

## RESEARCH ARTICLE

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## An *in silico* analysis of the glutamate dehydrogenases of *Teladorsagia circumcincta* and *Haemonchus contortus*

**ABSTRACT**

Nematode glutamate dehydrogenase (GDH) amino acid sequences are very highly conserved (68-99% identity) and are also very similar to those of the bovine and human enzymes (54-60% identity). The residues involved in binding nucleotides or substrates are completely conserved and tend to be located in highly conserved regions of the sequence. Based on the strong homology between the bovine, *Teladorsagia circumcincta* and *Haemonchus contortus* GDH sequences, models of the structure of the *T. circumcincta* and *H. contortus* monomers were constructed. The structure of the *T. circumcincta* monomer obtained using SWISS-MODEL was very similar to that of the bovine enzyme monomer and the backbone of the polypeptide deviated very little from that of the bovine enzyme monomer. Despite the sequence differences between the bovine and *T. circumcincta* enzymes, the relative positions and orientations of the residues involved in ligand binding were very similar. The reported  $K_m$  for  $\text{NADP}^+$  of *T. circumcincta* is about 35 and times that of the bovine enzyme, whereas the  $K_m$ s of the two enzymes for glutamate,  $\alpha$ -ketoglutarate and  $\text{NAD(P)H}$  are much more similar. The residue corresponding to S267 of the bovine enzyme is involved in binding the 2'-phosphate of  $\text{NADP}^+$  and is replaced in the *T. circumcincta* and *H. contortus* sequences by a tryptophan. The partial occlusion of the  $\text{NAD(P)}$ -binding site by the tryptophan sidechain and the loss of at least one potential H-bond provided by the serine may explain the lower affinity of the *T. circumcincta* for  $\text{NADP}^+$ .

**Key words:** glutamate dehydrogenase, structure, *Teladorsagia circumcincta*, parasite, nematode

**Introduction**

*Teladorsagia circumcincta* and *Haemonchus contortus* are common nematode parasites of sheep. In some regions the burden of parasitism by these species and their growing resistance to current anthelmintics has compromised the viability of sheep farming (Waller *et al.*, 1996; van Wyk *et al.*, 1997), but the welfare of the sheep is at risk without reliable control of the parasite burden. These, and other considerations, have motivated a search for new targets for anthelmintics.

One target that has been suggested (Umair *et al.*, 2011) is glutamate dehydrogenase (GDH, E.C. 1.4.1.3), which catalyses the reversible oxidative deamination of glutamate to  $\alpha$ -ketoglutarate using either  $\text{NAD}^+$  or  $\text{NADP}^+$  as the electron acceptor. The enzyme represents an important link between the tricarboxylic acid cycle and amino acid metabolism and has been extensively studied in mammals (Plaitakis & Zaganas, 2001; Owen *et al.*, 2002; Newsholme *et al.*, 2003; Frigerio *et al.*, 2008), plants (Mayashita & Good, 2008), fungi (Marzluf, 1981) and bacteria (Hudson & Daniel, 1993), but nematode GDHs have received relatively little attention.

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The suggestion that GDH might be a target for anthelmintics was based on undefined differences in amino acid sequence between the nematode and host enzymes and on their kinetic characteristics (Umair *et al.*, 2011). Such suggestions are not unique. For example, it has been suggested that the GDH of *Plasmodium* spp. might be a target for antimalarial therapy (Werner *et al.*, 2005). *Plasmodium falciparum* has three *gdh* genes, but it has been demonstrated that it can survive without GDH a (Storm *et al.*, 2011), whereas *Caenorhabditis elegans* has only one on chromosome 4. As we show, the *P. falciparum* enzyme is very different from that of either *T. circumcincta* or *H. contortus* and the lifestyles of the parasites are also dissimilar.

The three most significant features of the kinetic properties of the *T. circumcincta* GDH (*TcGDH*) are (i) that it is active with either NAD(H) or NADP(H) (Muhamad *et al.*, 2011), (ii) that the  $K_m$ s for the dinucleotides tend to be greater for the *TcGDH* than for the bovine enzyme (*BtGDH*) (Frieden, 1959; Engel & Dalziel, 1969; Rife & Cleland, 1980; McCarthy & Tipton, 1985), and (iii) the  $K_m(\text{NADP}^+):K_m(\text{NAD}^+)$  ratio is much greater for the *TcGDH* than is the case for *BtGDH* (Frieden, 1959; Engel & Dalziel, 1969; Rife & Cleland, 1980; McCarthy & Tipton, 1985), although the kinetics of the latter are known to be very complex. That the enzyme is able to use both NAD(H) and NADP(H) prompts the suggestion that it might have more in common with mammalian enzymes, which behave similarly (Rife & Cleland, 1980; McCarthy & Tipton, 1985), rather than the plant, bacterial or *Plasmodium* spp. enzymes, which tend to exhibit specificity for either NAD(H) or NADP(H) (Gore, 1981; Storm *et al.*, 2011). The high ratio of  $K_m(\text{NADP}^+):K_m(\text{NAD}^+)$  reported for *TcGDH* is quite unlike *BtGDH*, for example, for which the ratio is less than 1 (Frieden, 1959; Engel & Dalziel, 1969; Rife & Cleland, 1980; McCarthy & Tipton, 1985) and the difference appears to result from the very high  $K_m$  for  $\text{NADP}^+$  reported for the *TcGDH* (Muhamad *et al.*, 2011; Umair *et al.*, 2011). If the *H. contortus* GDH (*HcGDH*) has a similarly high  $K_m$  for  $\text{NADP}^+$ , it might explain the report by Rhodes and Ferguson (1973) that this enzyme could utilise only NAD(H). From these observations, we infer that there might be some significant structural difference between *TcGDH* and both *HcGDH* and *BtGDH*.

