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Characterisation and structural analysis of glyoxylate cycle enzymes of *Teladorsagia circumcincta*

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ABSTRACT

A 1332 bp full length cDNA encoding *Teladorsagia circumcincta* isocitrate lyase (*TciICL*) and a 1575 bp full length cDNA encoding *T. circumcincta* malate synthase (*TciMS*) were cloned, expressed in *Escherichia coli* and the recombinant proteins purified. The predicted *TciICL* protein of 444 amino acids was present as a single band of about 52 kDa on SDS-PAGE and the recombinant *TciMS* of 525 amino acids formed a single band about 62 kDa. Multiple alignments of the combined bifunctional *TciICL-MS* protein sequence with homologues from other nematodes showed that the greatest similarity (89-92%) to the homologues of *Ancylostoma ceylanicum*, *Haemonchus contortus* and *Haemonchus placei* and 71-87% similarity to the other nematode sequences. The 3-dimensional structures, binding and catalytic sites were determined for *TciICL* and *TciMS* and shown to be highly conserved. Substrate and metal ion binding sites were identified and were completely conserved in other homologues. *TciICL* was confirmed as a functional enzyme. At 30 °C, the optimum pH was pH 7.5, the $V_{\max}$ was 275 ± 23 nmoles.min⁻¹.mg⁻¹ protein and the apparent K_m for the substrate isocitrate was 0.7 ± 0.01 μ M (mean $\pm$ SEM, n = 3). Addition of 10 mM metal ions (except Mg²⁺) or 1 mM inhibitors reduced the recombinant *TciICL* activity by 60-90%. Antibodies in both serum and saliva from field-immune, but not nematode-naïve, sheep recognised recombinant *TciICL* in ELISA, supporting similar antigenicity to that of the native enzyme.

Keywords: Cloning; ELISA; Expression; Isocitrate lyase; Kinetic properties; Malate synthase; *Teladorsagia circumcincta*

1. Introduction

The glyoxylate cycle (GC) converts isocitrate to malate in a two enzyme shunt which is an alternative to the full TCA cycle [1]. The GC allows net conversion of lipid to carbohydrate, as it bypasses the two CO₂-generating decarboxylations in the conversion of isocitrate to succinate in the TCA cycle. The GC consists of the two enzymes isocitrate lyase (ICL) (EC 4.1.3.1) and malate synthase (MS) (EC 2.3.3.9): ICL cleaves isocitrate to glyoxylate and succinate, followed by MS condensing glyoxylate with acetyl CoA to form malate and coenzymeA [1]. In the TCA cycle, isocitrate is the substrate for isocitrate dehydrogenase (ICD) and later malate is generated by fumarase; CO₂ is generated at the steps catalysed by ICD and glutamate dehydrogenase (GDH).

This shunt is active in bacteria, fungi, nematodes and plants, and the enzymes are reported to be inducible under specific conditions in other groups of animals under stress conditions [2, 3]. In nematodes, enzyme activity has been measured in several free-living species [4-6] and the animal parasitic *Ascaris lumbricoides* [7], *Ascaris suum* [8] and *Haemonchus contortus* [9]. In nematodes, there is a bifunctional ICL-MS gene, which has been sequenced and expressed in a number of species, including *Caenorhabditis elegans* [10] and *Strongyloides stercoralis* [11] and other sequences are available in databases.

There is greater GC activity in nematodes when lipid reserves are being used in non-feeding stages and eggs [12, 13], during embryonic development [6, 9] and when trehalose accumulates to tolerate desiccation or freezing [14-16]. Knockdown of ICL by RNAi reduced egg production in the plant parasitic *Meloidogyne incognita* [17]. The relative flows of carbon in the mitochondria through the GC and TCA cycles are determined by competition for the substrate isocitrate by ICL and isocitrate

dehydrogenase (IDH) and the activities and properties of these two enzymes [2]. Coarse control of flux is determined by transcription and synthesis of the enzymes according to the metabolic environment, such as during development through the life-cycle [13] and formation of dauer larvae [18]. Fine rapid control is through variation in K_m , specific activity and number of multiple forms of the enzyme [14, 19]. Madin et al. [14] showed that early in desiccation the isocitrate concentration rose, saturating IDH with its 40-fold lower K_m than that of ICL. The V_{max} of IDH progressively fell, resulting in a reduced flux through IDH and the TCA cycle and a relatively higher flux through ICL and the GC shunt. Changes in cellular pH could affect enzyme activities, as the ICL of nematodes have pH optima of 7.4-7.7, lower than that for IDH [7, 20].

The aim of the present experiments was to determine the full length sequence of ICL-MS in the sheep abomasal nematode parasite *Teladorsagia circumcincta*, express the proteins and determine kinetic properties and antigenicity of the recombinantly produced enzyme.

2. Materials and methods

All chemicals were purchased from the Sigma Chemical Co. (Mo, USA) unless stated otherwise. Use of experimental animals for culturing and harvesting adult worms for RNA extraction has been approved by the AgResearch Grasslands Animal Ethics Committee (protocol #13052).

2.1. Parasites

Pure cultures of *T. circumcincta* were maintained in the laboratory by regular passage through sheep. Adult worms were recovered from the abomasa of infected sheep

as described previously [21]. Briefly, abomasal contents were mixed 2:1 with 3% agar and the solidified agar blocks incubated at 37 °C in a saline bath. Clumps of parasites were collected from the saline soon after emergence and frozen in Eppendorff tubes at -80 °C for molecular biology procedures.

2.2. RNA isolation and cDNA synthesis

Adult *T. circumcincta* (50-100 µl packed volume) in 1 ml Trizol (Life Technologies) were ground to a fine powder in a mortar under liquid N₂ and RNA extracted according to the manufacturer's instructions. The quality and concentration of the RNA was assessed using Nanodrop. First strand cDNA synthesised from 1 µg using the iScript Select cDNA Synthesis Kit (Bio-Rad) and a 1:1 mixture of Oligo (dT)₂₀ and random primers. As ICL-MS is one large bifunctional protein, ICL and MS were expressed and characterised as separate proteins. Primers were designed to obtain the full-length sequences using two partial *T. circumcincta* ICL-MS sequences: TDC00774 in NEMBASE and TELCIR_04692 in AgResearch's internal database.

