Abstract

Translationally controlled tumor protein (TCTP)¹ is often designated as a stress-related protein because of its highly regulated expression in stress conditions. Following a thermal shock, TCTP expression is highly up regulated in a variety of cells. However, at present it is not known whether this up regulation has any cell protective function similar to other heat shock proteins. In this study human TCTP (HuTCTP) and a TCTP homolog (SmTCTP) from *Schistosoma mansoni* were evaluated for heat shock protein-like function and molecular chaperone activity. Our results show that similar to other molecular chaperones, both human and parasite TCTPs can bind to a variety of denatured proteins and protect them from the harmful effects of thermal shock. An important observation was the ability of both HuTCTP and SmTCTP to bind to native protein and protect them from thermal denaturation. Over expression of TCTP in bacterial cells protected them from heat shock-induced death. These findings suggest that TCTP may belong to a novel small molecular weight heat shock protein.

Keywords

TCTP; chaperone; luciferase; heat shock protein

Introduction

TCTP was first identified in Ehrlich ascites tumor cell lines [1] and was later shown to be a growth-associated protein ubiquitously present in a variety of cells. TCTP is one of the 20 most abundantly expressed proteins in normal cells [2] and its function is described as a heat stable, calcium binding [3;4], antioxidant protein [5] that negatively regulates apoptosis [6;7] and cause release of histamine from basophils [3;4;8]. TCTP also gets transported to multi organelles within the cell.

TCTP expression is increased in response to a variety of stress conditions [9;10] and TCTP can prevent hydrogen peroxide induced cell death [11]. Similarly, Mak *et al* [12] showed that

¹Abbreviations used in this paper: TCTP, translationally controlled tumor protein; CS, citrullin synthase; HSPs, heat-shock proteins; PBMC, peripheral blood mononuclear cells

© 2009 Elsevier Inc. All rights reserved.

Address for correspondence and reprint requests to Dr K. Ramaswamy, Department of Biomedical Sciences, College of Medicine, University of Illinois, Rockford, IL 61107. E-mail address: E-mail: ramswamy@uic.edu, Phone: (815) 395-5696, Fax: (815) 395-5684.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

TCTP is one of three major genes that were up regulated in *Trichinella spiralis* larvae following a heat shock stimuli.

In cells that are heat stressed, there is an increase in heat shock protein (HSP) expression, which in turn protects several critical proteins inside the cells by acting as molecular chaperones [13]. Solution structure of TCTP show structural similarity to a family of guanine nucleotide-free chaperones that binds to the GDP/GTP free form of Rab proteins (members of the Ras superfamily) [14]. Similarly, TCTP can bind to and stabilize MCL1, a very labile antiapoptotic protein from protein degradation [15]. These findings suggest that TCTP can potentially function as molecular chaperone. To begin to understand the cellular function of this fascinating protein, in this study we evaluated whether TCTP is a heat shock protein and whether it can protect cellular proteins from heat shock damage by acting as a molecular chaperone.

Materials and methods

Evaluation of TCTP expression in parasites after a heat shock treatment

Cercariae released from snails (cold blooded) enter into the vertebrate (warm blooded) host through the skin to establish infection. This change from cold blooded to warm blooded host creates a heat shock that could trigger several heat shock proteins including probably TCTP. Therefore, we first evaluated the expression levels of TCTP in cercariae (the free-living form) and compared this to skin-stage schistosomula (the stage that are found in the skin of vertebrate host). Cercariae and schistosomula stages were prepared as described previously [4].

We also exposed cercariae and schistosomula to a heat shock stimuli *in vitro* and measured differences in TCTP expression. Briefly, 1000 cercariae or schistosomula suspended in 1 ml distilled water were incubated for one hour at 37°C or at 42°C. Larvae maintained at room temperature (25°C) served as controls. Following incubation, mRNA was isolated, cDNA prepared and expression levels of TCTP was determined by PCR using insert specific primers [4]. PCR conditions were denaturation at 95°C for 30S, primer annealing at 55°C for 30S, primer extension at 72°C for 30S and cycle repeated 30 times. Final extension was at 72°C for 5 min before storing the samples at 4°C.