There has been no consideration of the structure of the nematode GDH, other than in a very preliminary form based on a partial *T. circumcincta* sequence (Green *et al.*, 2004). In part this is due to the difficulty of obtaining sufficient

nematodes from which to purify the enzyme. However, cDNA sequences of both *HcGDH* and *TcGDH* have been reported (Skuce *et al.*, 1999; Umair *et al.*, 2011) and the latter has been expressed in *Escherichia coli*. This recombinant *TcGDH* (*rTcGDH*) appears to behave almost identically to the *TcGDH* in crude homogenates (Muhamad *et al.*, 2011). Curiously, the specific activity of the *rTcGDH* is only about 4 times that of the enzyme in crude homogenates (Muhamad *et al.*, 2011). We infer from this that either *TcGDH* is about 25% of the protein in *T. circumcincta*, which seems improbable, or that the *rTcGDH* was adversely affected by the six-histidine tag engineered into it, the expression in *E. coli* or the purification procedure. However, Kim *et al.* (2003) applied a very similar strategy to *BtGDH* and showed that the recombinant and the native enzyme had very similar kinetics, so the problem presumably lies elsewhere. Until this, and several others, issues are resolved, there is little point in attempting to determine the structure of the *rTcGDH*, but the sequence of *TcGDH* can provide some insight into the structural properties of the enzyme.

Here we report on the properties of the best of the models we have constructed using the *TcGDH* and *HcGDH* sequences and crystal structures of the homologous *BtGDH*. These models provide a possible explanation for the very high  $K_m(\text{NADP}^+)$  of *TcGDH*, but do not explain the lack of activity of *HcGDH* with  $\text{NADP}^+$ .

## Materials and Methods

Glutamate dehydrogenase amino acid sequences were obtained from GenBank for *Caenorhabditis elegans* (NP\_502267.1), *C. briggsae* (XP\_002633432.1), *C. brenneri* (EGT40056.1), *C. remanei* (XP\_003100701.1), *Ascaris suum* (ADY42913.1), *Brugia malayi* (XP\_001893113.1), *H. contortus* (AAC19750.1), *Neospora caninum* (CBZ49515.1), *Plasmodium falciparum* (XP\_001348337.1), *Toxoplasma gondii* (XP\_002370120.1) and *T. circumcincta* (AEO44571.1). The crystal structures of *BtGDH* (1HWY, 1HWZ and 3MW9), *P. falciparum* (2BMA) and human GDH (1L1F) were obtained from the Protein Databank (<http://www.pdb.org/pdb/home/home.do>) and the sequences used in the analysis are those of the crystals in order to maintain consistency between the sequence and structure analyses.

Sequences were aligned with ClustalX (Thompson *et al.*, 1997) using the Gonnet substitution matrix, a gap opening penalty of 10 and a gap extension penalty of 0.2.

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The mutability of the sequences was quantified at position  $i$  in the alignment using

$$M_i = \frac{1}{(2m+1)\ln(0.05)} \sum_{j=i-m}^{i+m} \sum_x P_j(\text{residue} = x) \ln P_j(\text{residue} = x)$$

where  $P_j(\cdot)$  is the probability of amino acid  $x$  appearing at position  $j$  and the inner summation is taken over all the amino acids aligned at position  $j$  and the values are averaged over a  $2m + 1$  residue window centred on residue  $i$  (Brown et al., 1993). If all the sequences are identical at position  $j$ , then  $P_j = 1$  and contributes nothing to  $M_i$ , but if two or more amino acids are aligned at position  $i$ , then  $P_j < 1$  and  $M_i$  is increased.

Theoretical structures of the monomers of *TcGDH* and *HcGDH* were calculated from amino acid sequences and the structure of *BtGDH* (1HWY) using Phyre [http://www.sbg.bio.ic.ac.uk/phyre (Kelley & Sternberg, 2009)], ESyPred3D [http://www.fundp.ac.be/sciences/biologie/urbm/bioinfo/esypred (Lambert et al., 2002)] and SWISS-MODEL [http://swissmodel.expasy.org (Kiefer et al., 2009)]. For comparison, a structure of *TcGDH* based on the *P. falciparum* structure (2BMA) was built using SWISS-MODEL. The quality of fit parameters were calculated using PDBeFold (Krissinel, 2007), which reports three specific scores:

1. The  $Q$  score measures the quality of the alignment of the  $C_{\alpha}$ s and is calculated from the alignment length ( $N_{\text{align}}$ ) compared with the number of residues in the two amino acid sequences considered ( $N_1$  and  $N_2$ ) and the root mean square deviation (RMSD) between the  $C_{\alpha}$ s

$$Q = \frac{N_{\text{align}}^2}{\left(1 + (\text{RMSD}/R_0)^2\right) N_1 N_2}$$

where  $R_0$  is empirically set to 3 Å. The value of  $Q$  ranges from 1 for identical structures downwards as RMSD rises and  $N_{\text{align}}$  falls.

2. The  $P$  score measures the probability ( $p$ ) of achieving the same or better quality of match by randomly picking structures from the database

$$P = -\log p$$

so the higher  $P$ , the more significant the match.

3. The  $Z$  score measures the probability ( $p_z$ ) that a match of at least the same quality could be obtained by

randomly picking structures from the database assuming a normal distribution so

$$p_z = \text{erfc}\left(\frac{Z}{2}\right)$$

If two structures are matched uniquely, then  $p_z = p$ . The higher  $Z$ -score, the higher is the statistical significance of the match.

The residues involved in ligating the reactants or effectors were identified from the relevant *BtGDH* crystal structures (1HWY, 1HWZ and 3MW9) using PDBsum [http://www.ebi.ac.uk/pdbsum (Laskowski, 2009)]. In each case all residues identified are given even if some are not identified in all the structures. Structural and functional domains were identified using CATH [http://www.cathdb.info (Cuff et al., 2011)] and SCOP [http://scop.mrc-lmb.cam.ac.uk/scop/index.html (Andreeva et al., 2008)].

