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A Functional Comparison of the Archaeon Tricorn Protease and Selected Serine Proteases from *Trypanosoma brucei brucei*

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Abstract: ***Trypanosomes are protozoan parasites causing African trypanosomiasis, a neglected tropical disease in Africa affecting humans and animals. Current control methods have focused on the use of drugs which have adverse effects and develop resistance and with no available conventional vaccine. Proteolysis is a key process in trypanosome survival in the mammalian host hence identification of other parasitic factors would lead to the development of new chemotherapeutic agents. This study investigated archaeon tricorn protease functional analogs in Trypanosoma brucei brucei through bioinformatics approaches. The protein sequences were retrieved from NCBI (http://www.ncbi.nlm.nih.gov/protein/) and position specific iterated basic local sequence alignment (PSI-BLAST) was performed using default parameters to determine patterns of conservation which aid in the recognition of distant similarities. The 3D models of the putative proteins were constructed using T. acidophilum tricorn protease. The constructed models were analyzed based on percentage identity, e-value and bit-score. Structural alignment was done using MATRAS (http://strcomp.protein.osaka-u.ac.jp/matras/) and PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC (https://www.pymol.org/) was used for viewing and structural analysis. The bioinformatics analysis revealed similarities in the catalytic core elements as well as the beta sheets serving as substrate entry and exit route to and from the catalytic chamber. Therefore, based on these similarities, this study reports the identification tricorn protease functional analogs in Trypanosoma brucei brucei.***

Keywords: Tricorn protease, trypanosomiasis, protein homology, serine peptidases, proteolysis

1. Overview

African Trypanosomiasis is a vector-borne disease caused by protozoan parasites of the genus *Trypanosoma* (Franco et al. 2014). There are two forms of the disease; Human African Trypanosomiasis (HAT) caused by *T.b gambiense* and *T.b rhodensiense* and Animal African Trypanosomiasis (AAT) caused by *T. b. brucei*, *T. congolense, T. vivax* among others. Wild and domestic animals can host the parasite hence act as a reservoir of infection for tsetse flies (Franco et al. 2014). The disease control strategies have relied on the use of drugs which have adverse effects and vector control methods which have proved in efficient (Simo et al. 2014). The development of a convectional vaccine has been hampered by the ability of the parasite to express variable surface glycoproteins (Simo et al. 2014). *T. b. brucei* is unable to infect primates due to its susceptibility to lysis by the human Trypanosome Lysis Factor-1 and is genotypically similar to the human pathogenic forms to *T. b. gambiense* and *T. b. rhodensiense* hence making it a good experimental model for human and animal infections studies (Simarro et al. 2011).

Initial intracellular protein degradation in the archaeon *Thermoplasma acidophilium*, is carried out by proteasome employing sieving mechanisms for substrate selection (Bochtler et al. 1999). Products of proteasomes are peptides of about 6–12 amino acid residues which are further degraded by tricorn protease and its interacting factors thus completing the proteasomal degradation pathway (Tamura et al. 1996). The C-terminal of tricorn protease consists of C1, PDZ,C2 domains and harbors the active site residues (S745, H746, S965, E 1023) (Brandstetter et al. 2001). Unlike the archaeon proteasome, the molecular protein degradation machinery in trypanosomes is thought to have trypsin-like, chymotrypsin-like and caspase-like proteolytic activity (Cardoso et al. 2011). This enables the trypanosome to degrade an array of peptides hence a vast products for further processing.

In this study, a structural analysis of tricorn protease was done with a view of identifying similarities with some *T. b. brucei* cytosolic serine proteases. The structural prediction of the putative *T. b. brucei* sequences was done based on homology modeling. Domain organization and composition were subsequently analyzed with focus on the active site residues and thus the study reports the functional similarities of tricorn protease and *T. b. brucei* serine proteases.

2. Methods

Sequence retrieval and analysis

The *T. acidophilum* tricorn protease (ACC4462.1) and the putative *T .b. brucei* protein sequences (EAN78104.1, EAN80234.1 and EAN78208.1) were retrieved from NCBI database (*https://www.ncbi.nlm.nih.gov/*). Sequence similarity searches were conducted in NCBI at *blast.ncbi.nlm.nih.gov/Blast.cgi* (Altschul et al. 1990).The sequences were analyzed through MAFFT v7 (http://mafft.cbrc.jp/alignment/server/index.html) (Katoh & Standley 2013) multiple sequence alignment tool to determine the conserved residues and viewed in Jalview (Waterhouse et al. 2009).