Expression of TCTP in human cells after a heat shock treatment

PBMCs were collected from two healthy donors after obtaining proper consent and approval from the Institutional Review Board of the University of Illinois, College of Medicine at Rockford. Approximately, 6×10^6 cells suspended in one ml were cultured at 37°C or at 42°C (for inducing heat shock) for 12 hrs. Samples were collected at various time intervals and processed for PCR analysis as described before [4] using primers that are specific for human TCTP.

In vitro peptide binding assay for heat shock protein

Recombinant SmTCTP or recombinant human TCTP was prepared as described previously [4] and was biotinylated using a kit (ThermoFisher Scientific, Rockford, IL). Concentration of biotinylated protein was estimated using a BCA kit (ThermoFisher Scientific). Binding of rSmTCTP or rHuTCTP to citrulline synthase (CS), luciferase and lysozyme was determined by ELISA [16]. BSA was used as the control. CS, luciferase and lysozyme were selected because these proteins are highly sensitive to heat and chemical denaturation. Wells of 96 well plates were coated overnight at 4°C with native proteins, proteins denatured by heat (42°C for 30 minutes) or proteins denatured by chemical (8M guanidium hydrochloride). After blocking non-specific sites with 3% BSA, wells were incubated with 1µg/ml of biotinylated rSmTCTP or biotinylated rHuTCTP for 30 minutes at room temperature in the presence or absence of ATP. After washing, the wells were incubated with horse radish peroxidase labeled streptavidin

(Sigma, 1:10000 dilution) for 15 minutes at room temperature. Color was developed with OPD substrate and OD₄₀₅ was measured in an ELISA plate reader (Dynatech MR4000).

In vitro peptide blocking assay

To determine specificity of binding, various dilutions of polyclonal mouse anti-SmTCTP antibodies (1:50 to 1:10000) were incubated with 1 µg of biotinylated rSmTCTP for 1h at room temperature. Mouse anti-SmHMGB1 antibodies were used as control antibodies. Following incubation, 100 µl of the TCTP:antibody complex were added to the wells coated with denatured or native substrate proteins and binding of rSmTCTP was determined as described above.

Chaperone assay

Chaperone assay was performed as described previously [17;18]. The assay measures thermally induced aggregation of CS and luciferase in the presence or absence of TCTP in 50 mM sodium phosphate buffer, pH 7.4 containing 100 mM NaCl. His-tagged rSmTCTP and rHuTCTP (GenBank accession # AY334563) were used in this study. BSA was used as a control protein. Briefly, CS and luciferase were incubated at 42°C with TCTP or BSA at a molar ratio of 1:2 for various time intervals from 0 to 30 minutes. Following treatment thermal denaturation (aggregation) was monitored spectrophotometrically at 360nm.

Thermal denaturation of luciferase

In some studies, luciferase protein was heat denatured first. Briefly, one micromolar luciferase was heated for 8 min at 39.5°C in the presence of a denaturing buffer (25 mM HEPES, 5 mM MgSO₄, 5mM DTT, 150 mM KCl, pH 7.5) containing either 20 µg/ml of rSmTCTP or 20 µg/ml of rHuTCTP or 20 µg/ml of GBF in the absence of ATP (in 50 µl). This was sufficient to denature luciferase to less than 2% of its original activity as determined in a luminescence reader (Biotek instruments Inc., Winooski, VT). After heat denaturing, samples were immediately cooled in an ice bath for 30 s and luciferase activity was measured.

