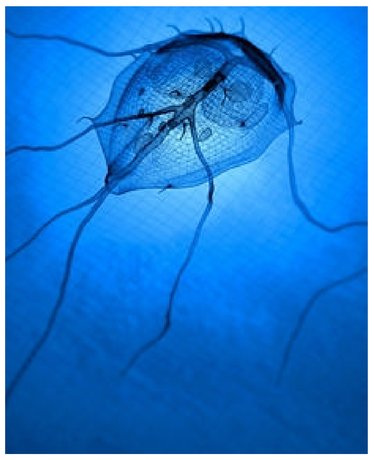

Point mutations in triose-phosphate isomerase of *Giardia duodenalis*

RedOx-dependency of catalytic activity and support for the neutral theory of evolution

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Abstract

Predicting how point mutations in genes alter the tertiary and quaternary structure of proteins is central to a number of areas of molecular biology and has implications in relation to the function and evolution of molecules. In the present study, we theoretically assessed the effects of 20 point mutations detected previously in a region of the triose-phosphate isomerase gene (p*tpi*) of the protozoan *Giardia duodenalis* on the three-dimensional structure of the 'wild-type' protein (TPI) [1].

Amino acid substitutions arising from codon variations were mainly located at surface-accessible sites or in hydrophobic pockets of TPI. None of the substitutions was predicted to exert a significant change to the fold or functionality of the enzyme, with the exception of one alteration (Arg100Cys).

It is tempting to speculate that Cys100 adds a particular functionality to TPI, which may comprise the intra-molecular disulphide formation between Cys100 and Cys127. Due to a concomitant conformational change, the catalytic activity of TPI is likely to be affected. This proposed mechanism shows similarity to the previously reported disulphide bond formation between Cys222 and Cys228 [2], and raises the hypothesis of RedOx regulation of TPI activity.

Assessment of amino acid substitutions

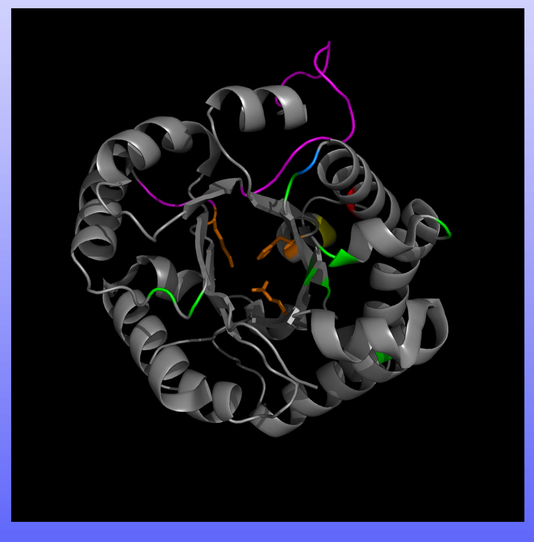
Previously, we detected point mutations in seven p*tpi* sequences from *G. duodenalis* from sheep (43 samples) and subsequently appraised the literature for polymorphic nucleotide positions in other p*tpi* sequences representing *Giardia* [1].

Using the amino acid sequences inferred by translation of the open reading frames (ORFs) of individual sequences, substitutions were identified by comparative analysis against a reference ('wild-type') sequence of the protein TPI for *G. duodenalis*.

Variation	TPI sequence	Topological element	Location	Structural effect
D35E	GQ444454, GQ444458-GQ444460, GQ444462	α1β2	Surface accessible	Conservative
V37I	GQ444454, GQ444459	α1β2	Hydrophobic pocket	Conservative
R100C	GQ444451	α4	Surface accessible	Destabilising; possible dithioether link with Cys127
S110G	GQ444451	α4	Partially buried	Maybe destabilising; interactions with E105 and T106-CO lost
G121E	GQ444460	α4 β5	Surface accessible	Stabilising; hydrophilic interactions with solvent
G121D	GQ444460	α4 β5	Surface accessible	Stabilising; hydrophilic interactions with solvent
M122V	GQ444459	α4 β5	Hydrophobic pocket	Semi-conservative
V128A	GQ444448	b5	Hydrophobic pocket	Conservative
M141V	GQ444451	α5	Surface accessible	Semi-conservative
E157K	EU518581	α5	Surface accessible	Change of electrostatic potential, hydrophilic interactions conserved
K163R	GQ444454, GQ444462	α5 β6	Surface accessible	Conservative
V166I	EU518537	β6	Hydrophobic pocket	Conservative

Table: A structural evaluation of amino acid variation as a consequence of individual and/or combined nucleotide polymorphism in the p*tpi* gene.
'βn' denotes the n-th beta-strand, 'αn' the helix following the n-th beta-strand, and 'αnβ(n+1)' denotes the linker peptide between the n-th alpha-helix and the subsequent beta-strand.