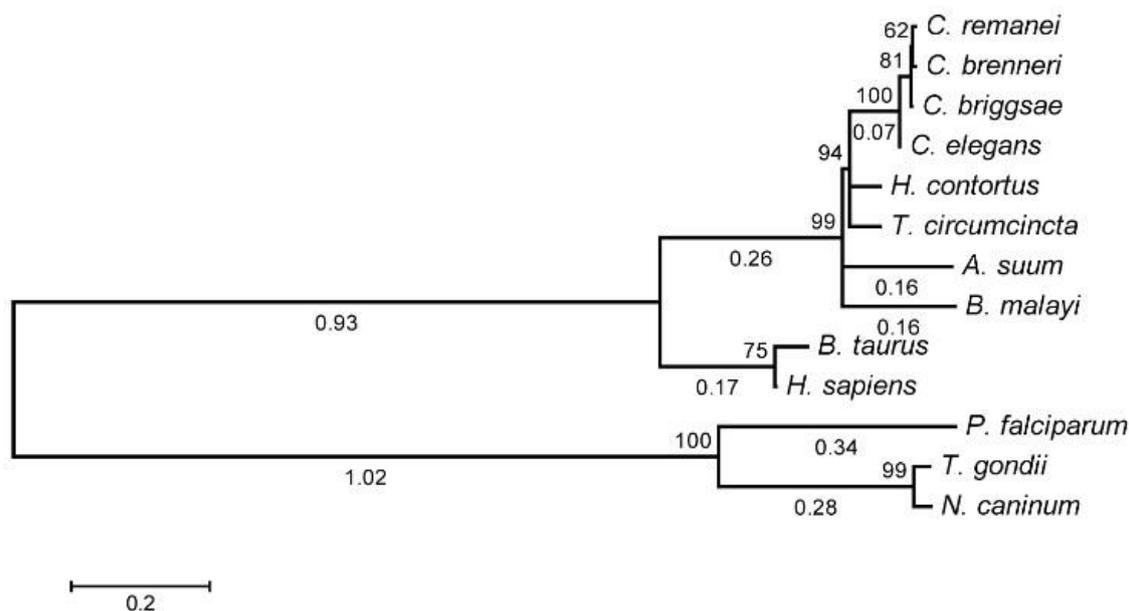
Figures showing molecular structures were generated using PyMOL [http://sourceforge.net/projects/pymol/].

## Results and Discussion

### Sequence analysis

A preliminary analysis of the available sequences included GDH sequences from a range of mammals, parasites and microorganisms. Based on this and the available crystal structures, we selected thirteen representative sequences to construct the phylogenetic tree shown in Figure 1. The nematode sequences are clustered together and are clearly distinct from the cluster of protozoan sequences. While there is argument concerning the interpretation of bootstrap probabilities (Soltis & Soltis, 2003), it is clear that the protozoan sequences form a group that is significantly distinct from the nematode and mammalian sequences (Felsenstein & Kishino, 1993; Hillis & Bull, 1993). However, the bovine and human sequences, which have 95% identity (Table 1), are clustered with the nematode sequences (Figure 1). For clarity, we have not included the three protozoan GDHs in the sequence alignment shown in Figure 2. We have also omitted from this analysis the sequence of the sheep GDH (NP\_001265496.1) which differs in only 7 positions over the length of the sequence of the bovine enzyme (86% identity, 88% similarity).

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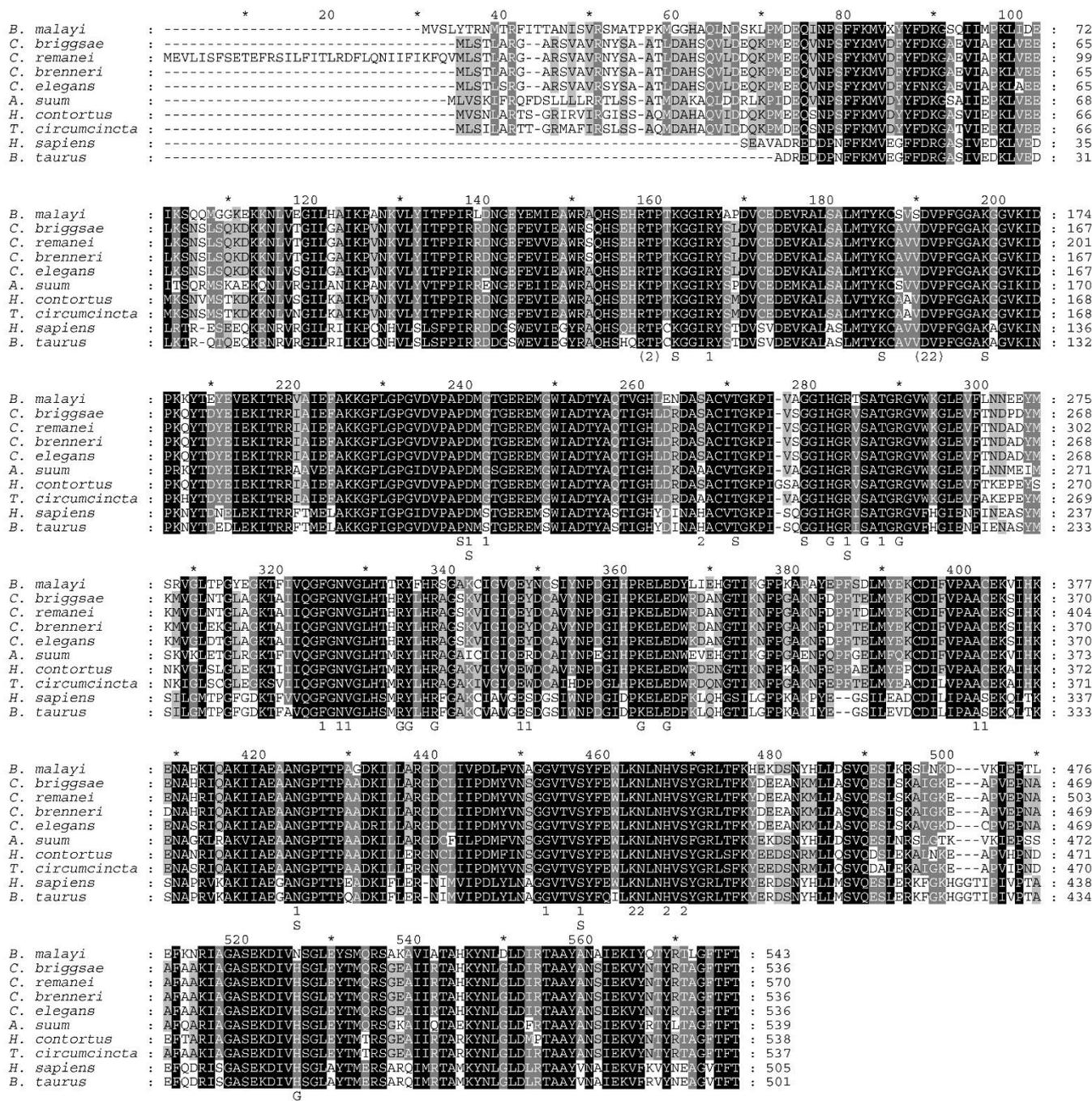