The full length *Tci*ICL or *Tci*MS cDNA was amplified from total *T. circumcincta* cDNA in a PCR containing either the oligonucleotide primers *Tci*ICL_FL-F (5'- CATGGGCGCGCCATGGCGCAGGCCGCTAAGAAC-3') and *Tci*ICL_FL-R (5'- CATGGCGGCCGCAAGAATCTCCTCATCTTCACTGCTG-3') or *Tci*MS_FL-F (5'- CATGAGGCCTATGACGCTGACTGCTCCTATGGCG-3') and *Tci*MS_FL-R (5'- CATGGCGGCCGCGTGTCTTCTGATACCAGCTTGTC-3') respectively. The full length genes were cloned, using standard protocols, into the expression vector AY2.4 [22], using the restriction enzymes *Ascl* and *NotI* for *Tci*ICL and *StuI* and *NOTI* for *Tci*MS (engineered into the forward and reverse primers,

underlined in the primer sequences) to allow the production of N-terminal His-tagged recombinant protein. The expression clones were sequenced to confirm identity.

Alignments were performed using the Muscle alignment option in Geneious Prime (Biomatters Ltd) with the Blosum 62 similarity matrix used to determine 100% similarity to helminth homologues.

2.3. Protein modelling and structural analysis of *TciICL* and *TciMS*

The Position-Specific Iterative Basic Local Alignment Search Tool (PSI-BLAST) [23] was used to compare the *TciICL* and *TciMS* protein sequences with deposited structures in the Protein Data Bank (PDB). The structural models of *TciICL* and *TciMS* were constructed by submitting the amino acid sequences obtained as described above, to the I-TASSER server [24]. For *TciMS*, only one model was obtained and for *TciICL* a model was selected from the best of five possible models generated. The structural model with the highest C-score was further validated using Procheck [25] and ProSA-web [26]. TM-score is a metric for measuring the similarity of two protein structures, or a global fold similarity between the generated model and the structure it was based on. Scores higher than 0.5 assumes the parent structure and modelled protein share the same fold while below 0.17 suggests a random nature to the produced model [27]. C-score is a confidence score for estimating the quality of predicted models by I-TASSER. It is calculated based on the significance of threading template alignments and the convergence parameters of the structure assembly simulations. C-score is typically in the range of -5 to 2, where a C-score of higher value signifies a model with a high confidence and vice-versa. The substrate binding domains were predicted, and active site residues deduced using the PyMOL Molecular Graphics System, Version 2.0 (Schrödinger, LLC).

2.4. Expression of *T. circumcincta* recombinant *TciICL* and *TciMS* in *E. coli*

For the expression of *TciICL*, *Escherichia coli* strain BL21 (DE3) was transformed with *E. coli* AY2.4 *TciICL* and grown in 10 ml Luria Broth (LB), supplemented with 100 µg/ml ampicillin, for 16 h at 30 °C and 250 rpm. The culture was diluted 20-fold in LB with 100 µg/ml ampicillin and grown at 37 °C and 250 rpm to an OD₆₀₀ of 0.6-0.8. L-arabinose was added to a final concentration of 0.2 % and the culture grown for an additional 4 h at 30 °C and 250 rpm. Bacteria were harvested by centrifugation at 5,000 g for 10 min at 4 °C. The pellet was weighed and the bacteria resuspended (10 g/ml) in equilibration buffer (20 mM sodium biphosphate, 0.5 M NaCl, 20 mM imidazole, pH 7.4). Protease inhibitors were added to the suspension, which was then passed through the chamber of a MP110 Microfluidizer® (Microfluidics, USA) seven times consecutively under ice at 20,000 psi to ensure the full lysis of *E.coli*, as recommended by the manufacturer. The crude lysate was centrifuged at 15,000 g for 20 min at 4 °C to remove cell debris and the supernatant filtered through a 0.22 µm filter prior to purification.

TciMS was expressed in *E. coli* strain BL21 (DE3), transformed with *E. coli* Rosetta gami *TciMS*, and grown in 10 ml LB supplemented with 100 µg/ml ampicillin, kanamycin 50 µg/ml, chloramphenicol 34 µg/ml and tetracycline 12.5 µg/ml for 16 h at 37 °C and 250 rpm. The culture was diluted 20-fold in LB with 100 µg/ml ampicillin, kanamycin 50 µg/ml, chloramphenicol 34 µg/ml and tetracycline 12.5 µg/ml and grown to an OD₆₀₀ of 0.6-0.8 at 37 °C and 250 rpm. L-arabinose was added to a final concentration of 0.2 % and the culture grown for an additional 16 h at 37 °C and 250 rpm.

Recombinant *TciMS* was expressed as a totally insoluble protein which was purified and folded as inclusion bodies. Briefly, bacteria were harvested by centrifugation, the

supernatant was discarded and the pellet resolubilised in binding buffer (1 ml/10mg) containing 8 M urea. Protease inhibitors were added at room temperature for 10 min. The lysate was centrifuged at 16,000 g for 10 min at 4 °C to remove cell debris and the supernatant was collected and filtered through a 0.45µm filter.

2.5. Purification of recombinant *Tci*ICL and *Tci*MS

Purified recombinant poly-histidine *Tci*ICL was obtained by Fast Protein Liquid Chromatography (FPLC) under native conditions using a Ni-NTA column (Qiagen) and a BIO-RAD chromatography system (Bio-Rad, USA). Sodium biphosphate buffer was used as an equilibration buffer, sodium biphosphate containing 20 mM imidazole as the also wash buffer and sodium biphosphate containing 500mM imidazole as the elution buffer. The protein was dialysed overnight following the elution and the concentration was determined by the Nanodrop A280 nm assay using the extinction coefficient 68215 M⁻¹cm⁻¹ and molecular weight 51.8 kDa.

Purified recombinant *Tci*MS was obtained under the same conditions as described above, except the wash and elution buffers also contained 8 M urea. The recombinant *Tci*MS was purified as inclusion bodies, therefore, refolded by dialysing in the buffer containing 8 M, 6 M, 4 M, 2 M or no urea for 12 h in each buffer at 4 °C. The protein concentration was determined by the Nanodrop A280 nm assay using the extinction coefficient 66155 M⁻¹cm⁻¹ and molecular weight 62.5 kDa.

2.6. Gel electrophoresis

SDS-PAGE was performed using NuPAGE Novex 4-12 % Bis-Tris gels according to the instructions of the manufacturer (Invitrogen). Gels were stained with SimplyBlue™ SafeStain (Invitrogen). A western blot was also performed on the protein

using a monoclonal anti-poly histidine-peroxidase antibody. Blots were incubated overnight in 1:2000 antibody in buffer (4 % skim milk powder in Tris-buffered saline and 0.1 % Tween-20) at room temperature and developed to detect His-tagged recombinant protein.

2.7. *TciICL* activity (E.C. 4.1.3.1)

TciICL enzyme activity was measured at 30 °C in a coupled assay in which isocitrate was converted to glyoxylate, which combined with phenylhydrazine. The increase in absorption was measured spectrophotometrically at 324 nm. The final reaction mixture (1 ml) contained 50 mM imidazole assay buffer, 10 mM MgCl₂, 1 mM EDTA, 1 mM isocitrate and 50 µg recombinant protein.