Re-activation of thermally inactivated luciferase

Refolding of the denatured luciferase was initiated by adding 1 µl of the denatured protein to 40 µl of refolding buffer containing 7.5 uM BSA, 50 mM KCl and 2 mM ATP, 50% rabbit reticulolysate (Promega, Madison, WI) in the presence or absence of 20 µg/ml of rSmTCTP. Refolding was allowed to proceed by incubation at 30°C for 3 hrs and luciferase activity was determined. The values are expressed as percentages activity relative to an equivalent amount of native luciferase.

Heat tolerance bioassay

The aim of these assay was to determine if higher expression of TCTP would confer thermal tolerance to SmTCTP transformed bacterial (BL21(DE3)) cells. SmTCTP transformed bacterial cells were grown until OD reading reached 0.6 nm and TCTP expression was induced with isopropyl-beta-D-thiogalactopyranoside (1 mM in LB medium containing ampicillin). Induced bacterial cells were then incubated at 42°C for 3 hours and serially diluted ranging from 1:10 to 1:100 and 10 µl droplet of each of the dilutions were plated on LB-agar. Plates were then incubated at 37°C for overnight. Bacterial cells expressing only the vector protein treated similarly served as controls. Following incubation, growth of bacterial cells was compared.

Statistical analysis

Statistical analysis was performed using Mann-Whitney U rank sum tests using Sigmastat 2.0 (Jandel Scientific Software, San Rafael, CA). $P < 0.05$ was considered statistically significant.

Results

TCTP expression is up regulated during heat shock

RT PCR analysis showed that following exposure of *S. mansoni* parasites or human PBMCs to 42°C there was a significant upregulation of TCTP (Fig 1A–C) compared to uninduced controls. However, there was a slight decrease in the TCTP mRNA expression at 42°C in schistosomula compared to schistosomula incubated at 37°C. This may be due to a decreased viability of schistosomula at 42°C.

Binding of SmTCTP to native and denatured protein substrates

rSmTCTP preferentially binds to denatured proteins substrates ($P < 0.01$) compared to native or control protein, BSA (Fig 2b). ATP is not essential for this binding (data not shown). TCTP binding to proteins was completely blocked when rSmTCTP was preincubated with mouse anti-SmTCTP antibodies (Fig 2a and 2b). This suggested that SmTCTP might belong to a novel class of small chaperones similar to other co-chaperones.

SmTCTP and HuTCTP prevents thermal aggregation of CS and luciferase

Potential role of TCTP as molecular chaperone was further analyzed using CS and luciferase as chaperone substrates. Subjecting CS to 42°C resulted in unfolding of the protein and significant aggregation within 10 minutes of incubation (Fig 2C). Addition of the non-chaperone control protein BSA had no effect on the heat-induced aggregation of CS. However, addition of rSmTCTP or rHuTCTP to the solution significantly ($P < 0.01$) inhibited the thermal aggregation of CS compared to BSA or rSmGBF proteins. In addition to CS, rSmTCTP and rHuTCTP also significantly ($P < 0.01$) prevented thermal aggregation of another classical chaperone substrate, luciferase for up to 30 minutes at 42°C (Fig 2D).

SmTCTP reactivates denatured luciferase

Only less than 7% of the thermally denatured luciferase recovered to its original activity after heat shock treatment at 42°C (Fig 3). However, >20% of luciferase enzyme activity was recovered when rSmTCTP and rabbit reticulolysate were added to the mixture. Addition of a control protein, rSmGBF had no effect. Interestingly, when rSmTCTP was added prior to heat denaturation, over 45% of the luciferase activity was protected suggesting that TCTP prevented irreversible denaturation of luciferase and maintained its correct folding competent state during thermal denaturation.

Over expression of SmTCTP conferred heat resistance

To determine if higher expression levels of TCTP will protect a cell *in vivo* from heat shock, we over expressed rSmTCTP in *E. coli* and subjected these cells to 42°C for 3 hours. The duration of heat shock used was sufficient enough to inhibit the recovery and growth of *E. coli* in LB agar as evidenced by the poor growth and smaller colonies of control cells exposed to heat shock. However, *E. coli* cells over expressing SmTCTP were able to rapidly recover and grow. Ability to recover was evident even after diluting the cultures to 1: 50. However, the recovery and growth of control *E. coli* cells containing the vector alone was substantially low even at a dilution of 1:10 (Fig 4). Cells transfected with the vector alone served as controls.