Protein modeling and analysis

The 3D models of the *T. brucei brucei* proteins were constructed using (PS)v2 (http://ps2.life.nctu.edu.tw/) (Huang et al. 2015). The constructed models were analyzed based on percentage identity, e-value and bit-score and

saved as pdb files. Structural alignment was done using MATRAS (http://strcomp.protein.osaka-u.ac.jp/matras/) (Kawabata 2003). PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC (https://www.pymol.org/) (Delano, 2002) was used for viewing and structural analysis.

3. Results and Discussion

The PSI-BLAST results revealed a weak homology between tricorn protease and *T. b. brucei* proteins including dipeptidyl peptidase IV (Tb927.10.6940), prolyl oligopeptidase (Tb927.10.8020) and oligopeptidase B (Tb927.11.12580. Structural analysis of the proteins revealed similarity with tricorn protease in that their C-terminal regions which harbour the active site residues (Figure 2a). These serine proteases also belong to the alpha-beta hydrolases. In all the structures, the catalytic core element (serine) was strictly conserved and was shown to lie at the entrance of a conserved helix (Figure 2b). The orientation of tricorn's 6- and 7-bladed beta propeller domains along the polypeptide seem to be similar to the orientation of the beta propeller sheets of the trypanosome proteins and both have been shown to act as channels for substrate exit and entry to the active site (Figure 2b). The active site residues were also oriented in a similar fashion along the polypeptide chain (Figure 2c).

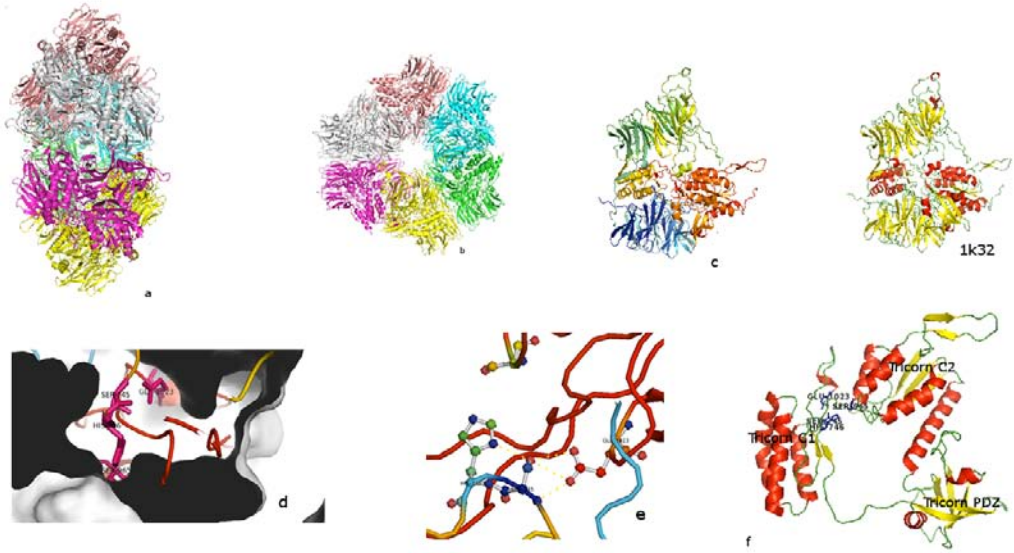

Figure 1: a; The hexameric 3D structure of Thermoplasma acidophilum tricorn protease coloured by chain, b; the oriented form of ' a' showing all the six chains, c: tricorn protease monomer coloured by spectrum from N- to C-terminal domain where blue reprsents the N-terminal domain (6-bladed beta propeller), green - tricorn protease domain 2 (7-bladed beta propeller), orange- PDZ domain, red: C-terminal domain, d: surface representation of tricorn protease active site with active site residues shown as purple sticks while the the main chain shown as ribbon, e: active site tetrad shown as ball and sticks, f; tricorn protease C-terminal domain, arranged as C1, PDZ, C2 in that order in the polypeptide chain.