(1) The optimum pH was determined with a substrate concentration of 1 mM isocitrate and a pH range of 6 to 9. Subsequent assays were carried out at pH 7.5.

(2) The apparent K_m for isocitrate was determined in reaction mixtures containing 0-5 mM of substrate. Subsequent assays were carried out at 1 mM isocitrate.

(3) The effects of bivalent metal ions and potential inhibitors on recombinant *TciICL* activity was tested with 10 mM Mg²⁺, Mn²⁺, Cu²⁺, Ca²⁺, Zn²⁺ and Ni²⁺ as chlorides, or 1 mM malonate, tartronate or tartrate.

2.8. Host recognition

Pooled serum and saliva samples taken from parasite-naive and parasite-exposed sheep were tested for the presence of antibodies that react with recombinant *TciICL* by ELISA, using standard procedures. Serum and saliva samples were collected from 18 male 6-7 months-old Romney lambs previously exposed to multiple species of parasite, including *T. circumcincta*, to which they had developed immunity. 100 µl per

well of the 5 µg/ml *Tci*ICL was immobilised onto ELISA plates (Maxisorp, Thermofisher Scientific), free binding sites were blocked with Superblock (Thermo Scientific), followed by incubation with serial dilutions of serum (200- to 12800-fold) or saliva (20- to 320-fold) in ELISA buffer for 2 h at room temperature. Bound serum immunoglobulins were detected by incubation for 2 h at 37 °C with 1:4000 diluted rabbit anti-sheep IgG-HRP and colour development with 3,3',5,5'-tetramethylbenzidine (TMB). Salivary IgA was similarly detected with rabbit anti-sheep IgA-HRP.

2.9. Data analysis

Replicate data are presented as mean ± SEM, n=3. Graph Prism v5 was used to plot and analyse the kinetic data and estimate K_m and V_{max} , using the non-linear fit function. The effects of metal ions and potential inhibitors was assessed using one-way ANOVA.

3. Results

3.1. *Tci*ICL and *Tci*MS gene sequences

The 1332 bp full length *T. circumcincta* cDNA ICL and the 1575 bp full length MS sequences, amplified from adult *T. circumcincta* cDNA, have been deposited in Genbank as Accession No SB7883248 and SB3620665 respectively. The nucleotide sequences were translated to amino acid sequence using Geneious Prime. The predicted proteins for *Tci*ICL and *Tci*MS consisted of 444 and 525 amino acids respectively. Although *Tci*ICL and *Tci*MS were cloned, expressed and purified as two separate proteins, the protein sequences from both of the proteins were joined for alignment and homology studies and comparing the substrate binding and active sites.

Multiple alignments of the amino acid sequence of *Tci*ICL-MS showed high % identity with nematode homologues in *Ancylostoma ceylanicum* (GI: ABO31935), *Ancylostoma caninum* (GI: ABU68338), *Haemonchus placei* (GI: VDO27591.1), *H. contortus* (GI: CDL95172.1), *Necator americanus* (GI: XP_013301212.1), *C. elegans* (GI: NP_001256387.1), *Dictyocaulus viviparus* (GI: KJH51538.1), *Caenorhabditis briggsae* (GI: CAP39673.2), *S. stercoralis* (GI: AAF00535.1) and *Toxocara canis* (GI: KHN81706.1). *A. ceylanicum*, *H. contortus* and *H. placei* ICL-MS were most closely related (90-92%), while *T. canis* ICL-MS showed least identity (71%). All active and binding sites are marked and completely conserved in *Tci*ICL-MS (Fig.1).

3.2. *Tci*ICL and *Tci*MS Structures

The predicted 3D structures of *Tci*ICL and *Tci*MS were modelled using the I-TASSER server to identify the active sites and structural characteristics (Fig. 2). From the best five models obtained for *Tci*ICL, the selected model had a C-score of 0.57, a TM value of 0.79 ± 0.09 , a RMSD (root-mean-square deviation) of 5.8 ± 3.7 Å, and normalized z-scores less than 9.14 (Fig. 2A). For *Tci*MS, only one possible model was produced with a C-score of 1.97, a TM value of 0.99 ± 0.04 , a RMSD of 3.4 ± 2.4 Å, with normalized z-scores less than 8.96 (Fig. 2B).

The 3-dimensional structures, binding and catalytic sites have been determined for *Tci*ICL and *Tci*MS over a wide range of ligands and shown to be highly conserved (Fig. 2). The protein structures for the *Tci*ICL and *Tci*MS superimposed best structural models corresponded to the monomers of [3P0X](#) [28] and [3S9Z](#) [29], associated with the ICL and MS from *Brucella abortus* strain 2308 and *Mycobacterium tuberculosis* strain H37Rv (ATCC 25618), respectively. The % identity of these sequences with the corresponding *T. circumcincta* sequences were 69% and 70%. The *Tci*ICL ligand binding

site (Ser-88, Gly-89, Trp-90, Asp-152, Cys-190, Gly-191, His-192, Arg-227, Glu-288, Asn-316, Ser-318, Ser-320, Thr-350, Leu-351) and the active site (His-179 and Arg-227) residues that fall within 4 Å of the substrate are shown in Fig. 2B, C. The catalytic residues of *TciMS* that fall within 4 Å of the substrate correspond to Thr-87, Arg-159, Glu-243, Gly-268, Arg-269, Arg-270, Asp-271, Met-323, Trp-359, Met-437, Asp-439, Ala-441, and the active site residues to Asp-109, Glu-111, and Arg-159 (Fig. 2E, F). The 3D-structures of *TciICL* and *TciMS* revealed an overall structural homology with the enzymes of other nematode species.

3.3. Recombinant protein expression

Maximal production of functional recombinant ICL was obtained in the *E. coli* strain BL21 (DE3), when expression was induced with 0.2 % L-arabinose for 16 h at 37 °C. The purified N-terminal PolyHistidine recombinant *TciICL* protein appeared as a single band of about 52 kDa on SDS-PAGE (Fig. 3). The presence of a His-tagged recombinant protein was confirmed by Western blotting (Fig. 3). Recombinant *TciMS* was expressed as inclusion bodies and attempts to solubilise the inclusion bodies with urea were unsuccessful. When the protein was dialysed in decreasing urea concentrations to facilitate the folding, the recombinant *TciMS* precipitated at 1 M urea.