Discussion

Results presented in the study show that TCTP is a heat inducible protein that can protect cells from thermal shock by potentially acting as a molecular chaperone. Our studies also show that overexpression of TCTP in cells can protect the cells from heat shock. These findings collectively suggest that TCTP may be a novel small molecular weight heat shock protein.

Expression of TCTP is known to be highly regulated, especially in response to a wide range of stress conditions such as ammonium starvation in fission yeast [9], in response to environmental pollutants such as dioxin in mouse embryonic cells [19], hypoxia conditions [20], DTT-induced oxidative stress [9], calcium perturbation [21], heavy metal toxicity [10] and heat shock [12]. In addition, one of the TCTP sequences deposited in the Genbank designates the protein as a pO₂ related protein in the hepato-carcinoma cells (Genbank accession number: AAM51565). These reports collectively support the notion that TCTP may be a stress-related protein.

Recently, we cloned TCTP homologues from several human parasites including *B. malayi*, *W. bancrofti* [3], *S. mansoni* [4] and *S. haematobium* (Genbank accession number: AY157847). All these parasites have life cycle stages inside an invertebrate host before infecting human. Analysis of the TCTP expression levels show that larval stages that enter the warm-blooded vertebrate host has significantly higher levels of TCTP compared to the stages in the cold-blooded invertebrate hosts. This observation did not surprise us because TCTP has been documented as a heat-induced protein [12]. In fact, following heat shock, TCTP was one of the three genes that were up regulated in the infective larvae of another human parasite *T. spiralis* [12]. In our study, larval stages of *S. mansoni* and human PBMCs subjected to thermal shock *in vitro* also showed higher TCTP expression. These findings thus confirmed previous finding that TCTP is a heat-inducible protein [12].

Heat shock proteins are a family of highly conserved molecules that are up regulated during heat or oxidative stress and protect the cellular proteins from denaturation [22]. Based on their molecular size, the heat shock proteins can be grouped into small molecular weight heat shock proteins (14–33 kDa) and large heat shock proteins (60–100 kDa). Given the molecular size of TCTP and the fact that TCTP is up regulated during heat stress, it is possible that TCTP is a small heat shock-like protein. One of the primary functions of heat shock proteins is to protect cells from stress-induced damages including inhibiting both apoptotic and necrotic pathways [23]. Several recent reports suggest that TCTP is an anti-apoptotic protein [6;14;24]. Heat shock proteins also function as molecular chaperon by modulating other protein functions by changing their conformation, promoting protein-protein interaction and disassembly, regulating protein degradation, facilitating protein translocation across membranes, and ensuring proper folding of newly synthesized proteins during translation [13;25]. A typical characteristics of molecular chaperones is that they can recognize proteins of non-native structure and prevent them from irreversible intracellular aggregation [26]. Results from our study show that TCTP can bind to a variety of proteins including CS, luciferase and lysozyme denatured by heat or by chemical. Another interesting finding in our study is that similar to small heat shock proteins [27], TCTP appears to have the ability to bind to denatured proteins in the absence of ATP. This suggests that TCTP may be a novel class of small heat shock chaperones.

By binding to denatured proteins, molecular chaperones prevent aggregation of proteins during heat stress [26]. Our study suggests that TCTP behaved similar to other classic molecular chaperones. In addition to binding and preventing thermal aggregation of denatured proteins, rSmTCTP can also reactivate the denatured proteins. These findings suggested that TCTP in addition to protecting the proteins from thermal damage could help reverse their function

during or following a heat shock. This property of TCTP may be thus potentially important in rescuing cells from the damaging effects of heat stress.