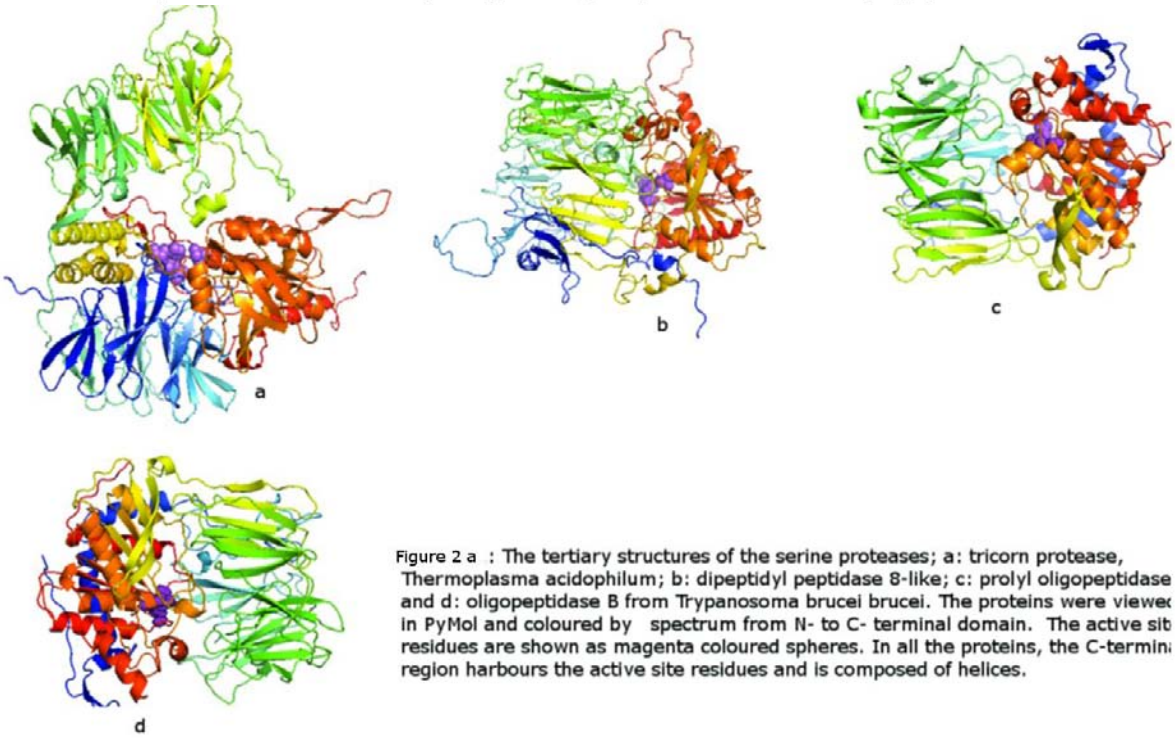

Figure 2 a : The tertiary structures of the serine proteases; a: tricorn protease, Thermoplasma acidophilum; b: dipeptidyl peptidase 8-like; c: prolyl oligopeptidase and d: oligopeptidase B from Trypanosoma brucei brucei. The proteins were viewed in PyMol and coloured by spectrum from N- to C- terminal domain. The active sit residues are shown as magenta coloured spheres. In all the proteins, the C-termini region harbours the active site residues and is composed of helices.