3.4. Enzyme assays

The optimum pH for recombinant *TciICL* activity at 30 °C was 7.5 (Fig. 4). The apparent K_m for isocitrate was $0.7 \pm 0.01 \mu\text{M}$ and the $V_{\max}$ was $275 \text{ nmoles min}^{-1} \text{ mg}^{-1}$ protein (mean $\pm$ SEM, $n=3$) (Fig. 5). The Hill Coefficient was calculated to be 1.55 for the one site binding hyperbola, which was the preferred fit over other models. The effects of metal ions and inhibitors on recombinant enzyme activity are shown in Fig. 6. Enzyme

activity was reduced by 60-90% when Mg^{2+} was replaced by the other bivalent ions and addition of 1 mM malonate, tartrate or tartronate inhibited the activity by 60-80% (Fig. 6). *TciMS* assays were not carried out as the recombinant protein precipitated without urea.

3.5. Host recognition

Recombinant *TciICL* was recognised in an ELISA by antibodies in both pooled serum and saliva collected from sheep exposed to nematodes in the field, but not from parasite-naïve animals (Fig. 7).

4. Discussion

The *T. circumcincta* GC enzymes ICL and MS were sequenced separately, as typically the two nematode enzymes form a bifunctional protein, too large to be expressed in *E. coli*. The probability of successfully expressing a soluble protein decreases considerably at molecular weights above ~60 kDa [30]. Proteins that do not express in soluble form may not be modified or folded properly or may precipitate within *E. coli* through the formation of inclusion bodies. Whereas the ICL protein was expressed and confirmed to be an active enzyme which was recognised in an ELISA by antibodies in serum and saliva of sheep exposed in the field to mixed nematode infections, MS was expressed as inclusion bodies and was unable to be obtained in solution in urea concentrations compatible with carrying out confirmatory biochemical assays.

Although the proteins were expressed separately, protein alignments were carried out on the combined ICL-MS sequence. A 1332 bp full length cDNA sequence encoding *T. circumcincta TciICL* was amplified from adult *T. circumcincta* cDNA, cloned and expressed in *E. coli*. The 444 amino acid *TciICL* protein expressed in *E. coli*

was functionally typical of isocitrate lyase of many species. A 1575 bp full length cDNA sequence encoding *T. circumcincta* *TciMS* was amplified, cloned and expressed in *E. coli*. The protein consists of 525 amino acids. The *TciICL*-MS protein sequence had 71-92% similarity to the enzyme homologues of other nematodes (Fig. 1), with all the tetramer interface and metal ion binding sites conserved.

The kinetic properties of the recombinant *TciICL* were generally similar to those of enzymes of other species. The optimum pH for *TciICL* activity at 30°C was pH 7.5 (Fig. 5), similar to that for the ICL of *H. contortus* [9] and *A. lumbricoides* [7]. The enzyme activity at 30 °C ($V_{\max}$ 275 ± 23 nmoles.min⁻¹.mg protein⁻¹), was much higher than that reported for *C. elegans* [18], possibly because tissue extracts were used rather than a recombinant protein. The K_m of *TciICL* for the substrate isocitrate was $0.7 \pm 0.01 \mu\text{M}$ (Fig. 5), higher than the $0.25 \mu\text{M}$ reported for recombinant *A. lumbricoides* ICL [7]. *TciICL* enzymatic activity was unchanged by the addition of 10 mM Mg^{2+} , indicating that the enzyme is dependent on the bivalent metal ion for its activity. However, the enzyme activity decreased dramatically when Mg^{2+} was replaced by other metal ion (Fig. 6). Addition of 1 mM inhibitor significantly inhibited recombinant *TciICL* activity (Fig. 6) as was the case for the free living nematode *Turbatrix aceti* [5, 31].

The recombinant *TciMS* protein was expressed in *E. coli* as inclusion bodies, which lack biological activity [32-34], but are often solubilised in high concentrations of chaotropic agents, such as urea [35]. Proteins expressed in inclusion bodies may not be folded correctly and be functional enzymes [36-38] as it depends on a number of factors such as pH, ionic strength, temperature or the presence of various solvent additives. *TciMS* was solubilised with 8 M urea, but precipitated in the buffer during subsequent dialysis at urea concentrations below 1 M. No confirmatory enzyme assays were

therefore performed. It is not surprising why *Tci*ICL was expressed as a soluble protein whereas *Tci*MS was not as there are a number of factors that make one protein soluble and not the other. It depends on whether the protein is membrane bound, which is the case for MS [39] and also on the size of the protein [30]. Further experiments are needed to optimise the expression including trying a *E. coli* cell- free system.

Recombinant *T. circumcincta* ICL was antigenic and antibodies in both serum and saliva from field-immune, but not nematode-naïve, sheep recognised *Tci*ICL in an ELISA (Fig. 7). These sheep had been exposed to a number of nematode parasites including *T. circumcincta* and to have developed immunity to this very common parasite in New Zealand. The lack of a functional GC in the mammalian host further supports exploring its possible use for biological control of nematode parasites.

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References

- [1] H.L. Kornberg, H.A. Krebs, Synthesis of cell constituents from C2-units by a modified tricarboxylic acid cycle, *Nature* 179 (1957) 988-991,
- [2] M. Cioni, P.V. Pinzauti, P. Vanni, Comparative biochemistry of the glyoxylate cycle, *Comp. Biochem. Physiol. B* 70 (1981) 1-26,
- [3] V.N. Popov, E.A. Moskalev, M.U. Shevchenko, A.T. Epeintsev, Comparative analysis of glyoxylate cycle key enzyme isocitrate lyase from organisms of different systematic groups, *J. Evol. Biochem. Physiol.* 41 (2005) 631-639,

- [4] M. Rothstein, H. Mayoh, Nematode biochemistry. VII. Presence of isocitrate lyase in *Panagrellus redivivus*, *Turbatrix aceti*, and *Rhabditis anomala*, Com. Biochem. Physiol. 16 (1965) 361-365.
- [5] M.P. McKinley, L.A. Field, R.N. Trelease, Multiple forms of isocitrate lyase in the matrix of *Turbatrix aceti* mitochondria, Arch. Biochem. Biophys. 197 (1979) 253-263,
- [6] F.R. Khan, B.A. McFadden, Embryogenesis and the glyoxylate cycle, FEBS Letters 115 (1980) 312-314,
- [7] J. Barrett, C.W. Ward, D.F. Fairbairn, The glyoxylate cycle and the conversion of triglycerides to carbohydrates in developing eggs of *Ascaris lumbricoides*, Comp. Biochem. Physiol. B 35 (1970) 577-586,
- [8] H. Rubin, R.N. Trelease, Subcellular localization of glyoxylate cycle enzymes in *Ascaris suum* larvae, J. Cell Biol. 70 (1976) 374-383,
- [9] M.J. Carrington, D.P. Jasmer, B.A. McFadden, Activities of isocitrate lyase and malate synthase during the development of free-living stages of *Haemonchus contortus* (Nematoda), Proc. Helminthol. Soc. Wash. 54 (1987) 277-279,
- [10] F. Liu, J.D. Thatcher, J.M. Barral, H.F. Epstein, Bifunctional glyoxylate cycle protein of *Caenorhabditis elegans*: a developmentally regulated protein of intestine and muscle, Develop. Biol. 169 (1995) 399-414,
- [11] A.A. Siddiqui, C.S. Stanley, S.L. Berk, Cloning and expression of isocitrate lyase from human round worm *Strongyloides stercoralis*, Parasite 7 (2000) 233-236,
- [12] C. A. Ash, H.J. Atkinson, Evidence for a temperature-dependent conversion of lipid reserves to carbohydrate in quiescent eggs of the nematode, *Nematodirus battus*. Comp. Biochem. Physiol. B 76 (1983) 603-610,