**Figure 1.** The bootstrap consensus phylogram derived by maximum likelihood from an alignment of 13 GDH amino acid sequences (432 gap-free positions). The maximum likelihood was based on the JTT matrix-based model (Jones *et al.*, 1992) using 500 bootstrap replicates (Felsenstein, 1985). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown as integers next to the branch points. The branch lengths (number of substitutions per site) are indicated below the branches unless the value was less than 0.05 in which case it is not shown. This analysis was conducted using MEGA5 (Tamura *et al.*, 2011).

**Table 1.** Conservation of the amino acid sequence alignment shown in Figure 2. The values are the identity (upper triangle) or the similarity (lower triangle) expressed as a percentage and they were calculated using Clustal X (Thompson *et al.*, 1997).

|                        | Identity (upper triangle) or similarity (lower triangle) (%) |                    |                   |                    |                   |                |                     |                        |                   |                  |
|------------------------|--|--------------------|-------------------|--------------------|-------------------|----------------|---------------------|------------------------|-------------------|------------------|
|                        | <i>B. malayi</i>   | <i>C. briggsae</i> | <i>C. remanei</i> | <i>C. brenneri</i> | <i>C. elegans</i> | <i>A. suum</i> | <i>H. contortus</i> | <i>T. circumcincta</i> | <i>H. sapiens</i> | <i>B. taurus</i> |
| <i>B. malayi</i>       |  | 72                 | 68                | 71                 | 72                | 75             | 72                  | 71                     | 58                | 57               |
| <i>C. briggsae</i>     | 86   |                    | 93                | 99                 | 97                | 75             | 85                  | 86                     | 58                | 58               |
| <i>C. remanei</i>      | 81   | 93                 |                   | 92                 | 91                | 70             | 79                  | 80                     | 55                | 54               |
| <i>C. brenneri</i>     | 86   | 99                 | 93                |                    | 97                | 75             | 84                  | 85                     | 58                | 58               |
| <i>C. elegans</i>      | 86   | 99                 | 93                | 99                 |                   | 75             | 84                  | 84                     | 58                | 58               |
| <i>A. suum</i>         | 87   | 88                 | 83                | 88                 | 89                |                | 75                  | 75                     | 59                | 57               |
| <i>H. contortus</i>    | 85   | 92                 | 86                | 91                 | 91                | 86             |                     | 91                     | 60                | 59               |
| <i>T. circumcincta</i> | 85   | 93                 | 87                | 93                 | 93                | 87             | 96                  |                        | 59                | 59               |
| <i>H. sapiens</i>      | 74   | 74                 | 69                | 74                 | 74                | 75             | 74                  | 74                     |                   | 95               |
| <i>B. taurus</i>       | 73   | 73                 | 68                | 73                 | 73                | 75             | 73                  | 73                     | 97                |                  |

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**Figure 2.** Alignment of nematode, bovine and human GDH amino acid sequences. The sequences were aligned with Clustal X (Thompson et al., 1997) using the Gonnet matrix, a gap opening penalty of 10 and a gap extension of 0.2. The letters below the alignment indicate the positions of residues involved in ligand binding in the crystal structures (1HWY, 1HWZ and 3MW9) of the bovine enzyme (S –  $\alpha$ -ketoglutarate or glutamate; G – GTP; 1 – NAD<sub>a</sub> or NADP; 2 – NAD<sub>b</sub>; symbols in brackets indicate residues in other subunits).

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As mentioned previously, the cDNA sequence of GDH from nematodes is very similar to that of mammals (Muhamad *et al.*, 2011). From Figure 2 it is clear that the amino acid sequences are not only highly conserved among nematodes (68-99% identity), but they are also very similar (54-60% identity) to the bovine and human sequences (Table 1).

An interesting feature of the sequence alignment shown in Figure 2 is that *HcGDH* appears to have a glycine (G240, using the numbering for the *H. contortus* sequence) that is absent from all of the other sequences shown (position 276 of the alignment). This residue forms part of a tripeptide that is relatively poorly conserved between two relatively extensive regions of highly conserved sequence.

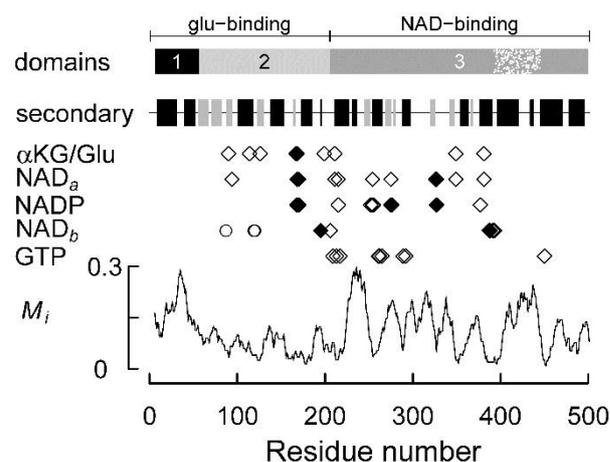
### Structural models

The human GDH and *BtGDH* differ very little (Figures 1 and 2) and that other considerations would determine which of them should be used as a basis for the construction of a theoretical model of the *TcGDH* and *HcGDH* monomers. Consequently, the availability of kinetic data and several different crystal structures persuaded us to employ *BtGDH*.