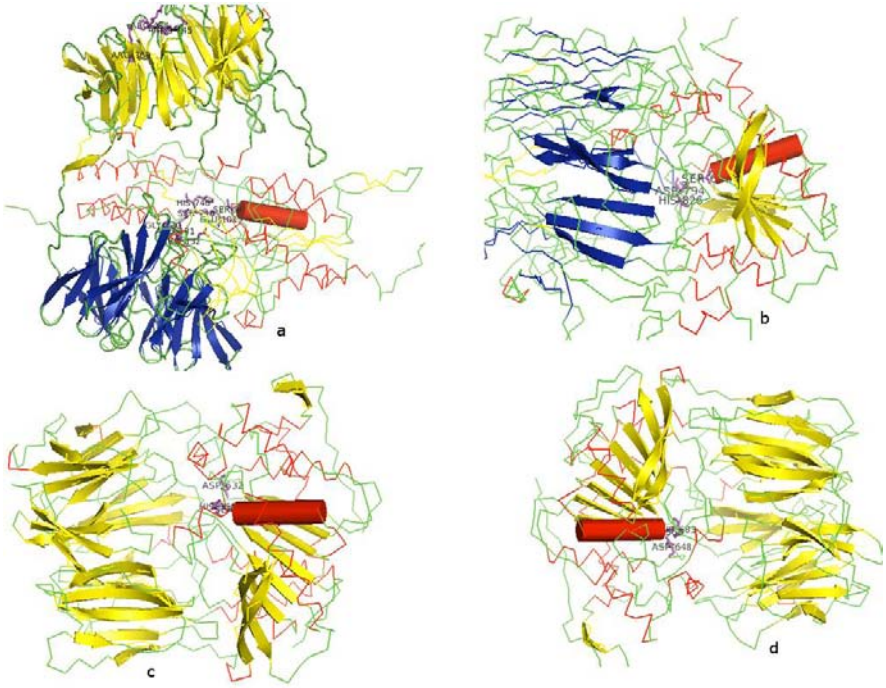

Figure 2b: The structural homology of the catalytic core element, serine following the strictly conserved helix shown as red cylindrical helix in the structure. The main chain is shown as ribbon coloured by secondary structure where red, yellow and green represents helices, sheets and coils respectively. The beta propeller sheets provide substrate entrance and exit routes to/from the active site. In tricorn protease (*T. acidophilum*), the serine is at position 965 as shown on "a"; S699, S549 and S563 in dipeptidyl peptidase IV, prolyl oligopeptidase and oligopetidase B (b, c and d) respectively. This is a striking similarity in these proteins which suggests a functional relationship.

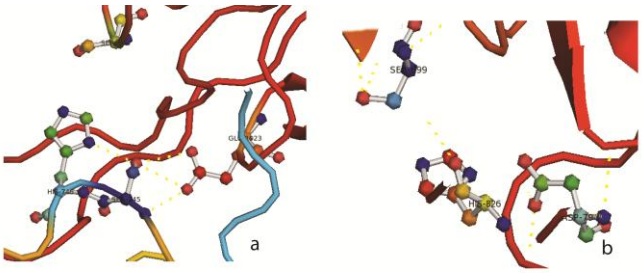

Figure 2 c: The orientation of the active site residues; a: tricorn protease and b: dipeptidyl peptidase 8-like which was chosen to represent the other trypanosome proteins due to their similarity in orientation.

Comparative genomics of serine peptidase in the two organisms in this study points out to unequal distribution of the serine peptidases where S41 seemed to lack in trypanosomes which seemed to have S8 and S9 peptidases (Appendix 1).

Appendix 1: Distribution of serine peptidase families in *Thermoplasma acidophilum* and *Trypanosoma brucei brucei*

MEROPS ID	Name	*T. acidophilum*	*T. brucei brucei*
S8 unassigned	subfamily S8A unassigned peptidases	0	2
S09.A29	At4g17150 (Arabidopsis thaliana)-type peptidase	0	1
S9 homologues	family S9 non-peptidase homologues	0	1
S9 unassigned	subfamily S9A unassigned peptidases	0	4
S9 unassigned	subfamily S9C unassigned peptidases	0	3
S9 unassigned	family S9 unassigned peptidases	1	2
S10 unassigned	family S10 unassigned peptidases	0	3
S16.A11	PF0467 (Pyrococcus furiosus)	1	0
S26 unassigned	subfamily S26A unassigned peptidases	0	1
S26 unassigned	subfamily S26B unassigned peptidases	0	1
S33.005	tricorn interacting factor F1	1	0
S33.010	SCO7095-type peptidase	1	0
S33 unassigned	family S33 unassigned peptidases	1	5
S41.005	tricorn core peptidase (archaea)	1	0
S45 unassigned	family S45 unassigned peptidases	2	0
S49 homologues	subfamily S49B non-peptidase homologues	1	0
S49 unassigned	subfamily S49A unassigned peptidases	1	0
S53 unassigned	family S53 unassigned peptidases	2	0
S59 homologues	family S59 non-peptidase homologues	0	1
S66.001	murein tetrapeptidase LD-carboxypeptidase	1	0

The S8 and S9 are well developed in *T. brucei brucei* while the S41 is well developed in *T. acidophilum.*

Studies have also shown that most of the higher eukaryotic organisms from yeast to mammals, utilize proteasomes as well as large enzyme complexes such as the cytosolic and lysosomal dipeptidyl and tripeptidyl peptidases (DPP and TPP) with functional analogies to tricorn (Tomkinson 1999, Geier, *et al.,* 1999). Studies have also shown that tricorn protease is patchily distributed and other archaeon such as Desulfurococcales, example *Pyrolobus*, *Desulfurococcus* lack tricorn protease but have tetrahedral aminopeptidase (TET) which also acts downstream of the proteasome assumes the role of tricorn protease (Borissenko & Groll 2005).

4. Conclusion

Based on the structural similarities, this study proposes the *T. b. brucei* dipeptidyl peptidase IV, oligopeptidase B and prolyl oligopeptidase as tricorn protease functional analogs.

5. Declaration

The authors declare that there is no conflict of interests regarding the publication of this paper.

6. Acknowledgements

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