- [13] F. Liu, J.D. Thatcher, H.F. Epstein, Induction of glyoxylate cycle expression in *Caenorhabditis elegans*: a fasting response throughout larval development, *Biochemistry* 36 (1997) 255-260,
- [14] K.A.C. Madin, S.H. Loomis, J.H. Crowe, Anhydrobiosis in nematodes: Control of carbon flow through the glyoxylate cycle, *J. Exp. Zool.* 234 (1985) 341-350,
- [15] B.H. Adhikari, D.H. Wall, B.J. Adams, Effect of slow desiccation and freezing on gene transcription and stress survival of an Antarctic nematode, *J. Exp. Biol.* 213 (2010) 1803-1812,
- [16] C. Erkut, V.P. Gade, S. Laxman, T.V. Kurzchalia, The glyoxylate shunt is essential for desiccation tolerance in *Caenorhabditis elegans* and budding yeast, *Biochem. Chem. Biol. Cell Biol. eLife* 5 (2016) e13614,
- [17] I.T. Lourenço-Tessutti, J.D.A. Souza, D. Martins-de-Sa, A.A.B. Viana, R.M.D.G. Carneiro, R.C. Togawa, J. de Almeida-Engler, J.A.N. Batista, M.C.M. Silva, R.R. Fragoso, M.F. Grossi-de-Sa, Knock-down of heat-shock protein 90 and isocitrate lyase gene expression reduced root-knot nematode reproduction, *Phytopathology* 105 (2015) 628-637,
- [18] V.B. O’Riordan, A.M. Burnell, Intermediary metabolism in the dauer larva of the nematode *Caenorhabditis elegans*—II. The glyoxylate cycle and fatty-acid oxidation, *Comp. Biochem. Physiol. B* 95 (1990) 125-130,
- [19] M.P. McKinley, R.N. Trelease, Regulation of carbon flow through the glyoxylate and tricarboxylic acid cycles in the mitochondria of *Turbatrix aceti* - I. Coarse and fine controls, *Comp. Biochem. Physiol. B* 67 (1980) 17-26,
- [20] W.J. Colonna, B.A. McFadden, Isocitrate lyase from parasitic and free-living nematodes, *Arch. Biochem. Biophys.* 170 (1975) 608-619,

- [21] S. Umair, C. Ria, J.S. Knight, H.V. Simpson, Sarcosine metabolism in *Haemonchus contortus* and *Teladorsagia circumcincta*, *Exp. Parasitol.* 134 (2013) 1–6.
- [22] J.S. Knight, A.H. Broadwell, W.N. Grant, C.B. Shoemaker, A strategy for shuffling numerous *Bacillus thuringiensis* crystal protein domains, *J. Econ. Entomol.* 9 (2004) 1805-1813,
- [23] S.F. Altschul, T.L. Madden, A.A. Schäffer, J. Zhang, Z. Zhang, W. Miller, D.J. Lipman, Gapped BLAST and PSI-BLAST: a new generation of protein database search programs, *Nucleic Acids Res.* 25 (1997) 3389-3402,
- [24] J. Yang, R. Yaan, A. Roy, D. Xu, J. Poisson, Y. Zhang, The I-TASSER Suite: Protein structure and function prediction, *Nat. Methods* 12 (2015) 7-8,
- [25] R.A. Laskowski, J.A.C. Rullmann, M.W. MacArthur, R. Kaptein, J.M. Thornton, AQUA and PROCHECK-NMR: programs for checking the quality of protein structures solved by NMR, *J. Biomol. NMR* 8 (1996) 477–486,
- [26] M. Wiederstein, M.J. Sippl, ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins, *Nucleic Acids Res.* 35 (2007) W407–W410,
- [27] Y. Zhang, J. Skolnick, Scoring function for automated assessment of protein structure template quality, *Proteins* 57 (2004) 702-710.
- [28] R.P. Bhusal, W. Jiao, B.X.C. Kwai, Acetyl-CoA-mediated activation of *Mycobacterium tuberculosis* isocitrate lyase 2, *Nat. Commun.* 10 (2019) 4639,
- [29] I.V. Krieger, J.S. Freundlich, V.B. Gawandi, J.P. Roberts, V.B. Gawandi, Q. Sun, T.R. Ioerger, Structure-guided discovery of phenyl-diketo acids as potent inhibitors of *Mycobacterium tuberculosis* malate synthase, *Chem. Biol.* 19 (2012) 1556-1567,
- [30] S. Gräslund, P. Nordlund, J. Weigelt, B.M. Hallberg, J. Bray, O. Gileadi, S. Knapp, Protein production and purification, *Nat. Method.* 5 (2018) 135–146,

- [31] U. Reiss, M. Rothstein, Isocitrate lyase from the free-living nematode *Turbatrix aceti*: Purification and properties, *Biochemistry* 9 (1974) 1796-1800.
- [32] S.M. Singh, A.K. Panda, Solubilization and refolding of bacterial inclusion body proteins, *J. Biosci. Bioengineer.* 4 (2005) 303–310,
- [33] S. Ventura, A. Villaverde, Protein quality in bacterial inclusion bodies, *Trend. Biotech.* 24 (2006) 179–185,
- [34] V. Upadhyay, A. Singh, D. Jha, A. Singh, A.K. Panda, Recovery of bioactive protein from bacterial inclusion bodies using trifluoroethanol as solubilization agent, *Microb. Cell Fact.* 15 (2016) 100,
- [35] A. Mohammadain, H. Kaghazian, A. Kavianpour, R. Jalalired, Solubilization of inclusion body proteins using low and very low concentrations of chemicals: implications of novel combined chemical treatment designs in enhancement of post-solubilization target protein purity and biological activity, *J. Chem. Tech. Biotech.* 93 (2018) 1579-1587,
- [36] N.K. Puri, E. Crivelli, M. Cardamone, R. Fiddes, J. Bertolini, B. Ninham, Solubilization of growth hormone and other recombinant proteins from *Escherichia coli* inclusion bodies by using a cationic surfactant, *Biochemic. J.* 285 (1992) 871–879,
- [37] R.R. Burgess, Purification of overproduced *Escherichia coli* RNA polymerase sigma factors by solubilizing inclusion bodies and refolding from Sarkosyl, *Method. Enzymol.* 273 (1996) 145–149,
- [38] M. Kudou, D. Ejima, H. Sato, R. Yumioka, T. Arakawa, K. Tsumoto, Refolding single-chain antibody (scFv) using lauroyl-l-glutamate as a solubilization detergent and arginine as a refolding additive, *Protein Express. Purif.* 77 (2011) 68–74,