The essential properties of *BtGDH* are summarised in Figure 3. The glu-binding domain consists of a helical hairpin and a 3-layer(aba) sandwich (structural domains 1 and 2, respectively, in Figure 3) and the NAD-binding domain is a Rossmann fold with an internal region that forms the antenna domain (the stippled region in structural domain 3, Figure 3). Based on the PDBsum analysis of the *BtGDH* structures, residues involved in binding glutamate and  $\alpha$ -ketoglutarate are mostly located in domain 2, but residues in domain 3 are also involved. Similarly, NAD(P) (NAD<sub>a</sub> and NADP) binding in the cleft between the glu- and NAD-binding domains involves residues from both domains 2 and 3, although most of the ligands are located in domain 3. Of particular note is R211 in *BtGDH* that is involved in binding both glutamate/ $\alpha$ -ketoglutarate and NAD(P)(H). A second NAD-binding site (NAD<sub>b</sub>) involves other residues in domains 2 and 3, as well as three residues in domain 2 of an adjacent subunit. Most of the residues involved in GTP binding are located in domain 3. All of these residues are located in relatively well conserved regions of the sequence and both the N-terminal portion of the NAD-binding domain and the antenna domain are relatively poorly conserved (Figure 3).

Of all the residues involved in binding NAD(P),  $\alpha$ -ketoglutarate, glutamate or GTP, only six in *BtGDH* (N168,

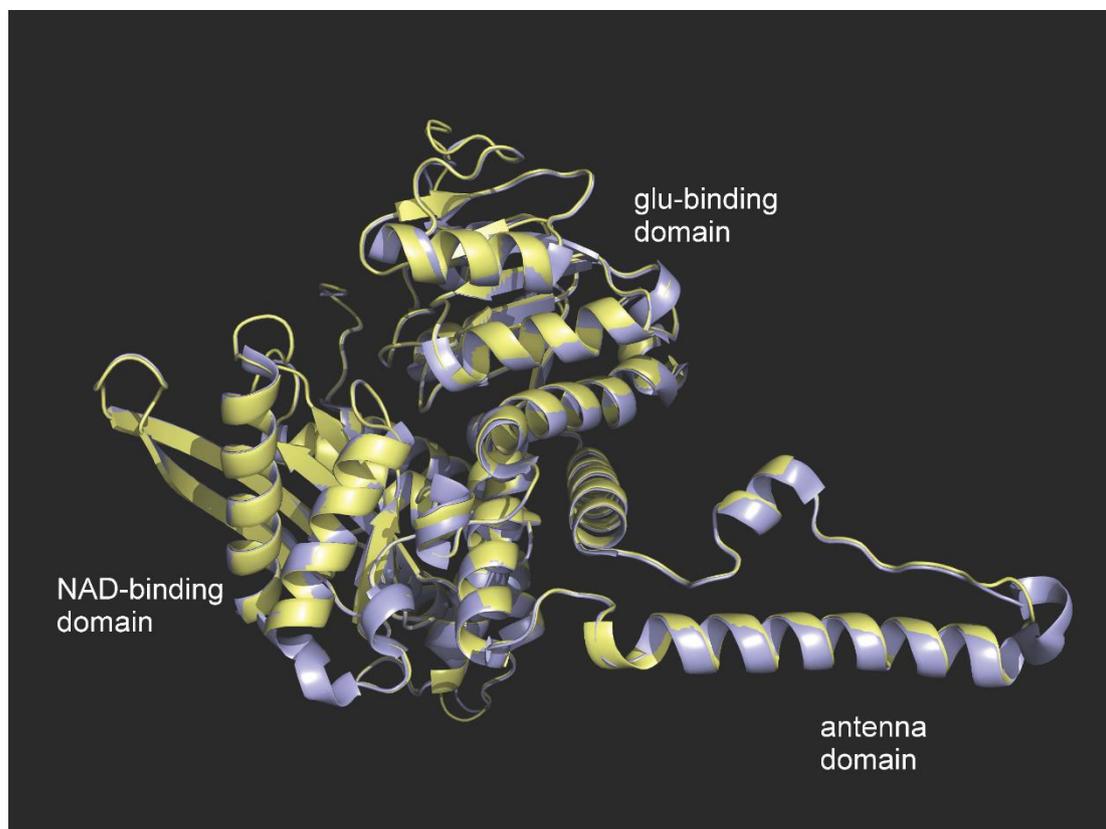
S170, H195, S276, S327, D388) differ from the corresponding residues in *TcGDH* (D, G, A, W, C, N) or *HcGDH* (D, G, S, W, C, N), as shown in Figures 2 and 3. Of these, two (H195  $\leftrightarrow$  A/S and D388  $\leftrightarrow$  N) ligate NAD<sub>b</sub> and will not be considered further, and another three are relatively conservative substitutions (N168  $\leftrightarrow$  D, S170  $\leftrightarrow$  G, S327  $\leftrightarrow$  C). The sixth difference (S276  $\leftrightarrow$  W) is more significant because a small polar residue (S) is replaced with a larger hydrophobic residue (W), but it is especially interesting as S276 in *BtGDH* (1HWZ) ligates the 2'-phosphate of NADP(H).



**Figure 3.** Summary of the structure and ligand binding sites of *BtGDH* and sequence mutability ( $M_i$ ) of selected GDHs. The symbols ( $\diamond$ ,  $\blacklozenge$ ,  $\circ$ ) indicate residues that are hydrogen bonded to the substrate, cofactor or effector. The residues at positions 87, 119 and 120, indicated by open circles ( $\circ$ ), are located in another subunit. Those residues indicated by solid diamonds ( $\blacklozenge$ ) differ between *BtGDH* and *TcGDH* or *HcGDH*. The three structural domains (1-3) were identified using CATH (<http://www.cathdb.info> (Cuff *et al.*, 2011)) and the two functional domains were identified using SCOP [<http://scop.mrc-lmb.cam.ac.uk/scop/index.html> (Andreeva *et al.*, 2008)]. The stippled region in domain 3 represents the 'antenna' domain. The secondary structure indicates the positions of  $\alpha$ -helices and  $\beta$ -strands (black and grey rectangles, respectively).

Three model structures were constructed for each of the *TcGDH* and *HcGDH* amino acid sequences. In each case, the models had the glu- and NAD-binding domains, as well as the antenna domain like *BtGDH* (Figure 4), but unlike the *P. falciparum* GDH (2BMA, (Werner *et al.*, 2005)) and the bacterial enzyme (Peterson & Smith, 1999).