Further confirmation on the cell protective role of TCTP during heat shock came from studies using *E. coli* cells over expressed with TCTP. This implies that SmTCTP may have an important role in protecting *S. mansoni* from harmful effects of heat shock that they encounter in the vertebrate host. In summary, the observation that the expression of TCTP is increased several fold during a heat shock plus the findings that TCTP can potentially act as a molecular chaperone by protecting the cellular proteins from the damaging effects of thermal shock and are capable of reactivating thermally damaged proteins suggest that TCTP is a novel small molecular weight heat shock protein.

Acknowledgement

This work was supported by Public Health Service grants AI-39066 and AI-064745 from NIAID.

References

1. Bohm H, Benndorf R, Gaestel M, Gross B, Nurnberg P, Kraft R, Otto A, Bielka H. The growth-related protein P23 of the Ehrlich ascites tumor: translational control, cloning and primary structure. *Biochem Int* 1989;19:277–286. [PubMed: 2479380]
2. Bommer UA, Thiele BJ. The translationally controlled tumour protein (TCTP). *Int J Biochem Cell Biol* 2004;36:379–385. [PubMed: 14687915]
3. Gnanasekar M, Rao KV, Chen L, Narayanan RB, Geetha M, Scott AL, Ramaswamy K, Kaliraj P. Molecular characterization of a calcium binding translationally controlled tumor protein homologue from the filarial parasites *Brugia malayi* and *Wuchereria bancrofti*. *Mol Biochem Parasitol* 2002;121:107–118. [PubMed: 11985867]
4. Rao KV, Chen L, Gnanasekar M, Ramaswamy K. Cloning and characterization of a calcium-binding, histamine-releasing protein from *Schistosoma mansoni*. *J Biol Chem* 2002;277:31207–31213. [PubMed: 12050167]
5. Gnanasekar M, Ramaswamy K. Translationally controlled tumor protein of *Brugia malayi* functions as an antioxidant protein. *Parasitol Res* 2007;101:1533–1540. [PubMed: 17687568]
6. Li F, Zhang D, Fujise K. Characterization of fortilin, a novel antiapoptotic protein. *J Biol Chem* 2001;276:47542–47549. [PubMed: 11598139]
7. Gnanasekar M, Thirugnanam S, Zheng G, Chen A, Ramaswamy K. Gene silencing of translationally controlled tumor protein (TCTP) by siRNA inhibits cell growth and induces apoptosis of human prostate cancer cells. *Int J Oncol* 2009;34:1241–1246. [PubMed: 19360337]
8. MacDonald SM, Rafnar T, Langdon J, Lichtenstein LM. Molecular identification of an IgE-dependent histamine-releasing factor. *Science* 1995;269:688–690. [PubMed: 7542803]
9. Bonnet C, Perret E, Dumont X, Picard A, Caput D, Lenaers G. Identification and transcription control of fission yeast genes repressed by an ammonium starvation growth arrest. *Yeast* 2000;16:23–33. [PubMed: 10620772]
10. Sturzenbaum SR, Kille P, Morgan AJ. Identification of heavy metal induced changes in the expression patterns of the translationally controlled tumour protein (TCTP) in the earthworm *Lumbricus rubellus*. *Biochim Biophys Acta* 1998;1398:294–304. [PubMed: 9655922]
11. Nagano-Ito M, Banba A, Ichikawa S. Functional cloning of genes that suppress oxidative stress-induced cell death: TCTP prevents hydrogen peroxide-induced cell death. *FEBS Lett* 2009;583:1363–1367. [PubMed: 19328788]
12. Mak CH, Su KW, Ko RC. Identification of some heat-induced genes of *Trichinella spiralis*. *Parasitology* 2001;123:293–300. [PubMed: 11578093]
13. Weibezahn J, Tessarz P, Schlieker C, Zahn R, Maglica Z, Lee S, Zentgraf H, Weber-Ban EU, Dougan DA, Tsai FT, Mogk A, Bukau B. Thermotolerance requires refolding of aggregated proteins by substrate translocation through the central pore of ClpB. *Cell* 2004;119:653–665. [PubMed: 15550247]