[39] A.G. Kinhikar, D. Vargas, H. Li, S.B. Mahaffey, L. Hinds, J.T. Belisle, S. Laal, *Mycobacterium tuberculosis* malate synthase is a laminin- binding adhesin, Mol. Microbiol. 60 (2006) 999-1013,

Figure Legends

Fig. 1. Multiple protein sequence alignment of *Tci*ICL-MS, *Ancylostoma ceylanicum* ICL-MS (GI: ABO31935), *Ancylostoma caninum* ICL-MS (GI: ABU68338), *Haemonchus placei* ICL-MS (GI: VDO27591.1), *Haemonchus contortus* ICL-MS (GI: CDL95172.1), *Necator americanus* ICL-MS (GI: XP_013301212.1), *Caenorhabditis elegans* ICL-MS (GI: NP_001256387.1), *Dictyocaulus viviparus* ICL-MS (GI: KJH51538.1), *Caenorhabditis briggsae* ICL-MS (GI: CAP39673.2), *Strongyloides stercoralis* ICL-MS (GI: AAF00535.1) and *Toxocara canis* ICL-MS (GI: KHN81706.1). Active and ligand binding sites are marked by Δ. The amino acid residues in grey represent ICL and the rest MS. The % homology of each sequence with *Tci*ICL-MS is shown at the end of the alignment.

Fig. 2. The predicted tertiary structures of the *Tci*ICL (left) and *Tci*MS (right) monomers. Locations are shown of the C- and N-terminus in the predicted tertiary structure of *Tci*ICL (A) and *Tci*MS (D). Superposition of the predicted tertiary structures showing the location of the active sites (mesh) within *Tci*ICL (B) and *Tci*MS (E). The active sites of *Tci*ICL are shown in green (C) and *Tci*MS in salmon (F) within 4Å of the superimposed 3P0X and 3S9Z active sites (mesh), respectively.

Fig. 3. A: Purified recombinant *Tci*ICL on a NuPage™ 4 - 12% Bis-Tris protein gel stained with SimplyBlue safe stain. Lane 1: Seeblue™ plus2 pre-stained protein standard

in kDa; Lane 2: filtered soluble bacterial lysate; Lane 3: unbound material; Lane 4: elution fraction (1 µg of purified recombinant *TciICL* indicated by the black arrow). **B:** Western blot of recombinant purified *TciICL*. Lane 1: Seeblue™ plus2 pre-stained protein standard in kDa; Lane 2: 1 µg of recombinant *TciICL* detected by a monoclonal anti-polyhistidine-peroxidase antibody. **C:** Purified recombinant *TciMS* on a NuPage™ 4 – 12 % Bis-Tris protein gel stained with SimplyBlue safe stain. Lane 1: Seeblue™ plus2 pre-stained protein standard in kDa; Lane 2: non-induced fraction; Lane 3: induced fraction; Lane 4: filtered soluble bacterial lysate; Lane 5: solubilised inclusion bodies; Lane 6: elution fraction pre-refolding of purified recombinant *TciMS*; Lane 7: purified recombinant *TciMS*. **D:** Western blot of recombinant purified *TciMS*. Lane 1: Seeblue™ plus2 pre-stained protein standard in kDa; Lane 2: 1 µg of recombinant *TciMS* detected by a monoclonal anti-polyhistidine-peroxidase antibody.

Fig. 4. Effects of pH on the activity of recombinant *TciICL* at 30 °C (mean ± SEM, n = 3). Enzyme activity was measured in a coupled assay in which isocitrate was converted to glyoxylate, which combined with phenyldrazine; the increase in absorption was measured spectrophotometrically at 324 nm.

Fig. 5. Effects of varying the substrate concentration on the activity of recombinant *TciICL* at 30 °C (mean ± SEM, n = 3). Enzyme activity was measured in a coupled assay in which isocitrate was converted to glyoxylate, which combined with phenyldrazine; the increase in absorption was measured spectrophotometrically at 324 nm.

Fig. 6. The effects of bivalent metal ions and potential inhibitors on recombinant *TciICL* activity (relative activity, mean ± SEM, n = 3) at 30 °C. Enzyme activity was measured in a coupled assay in which isocitrate was converted to glyoxylate, which combined with

504 phenyldrazine; the increase in absorption was measured spectrophotometrically at 324
505 nm.

506 **Fig. 7.** Recognition of recombinant *Tci*ICL by serially diluted immune serum (IgG) (top)
507 or saliva (IgA) (bottom) (■), but not by parasite-naïve serum or saliva (●).

Figure 1

		1	60
			*
	TcICL-MS	-----MAQAAKNWYQVVKAAPKGRFQG	IKRDYQVEDVLKLRGSVEIEYTLATRGANKLWQ
	A. ceylanicum ICL-MS	-----MAQAAKNWYQVIKAAPKGRFQG	IKRDYQVEDVLKLRGSVEIEYTLATRGANKLWQ
	A. caninum ICL-MS	-----MAQAAKNWYQVIKAAPKGRFQG	IKRDYQVEDVLKLRGSVEIEYTLATRGANKLWQ
	H. placei ICL-MS	-----MTQVAKNWYQVVKAAPKGRFQGI	IRDYHVEDVLKLRGSVEIEYTLATRGANKLWQ
	H. contortus ICL-MS	-----MTQVAKNWYQVVKAAPKGRFQGI	IRDYHVEDVLKLRGSVEIEYTLATRGANKLWQ
	N. americanus ICL-MS	-----VIRAAPKGRFQGI	KRDYQVEDVLKLRGSIEIEYTLASRGANKLWQ
	C. elegans ICL-MS	-----MSSAAKNFYQVKSAPKGRFKGI	KRDYTVEDVLKLRGSIDIDYTLATRGANKLWQ
	D. viviparus ICL-MS	-----MSSAAKNFYQVKSAPKGRFKGI	KRDYTVEDVLKLRGSIDIDYTLATRGANKLWQ
	C. briggsae ICL-MS	MTTPAAGAAKNFYQVKSAPKGRFKGI	KRDYTVEDVLKLRGSIDIDYTLATRGANKLWQ
	S. stercoralis ICL-MS	-----MRLKASNFYHIVKNSPKGRWTGI	KRNYEIKDVLKLRGSLNIEYTLANKTSNKLWH
	T. canis ICL-MS	----MSQQIAKNFYSI	IKSAPKGRFAGLKRNYEVEDVLKLRGSIEIEYTLATRGANKLWQ