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**Figure 4.** Superimposition of BtGDH (1HWY in blue) and the model of TcGDH generated using SWISS-MODEL (in yellow).

**Table 2.** Quality of fit parameters for predicted structures obtained for the *T. circumcincta* and *H. contortus* sequences (AEO44571.1 and AAC19750.1, respectively) based on the BtGDH structure (1HWY). Structures were calculated using Phyre (Kelley & Sternberg, 2009), ESyPred3D (Lambert *et al.*, 2002) and SWISS-MODEL (Kiefer *et al.*, 2009) and the quality of fit parameters were calculated using PDBeFold (Krissinel, 2007).

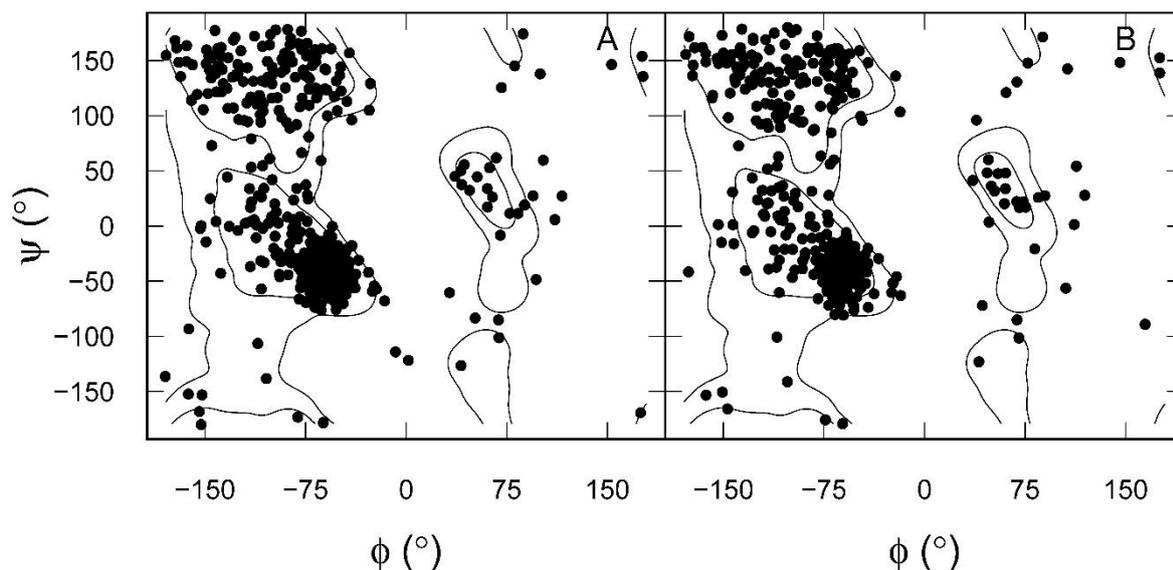
|                                | Phyre  | ESyPred3D | SWISS-MODEL |
|--------------------------------|--------|-----------|-------------|
| <i>T. circumcincta</i>         |        |           |             |
| <i>Q</i>                       | 0.5529 | 0.8863    | 0.9698      |
| <i>P</i>                       | 23.97  | 54.23     | 81.84       |
| <i>Z</i>                       | 15.34  | 22.26     | 27.34       |
| RMSD (Å)                       | 2.001  | 0.551     | 0.364       |
| Number of residues aligned     | 446    | 480       | 497         |
| Identical residues aligned (%) | 58.74  | 65        | 63.78       |
| <i>H. contortus</i>            |        |           |             |
| <i>Q</i>                       | 0.5542 | 0.8757    | 0.9753      |
| <i>P</i>                       | 22.92  | 54.58     | 77.77       |
| <i>Z</i>                       | 14.78  | 22.33     | 26.72       |
| RMSD (Å)                       | 2.016  | 0.404     | 0.251       |
| Number of residues aligned     | 448    | 474       | 497         |
| Identical residues aligned (%) | 58.04  | 64.56     | 64.19       |

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While all these structures were similar, those generated using SWISS-MODEL appeared to be the best (Table 2) because they had the smallest root mean square deviation (RMSD) from 1HWY, and the largest quality measures ( $Q$ ,  $P$  and  $Z$ ). For comparison, a theoretical structure of the *TcGDH* monomer based on the *P. falciparum* structure (2BMA) was built using SWISS-MODEL and this was clearly inferior ( $Q = 0.7618$ ,  $P = 57.99$ ,  $Z = 24.05$ ,  $\text{RMSD} = 0.513 \text{ \AA}$ ) to those based on the *BtGDH* structure (Table 2). Moreover, the models based on *BtGDH* yielded Ramachandran plots that were very similar to that of the bovine enzyme (Figure 5, A and B). This indicates that the backbone dihedral angles had not been greatly distorted in the model, which can be confirmed by inspection of Figure 4 in which the backbones of the aligned *BtGDH* and model *T. circumcincta* structures are superimposed. Naturally, there is rather more variation in the positions of the side chains, but those residues involved in ligand binding are largely in very similar positions even where the residues differ (Figure 6). The most significant exception to this generalisation is S276, which is located adjacent to the adenine ring of  $\text{NAD(P)}^+$  and provides three H-bonds to the 2'-phosphate of  $\text{NADP}^+$  in *BtGDH* (1HWZ). In the model *TcGDH* and *HcGDH* structures S276 is replaced with a tryptophan, the sidechain of which lies outside the electron density of the serine sidechain (Figure 6). This