14. Thaw P, Baxter NJ, Hounslow AM, Price C, Waltho JP, Craven CJ. Structure of TCTP reveals unexpected relationship with guanine nucleotide-free chaperones. *Nat Struct Biol* 2001;8:701–704. [PubMed: 11473261]
15. Liu H, Peng HW, Cheng YS, Yuan HS, Yang-Yen HF. Stabilization and enhancement of the antiapoptotic activity of mcl-1 by TCTP. *Mol Cell Biol* 2005;25:3117–3126. [PubMed: 15798198]
16. Thomson CA, Tenni R, Ananthanarayanan VS. Mapping Hsp47 binding site(s) using CNBr peptides derived from type I and type II collagen. *Protein Sci* 2003;12:1792–1800. [PubMed: 12876328]
17. Jakob U, Muse W, Eser M, Bardwell JC. Chaperone activity with a redox switch. *Cell* 1999;96:341–352. [PubMed: 10025400]
18. Reddy GB, Narayanan S, Reddy PY, Surolia I. Suppression of DTT-induced aggregation of abrin by alphaA- and alphaB-crystallins: a model aggregation assay for alpha-crystallin chaperone activity in vitro. *FEBS Lett* 2002;522:59–64. [PubMed: 12095619]
19. Oikawa K, Ohbayashi T, Mimura J, Fujii-Kuriyama Y, Teshima S, Rokutan K, Mukai K, Kuroda M. Dioxin stimulates synthesis and secretion of IgE-dependent histamine-releasing factor. *Biochem Biophys Res Commun* 2002;290:984–987. [PubMed: 11798171]
20. Rupec RA, Poujol D, Kaltschmidt C, Messer G. Isolation of a hypoxia-induced cDNA with homology to the mammalian growth-related protein p23. *Oncol Res* 1998;10:69–74. [PubMed: 9666514]
21. Xu A, Bellamy AR, Taylor JA. Expression of translationally controlled tumour protein is regulated by calcium at both the transcriptional and post-transcriptional level. *Biochem J* 1999;342(Pt 3):683–689. [PubMed: 10477280]
22. Harndahl U, Hall RB, Osteryoung KW, Vierling E, Bornman JF, Sundby C. The chloroplast small heat shock protein undergoes oxidation-dependent conformational changes and may protect plants from oxidative stress. *Cell Stress Chaperones* 1999;4:129–138. [PubMed: 10547062]
23. Takayama S, Reed JC, Homma S. Heat-shock proteins as regulators of apoptosis. *Oncogene* 2003;22:9041–9047. [PubMed: 14663482]
24. Graidist P, Phongdara A, Fujise K. Antiapoptotic protein partners fortilin and MCL1 independently protect cells from 5-fluorouracil-induced cytotoxicity. *J Biol Chem* 2004;279:40868–40875. [PubMed: 15262975]
25. Young JC, Agashe VR, Siegers K, Hartl FU. Pathways of chaperone-mediated protein folding in the cytosol. *Nat Rev Mol Cell Biol* 2004;5:781–791. [PubMed: 15459659]
26. Oh HJ, Chen X, Subjeck JR. Hsp110 protects heat-denatured proteins and confers cellular thermoresistance. *J Biol Chem* 1997;272:31636–31640. [PubMed: 9395504]
27. Basha E, Lee GJ, Demeler B, Vierling E. Chaperone activity of cytosolic small heat shock proteins from wheat. *Eur J Biochem* 2004;271:1426–1436. [PubMed: 15066169]

Figure 1.