			120
		ΔΔΔ	
	TcICL-MS	LLHTEPFVPALGAQTGNQAVQMVRAGL	KAIYLSGWQVAADANTAGDMYPDQSLYPANSGP
	A. ceylanicum ICL-MS	LLHTEPFVPALGAQTGNQAVQMVRAGL	KAIYLSGWQVAADANTAGDMYPDQSLYPANSGP
	A. caninum ICL-MS	LLHTEPFVPALGAQTGNQAVQMVRAGL	KAIYLSGWQVAADANTAGDMYPDQSLYPANSGP
	H. placei ICL-MS	LLHTEPFVPALGAQTGNQAVQMVRAGL	KAIYLSGWQVAADANTAGDMYPDQSLYPANSGP
	H. contortus ICL-MS	LLHTEPFVPALGAQTGNQAVQMVRAGL	KAIYLSGWQVAADANTAGDMYPDQSLYPANSGP
	N. americanus ICL-MS	LLHTEPFVPALGAQTGNQAVQMVRAGL	KAIYLSGWQVAADANAAGDMYPDQSLYPANSGP
	C. elegans ICL-MS	LLHTEPFVPALGAQTGNQAVQMVRAGL	KAIYLSGWQVAADANSAGDMYPDQSLYPANSGP
	D. viviparus ICL-MS	LLHTEPFVPALGAQTGNQAVQMVRAGL	KAIYLSGWQVAADANSAGDMYPDQSLYPANSGP
	C. briggsae ICL-MS	LLHTEPFVPALGAQTGNQAVQMVRAGL	KAIYLSGWQVAADANSAGDMYPDQSLYPANSGP
	S. stercoralis ICL-MS	LIHTEPYVAALGAQTGNQAVQMIKAGL	KAIYLSGWQVAADGNTAGDMYPDQSLYPSNSGP
	T. canis ICL-MS	LLHTEPFIAALGAQTGNQAVQMVRAGL	PAIYLSGWQVAADMNCAGEMYPDQSLYPANSGP

			180
		Δ	
	TcICL-MS	ELCRRINRSLRRADQIEAVEAEDYLAQRD	WYAPIVADAEAGFGGALNCFELMKSYIEAGA
	A. ceylanicum ICL-MS	ELCRRINRSLRRADQIEAVEAEDYLAQRD	WYAPIVADAEAGFGGALNCFELMKAYIEAGA
	A. caninum ICL-MS	ELCRRINRSLRRADQIEAVEAEDYLAQRD	WYAPIVADAEAGFGGALNCFELMKAYIEAGA
	H. placei ICL-MS	ELCRRINRSLRRADQIEAVEAEDYLAQRD	WYAPIVADAEAGFGGALNCFELMKAYIEAGA
	H. contortus ICL-MS	ELCRRINRSLRRADQIEAVEAEDYLAQRD	WYAPIVADAEAGFGGALNCFELMKAYIEAGA
	N. americanus ICL-MS	ELCRRINRSLRRADQIDAVEAEDYLAQRD	WYAPIVADAEAGFGGALNCFELMKAYIEAGA

<i>C. elegans</i>	ICL-MS	ELAKRINRSLRRADQIEACEAEDYLAQRDWDYAPIVADAEAGFGGALNCFELMKAYIEAGA
<i>D. viviparus</i>	ICL-MS	ELAKRINRSLRRADQIEACEAEDYLAQRDWDYAPIVADAEAGFGGALNCFELMKAYIEAGA
<i>C. briggsae</i>	ICL-MS	ELARRINRSLRRADQIEACEAEDYLAQRDWDYAPIVADAEAGFGGALNCFELMKAYIEAGA
<i>S. stercoralis</i>	ICL-MS	ELARRINKSLRRADQIECAESDDMQPYRDYAPIVADCEAGFGGSLNCFEITKSYIESGV
<i>T. canis</i>	ICL-MS	ELCRRINKSLRRADQVEAVEAEDYRSSRDWDYAPIVADAEAGFGGALNCFELMKAYIEAGA

			240
		Δ ΔΔ Δ	
<i>Tc</i>	ICL-MS	AGVHFEDRLGSEKKCGHMGKVLIPTAQHIRHLNAARLAADVCGAPTIVVARTDAESSRL	
<i>A. ceylanicum</i>	ICL-MS	AGVHFEDQLGSEKKCGHMGKVLIPTAQHIRHLNAARLAADVCGAPTIIIVARTDAESSRL	
<i>A. caninum</i>	ICL-MS	SGVHFEDQLGSEKKCGHMGKVLIPTAQHIRHLNAARLAADVCGAPTIIIVARTDAESSRL	
<i>H. placei</i>	ICL-MS	AGVHFEDQLGSEKKCGHMGKVLIPTAQHIRHLNAARLAADVCGAPTIVVARTDAESSRL	
<i>H. contortus</i>	ICL-MS	AGVHFEDQLGSEKKCGHMGKVLIPTAQHIRHLNAARLAADVCGAPTIVVARTDAESSRL	
<i>N. americanus</i>	ICL-MS	AGVHFEDQLGSEKKCGHMGKVLIPTAQHIRHLNAARLAADVCGTPTIIIVARTDAESSRL	
<i>C. elegans</i>	ICL-MS	AGVHYEDQLGSEKKCGHMGKVLIPTAQHIRHLNASRLAADVCGVPTIIIVARTDAESSRL	
<i>D. viviparus</i>	ICL-MS	AGVHYEDQLGSEKKCGHMGKVLIPTAQHIRHLNASRLAADVCGVPTIIIVARTDAESSRL	
<i>C. briggsae</i>	ICL-MS	AGVHYEDQLGSEKKCGHMGKVLIPTAQHIRHLNASRLAADVCGVPTIIIVARTDAESSRL	
<i>S. stercoralis</i>	ICL-MS	AGVHFEDQLGSEKKCGHMGKVLIPISEHIRHLNAARLAADVCDTPTIIIVARTDAESARL	
<i>T. canis</i>	ICL-MS	AGVHYEDQLGSEKKCGHMGKVLIPTEQHIRHLNAARLAADVCGVPTIIIVARTDAESARL	