Amino acid variations due to nucleotide polymorphism were mapped onto the three-dimensional fold of *G. duodenalis* TPI (PDB accession code 2dp3) using the following colour code: conservative substitution (green), stabilising substitution (blue), destabilising substitution (red). The variation of Arg100 to Cys is indicated in yellow. Catalytic residues are drawn explicitly and coloured orange. The loops involved in dimer formation (loop 1, left; loop3, right) are shown in magenta.



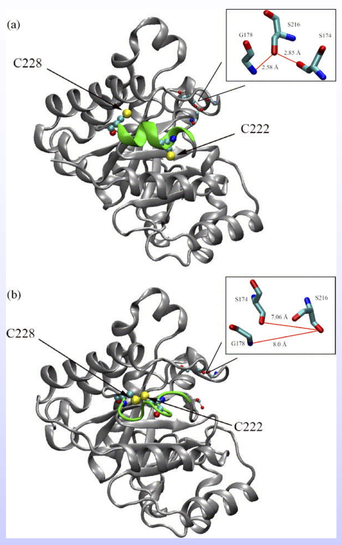
Support for the neutral theory

The neutral theory contends that evolution at the molecular level is not solely shaped by "Darwinian selection but also by random drift of selectively neutral or nearly neutral mutants". This theory does not deny the role of natural selection, rather it assumes that only a small proportion of changes is adaptive, whereas the "majority of phenotypically silent molecular substitutions exert no significant influence on survival and reproduction and drift randomly through a species" [3].

It is noteworthy that amino acid substitutions observed in this study also occur in the non-solvent exposed areas of TPI, but these substitutions retain the local 'packing effects'. The amino acid variations are thus in agreement with some of the current theories of molecular evolution, but in particular fit the idea of the neutral theory.

Enzymatic activity may be RedOx-dependent

The only amino acid substitution predicted to cause an alteration of the conformation of *Giardia* TPI and enzymatic activity was Arg100Cys from the sequence represented by accession number GQ444451. Cys127(β5) is positioned not too far from residue 100(α4). At a first glance, it seemed unlikely that Cys100 and Cys127 could engage in an intra-molecular dithioether-bond, since the distance between Cβ(100) and Cβ(127) was inferred to be 7.7 Å. Formation of a covalent bond between both cysteine residues would thus require a significant conformational change in the core of protein, pulling together α4 and α5. The covalent link would also force a re-arrangement of the N-terminus of helix α4, in which the catalytic residue His96 is located.



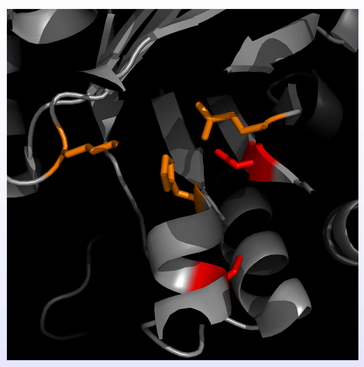


Figure: Dithioether linkages in *Giardia* TPI. ***Left***: Molecular dynamics indicate the possibility of disulfide bond formation between C222 and C228 [2]. ***Right***: The polymorphism resulting in the Arg100Cys mutation is likely to confer further RedOx-dependency on the enzymatic activity of *Giardia* TPI.

However, a similar situation has recently been reported based on results of enzyme assays and mutagenesis [2]. Native *Giardia* TPI was found to form an intra-molecular disulfide-bridge with residues Cys222 and Cys228. The Cα-Cα distance of 10.8 Å between these two residues is even greater than that between Arg100 and Cys127. Additionally, Cys228 is positioned 1.5 turns higher on helix α7 and points in the opposite direction to Cys222. The formation of an intramolecular disulfide link between these two residues requires an almost complete unfolding of helix α7, which is also visible in the model obtained using a molecular dynamic simulation [2]. If an internal disulfide link is indeed formed between Cys100 and Cys127, it is very likely that this oxidised form of the *Giardia* TPI Arg100Cys variant would possess a greatly reduced catalytic activity.

Acknowledgements

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