Heat shock treatment upregulates TCTP expression. **A.** Approximately 1000 schistosomula were incubated at 25°C, 37°C or at 42°C for 1 h. Following incubation, expression of SmTCTP was analyzed by RT-PCR using sequence specific primers. Results show that SmTCTP expression is significantly up regulated in schistosomula incubated at 37°C and 42°C compared to schistosomula incubated at 25°C. Expression of actin gene remained unchanged at all incubation temperatures. Levels of human TCTP was also increased in human PBMC (6×10^6) incubated at 42°C for 12 hrs compared to those incubated at 37°C. Human actin was used as an internal control. **B.** Normalized expression of SmTCTP to actin and **C.** Normalized expression of Human TCTP to actin. Results show that SmTCTP and Human TCTP expression is significantly elevated ($P < 0.05$) after thermal shock.

Figure 2.

A. rSmTCTP binding to citrulline synthase (CS) can be blocked by anti-SmTCTP. 1 μ g of biotinylated rSmTCTP was preincubated with various dilutions of mouse anti-SmTCTP or a control antibody (antiHMGB-1) and then added to the wells coated with 1 μ g of heat denatured CS. Results show that 1:500 dilution of anti-SmTCTP was sufficient to block binding of rSmTCTP to CS.

B. SmTCTP binds to classical chaperone substrates. 1 μ g of CS, luciferase (LUC), lysozyme (LYS) or BSA was denatured by heat or with guanidium hydrochloride. Denatured and native substrate proteins were then coated on to 96 well plates. Binding of rSmTCTP to the coated proteins were analyzed by ELISA. Results show that rSmTCTP bound to all the proteins that were denatured by heat (H) or guanidium hydrochloride (G) but not to native substrate proteins (N). Preincubation of rSmTCTP with mouse anti-SmTCTP antibodies (1: 500) blocked the binding of rSmTCTP to proteins that were denatured by heat (Hb), guanidium hydrochloride (Gb) or native (Nb). * $P < 0.01$ compared to rSmTCTP treated with anti-SmTCTP.

# $p < 0.05$ compared to native substrate proteins. ** $p < 0.05$ compared to SmTCTP treated with anti-SmTCTP.

C and D. TCTP prevents thermal aggregation of proteins. Citrulline synthase (C) and luciferase (D) were incubated at 42°C in the presence of two fold molar concentration of rSmTCTP or rHuTCTP for different time intervals (0–30min). Thermal aggregation of proteins was determined spectrophotometrically by measuring the light scatter at 360nm. Results show that both rHuTCTP and rSmTCTP efficiently prevented thermal aggregation of CS and Luciferase. Data presented are representative of three similar experiments. * Statistically significant $p < 0.01$.

Figure 3.

TCTP protects and reactivates thermally denatured luciferase (LUC). Luciferase was heat denatured at 42°C for 3 hrs in the presence or absence of rSmTCTP. Following denaturation luciferase activity was determined in a fluorimeter. Results show that in the presence of rSmTCTP, heat denatured luciferase regained significant ($p < 0.001$) amounts of luciferase activity when allowed to renature (LUC+TCTP). Similarly, addition of rSmTCTP after heat denaturation of luciferase (DnLUC+TCTP) also significantly ($p < 0.01$) recovered the luciferase activity. A control recombinant protein, rGBF failed to protect or reactivate luciferase under similar conditions. Heat denatured luciferase allowed to reactivate by itself served as control (LUCI). Data presented are representative of three similar experiments. * Statistically significant $p < 0.01$.

Figure 4.

E. coli cells over expressing SmTCTP were protected from heat-induced cell death. *E. coli* cells over expressing TCTP or transfected with blank vector (pRSET A) were exposed to 42°C for 3 hours. Following heat shock exposure, cells were serially diluted from 1:10 to 1:100 and plated on to LB agar. Plates were incubated for 12h at 37°C and growth of bacterial cells was evaluated. Results show that cells transfected with the blank vector had markedly reduced growth, whereas, cells transfected with *SmTCTP* showed greater recovery from heat shock. SmTCTP expressing cells grew even up to a dilution of 1:50. Cells not exposed to heat served as control. The data presented is representative of two experiments with similar results.