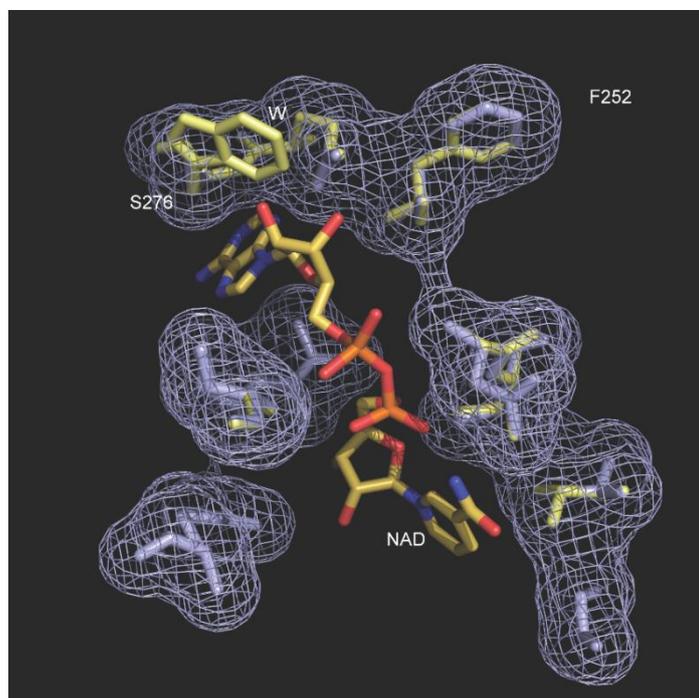
difference involves the replacement of the polar sidechain with a hydrophobic sidechain that is significantly larger, resulting in the partial occlusion of the  $\text{NAD(P)}$ -binding site and removal of at least one potential H-bond to the 2'-phosphate group.

The glycine residue that appears to be specific to *HcGDH* (G240) forms part of a loop that is distinct from the corresponding helix fragments found in *TcGDH* and *BtGDH* (indicated by G in Figure 7). This loop is exposed at the surface of the structure and is adjacent to the  $\alpha$ -ketoglutarate/glu-binding site (indicated by S in Figure 7) and is located in the vicinity of three ligands (Figure 2). In Figure 7 it is clear that the  $\alpha$ -ketoglutarate is parallel to a helix that is essentially identical in *BtGDH* and the model structures of *TcGDH* and *HcGDH*. The other side of the binding site is formed by loops that appear to protrude further into the site in the model *TcGDH* and *HcGDH* structures than in the *BtGDH* structure (indicated by the three asterisks in Figure 7). The residues directly involved in binding  $\alpha$ -ketoglutarate or glutamate, based on the PDBsum analysis, are essentially identical in all three structures except for N168, which is replaced with an aspartate in both nematode sequences (Figure 2). It is clear from Figure 8 that the orientation of the sidechain differs between the *BtGDH* and the model structures and that this results in the loss of an H-bond.

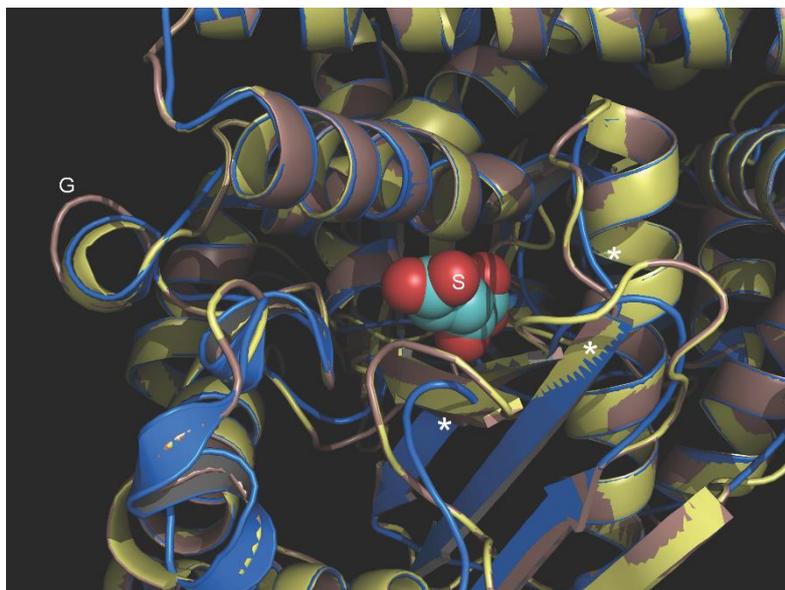


**Figure 5.** Ramachandran plots for *BtGDH* (A, 1HWY) and SWISS-MODEL derived *TcGDH* (B) structures. The contours are based on those of Lovell *et al.* (2003).

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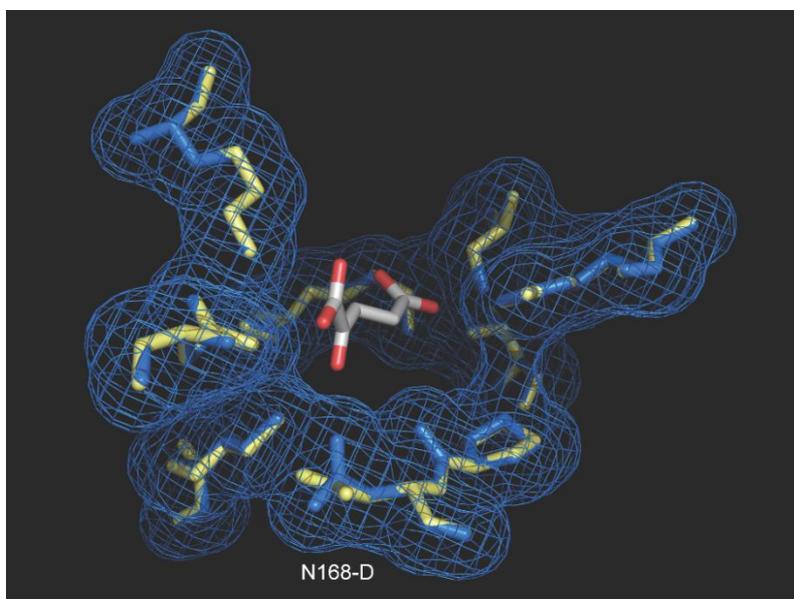


**Figure 6.** Structural alignment showing the NAD(P)-binding residues of BtGDH (1HWY, in blue) and the corresponding residues in the model of TcGDH generated using SWISS-MODEL (yellow). Also shown is the NAD<sup>+</sup> bound in the BtGDH structure. Note that the tryptophan corresponding to S276 in BtGDH is very close to the 2'-OH of NAD<sup>+</sup> and that the tryptophan side-chain lies outside the electron density of S276.



**Figure 7.** The structure of the  $\alpha$ -ketoglutarate/glu-binding site in BtGDH (1HWY, blue) and in the model TcGDH (yellow) and HcGDH (red) structures. The bovine structure has  $\alpha$ -ketoglutarate bound at this site (S) and G240 in the HcGDH sequence (G) and the three loops (\*) that protrude more into the binding site in the model structures than in BtGDH are also indicated (towards the lower right of the  $\alpha$ -ketoglutarate).

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**Figure 8.** Details of the ligands forming the  $\alpha$ -ketoglutarate/glu-binding site in *BtGDH* (1HWY, blue) and in the model *TcGDH* (yellow).

### Structural implications

Both the structural and kinetic properties of *TcGDH* and *HcGDH* are more like those of the bovine enzyme than those of the *P. falciparum* GDH. The ligand binding residues are similar and the backbone of the monomer is not distorted relative to the bovine enzyme. However, six residues involved in ligating substrates or cofactors differ between *BtGDH* and *TcGDH* (Figures 2 and 3). The  $K_m$  for NAD(P)H is about 0.02 mM for the bovine enzyme (Rife & Cleland, 1980; McCarthy & Tipton, 1985) and only very slightly larger (0.03-0.05 mM) for rGDH and *HcGDH* (Rhodes & Ferguson, 1973; Umair *et al.*, 2011).

The most significant kinetic difference between *BtGDH* and the *TcGDH* is the  $K_m$  for NADP<sup>+</sup> (Table 3). This has been reported to be 0.028 mM for the bovine enzyme (Rife & Cleland, 1980; McCarthy & Tipton, 1985) and 1 mM for *TcGDH* (Umair *et al.*, 2011). The 2'-phosphate group of NADP<sup>+</sup> is ligated by S276 in the bovine enzyme, but this is replaced by a tryptophan in r*TcGDH* (Figure 1A). This has two effects: (a) the terminal hydroxyl group is absent from the side chain, removing one possible H-bond and (b) there is less space available for the NADP<sup>+</sup>. It might be speculated that the steric constraint is especially likely to provide some rationalisation for the lower affinity of *TcGDH* for NADP<sup>+</sup>, which might also explain the small decrease in the affinity of

*TcGDH* for NAD(H). Some limited support for this speculation is provided by a report (Yoon *et al.*, 2002) with human GDH mutants of a residue equivalent to E275, in each of which the  $K_m$ (NADH) was increased about 10-fold. Unfortunately, the similarity between *TcGDH* and *HcGDH* means that only one ligand binding residue is different (H195 is replaced with S rather than A). This does not explain the lack of activity of *HcGDH* with NADP(H) (Rhodes & Ferguson, 1973).

A less significant difference in the reported kinetics of the three enzymes is that  $K_m$ (glutamate) and  $K_m$ ( $\alpha$ -ketoglutarate) are much smaller in *TcGDH* than in *HcGDH* (Table 3). While this might relate to the assay conditions, the model structures of the binding site shown in Figure 7 prompt the speculation that the extra residue in *HcGDH* (G240) might make the loop in which it is located more flexible than the corresponding helix-fragments in *BtGDH* and the model *TcGDH*. Perhaps this possible flexibility makes access to the site slightly more difficult. In contrast, the corresponding  $K_m$ s of *TcGDH* are smaller than those reported for *BtGDH* (Table 3). This could relate to the loops that protude into the site more in *TcGDH* (and *HcGDH*) than they do in *BtGDH* (Figure 7). If this is the case, then the larger  $K_m$ (glutamate) and  $K_m$ ( $\alpha$ -ketoglutarate) of *HcGDH* are even more significant.

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**Table 3.** Values of the  $K_m$ s reported for *BtGDH*, *rTcGDH*, *TcGDH* and *HcGDH*.

|                         | $K_m$ (mM)                     |                            |                           |                                  |
|-------------------------|--------------------------------|----------------------------|---------------------------|----------------------------------|
|                         | <i>Bos taurus</i> <sup>a</sup> | <i>T. circumcincta</i>     |                           | <i>H. contortus</i> <sup>d</sup> |
|                         |                                | <i>rTcGDH</i> <sup>b</sup> | <i>TcGDH</i> <sup>c</sup> |                                  |
| $\alpha$ -ketoglutarate | 0.36-2.4                       | 0.07-0.1                   | 0.06-0.09                 | 0.74                             |
| glutamate               | 0.74-3                         | 0.35-0.45                  | 0.15-0.7                  | 3.3                              |
| NAD <sup>+</sup>        | 0.076-0.22                     | 0.7                        | 0.7                       | 0.31                             |
| NADP <sup>+</sup>       | 0.028                          | 1                          | 3                         | —                                |
| NADH                    | 0.02                           | 0.05                       | 0.025                     | 0.033                            |
| NADPH                   | 0.02-0.022                     | 0.03                       | 0.10                      | —                                |
| NH <sub>3</sub>         | 6.5-50                         | 37-40                      | 18                        | 42                               |

<sup>a</sup> (Frieden, 1959; Engel & Dalziel, 1969; Rife & Cleland, 1980; McCarthy & Tipton, 1985) <sup>b</sup> (Umair et al., 2011) <sup>c</sup> (Muhamad et al., 2011)

<sup>d</sup> (Rhodes & Ferguson, 1973)

## Conclusion

It has been suggested that GDH is a potential target for anthelmintics (Umair et al., 2011) because there are “significant differences” in amino acid sequence between the host and the parasite enzyme. The structural models of *TcGDH* and *HcGDH* described here were based on the bovine enzyme, which differs in only 7 positions from the sequence of the sheep enzyme. We infer from this conservation of sequence (Figure 2) and, consequently, of model structure (Figure 4) that the “significant differences” to which Umair et al. (2011) refer, but do not define, are unlikely to render the parasite GDHs sufficiently different from that of the host to make them viable therapeutic targets. However, the replacement of S276 with a tryptophan in the nematode GDHs (Figure 6) provides a plausible explanation of their reduced affinity for NADP<sup>+</sup> (Table 3) and this may have significant implications for amino acid metabolism.

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