			300
		Δ	
<i>Tc</i>	ICL-MS	LTSDVDERDHPYIDRQAGRTVEGFHRLKDESTALQYCIDRAINYPYCDLIWMETSHPTIA	
<i>A. ceylanicum</i>	ICL-MS	LTSDVDERDHPFIDRAAGRTVEGFYRLKDESTALQYCIDRAINYPYCDLIWMETSHPTIA	
<i>A. caninum</i>	ICL-MS	LTSDVDERDHPFIDRAAGRTVEGFYRLKDESTALQYCIERAINYPYCDLIWMETSHPTIA	
<i>H. placei</i>	ICL-MS	LTSDVDERDHPFIDRDAGRTVEGFYRLKDSMALQYCIERAINYPYCDLIWMETSHPTIA	
<i>H. contortus</i>	ICL-MS	LTSDVDERDHPFIDRDAGRTVEGFYRLKDSMALQYCIERAINYPYCDLIWMETSHPTIA	
<i>N. americanus</i>	ICL-MS	LTSDVDERDHPFIDRDSGRTVEGFYRLKNSTALQYCIERAINYPYCDLIWMETSHPTIA	
<i>C. elegans</i>	ICL-MS	LTSDIDPRDHPYIDYEAGRTIEGFYRLKDESTAIQYCIDRAIQYAPYTDLIWMETSHPTIA	
<i>D. viviparus</i>	ICL-MS	LTSDIDPRDHPYIDYEAGRTIEGFYRLKDESTAIQYCIDRAIQYAPYTDLIWMETSHPTIA	
<i>C. briggsae</i>	ICL-MS	LTSDIDPRDHPYIDYDAGRTIEGFYRLKDESTAIQYCVDRAIQYAPYCDLIWMETSHPTIA	
<i>S. stercoralis</i>	ICL-MS	LTSDIDERDHPFIDRKAGRTSEGFYRLKDESTSMEACIQRGIAYPYCDMIWMETSYP SLS	
<i>T. canis</i>	ICL-MS	LTSDIDERDHPYIDRQAGRTTEGFYRLKNETALKYCIERAISYAPYCDLIWMETSHPTLS	

360

			Δ Δ Δ	ΔΔ	
	TcICL-MS		DAREFAEGVRKVYPDKMFAYNCSPSFNWKQHLSP	TQLEKFQKELGALGFKYQF	ITLAGFH
A. ceylanicum	ICL-MS		DAREFAEGVRKVYPDKMFAYNCSPSFNWKHLSPA	QMEKFQKELGALGFKYQF	ITLAGFH
A. caninum	ICL-MS		DAREFSEGVVRKVYPDKMFAYNCSPSFNWKHLSPA	QMEKFQKELGALGFKYQF	ITLAGFH
H. placei	ICL-MS		DAREFAEGVRKVYPDKMFAYNCSPSFNWKHLSSSQ	LEKFQKELGALGFKYQF	ITLAGFH
H. contortus	ICL-MS		DAREFAEGVRKVYPDKMFAYNCSPSFNWKHLSSSQ	LEKFQKELGALGFKYQF	ITLAGFH
N. americanus	ICL-MS		DAREFAEGVRKVYPDKMFAYNCSPSFNWKHLSPA	QMEKFQKELGALGFKYQF	ITLAGFH
C. elegans	ICL-MS		DAREFAEGVHKQYPDKMFAYNCSPSFNWKHLSPSQ	MEKFQKELGAMGFKYQF	ITLAGYH
D. viviparus	ICL-MS		DAREFAEGVHKQYPDKMFAYNCSPSFNWKHLSPSQ	MEKFQKELGAMGFKYQF	ITLAGYH
C. briggsae	ICL-MS		DAREFAEGVHKQYPDKMFAYNCSPSFNWKHLSPSQ	MEKFQKELGAMGFKYQF	ITLAGFH
S. stercoralis	ICL-MS		QAKEFAEGVKREFPDKLFAYNCSPSFNWKHLKKS	DMEKYQRELGAMGFKYQF	ITLAGYH
T. canis	ICL-MS		DAKEFAEGVRKVYPDKLFAYNCSPSFNWRKHLRPV	DLEKFQKELGAMGFKYQF	ITLAGFH

420

	TcICL-MS		ANSYSMFDLARNYKEKGMLAYSSSQQEF	AAEQHGYSAVKHQREVGTGYFDHISNA	VTGG
A. ceylanicum	ICL-MS		ANSYSMFDLARNYKDKGMLAYSQ	LQEAEFSEKHGYSAVKHQREVGTGYFDHISNA	VTGG
A. caninum	ICL-MS		ANSYSMFDLARNYKDKGMLAYSQ	LQEAEFSEKHGYSAVKHQREVGTGYFDHISNA	VTGG
H. placei	ICL-MS		ANSFSMFDLARNYREKGMLAYSNLQEA	EFAEQHGYSAVKHQREVGTGYFDHISNA	VTGG
H. contortus	ICL-MS		ANSFSMFDLARNYREKGMLAYSNLQEA	EFAEQHGYSAVKHQREVGTGYFDHISNA	VTGG
N. americanus	ICL-MS		ANSFSMFDLARNYKDKGMLAYSQ	LQEAEFSEKYGYS	SAVKHQREVGTGYFDHISNA
C. elegans	ICL-MS		ANSYSMFDLARNYKEKGMLAYSG	LQEGEF	AAEKHGYTAVKHQREVGTGYFDAVSR
D. viviparus	ICL-MS		ANSYSMFDLARNYKEKGMLAYSG	LQEGEF	AAEKHGYTAVKHQREVGTGYFDAVSR
C. briggsae	ICL-MS		ANSYSMFDLAKNYKETGMLAYSALQ	EGEF	AAEKHGYTAVKHQREVGTGYFDAVSR
S. stercoralis	ICL-MS		TNSFSIFDLAKNYRERGM	AAAYAE	LQKAEFDAEKSGYTAVKHQREVGTGYFDVLGNACAGG
T. canis	ICL-MS		CNSFSVYDLARKYRERGM	AAAYSELQ	ENEFDSEKHGYSAVKHQREVGTGYFDHVANA

480

	TcICL-MS		QSSTTALAGSTEEAQF--HT-ATAS-SEDEE	IMTLTAPMAAGDEKILTPDALRFIKDLNK	
A. ceylanicum	ICL-MS		QSSTTALTGSTEEAQF--FT-ATAS-SEDEE	IMTLTAPTLAGDEKILTPDALRFIKELNK	
A. caninum	ICL-MS		QSSTTALTGSTEEAQF--FT-ATAS-SEDEE	IMTLTAPTLAGDEKILTPDALRFIKDLNK	
H. placei	ICL-MS		MSSTTALTGSTEEAQF--KT-ITAS-SDDEE	ILVLTAPLNTGDEKILTPDALRFIKDLNL	
H. contortus	ICL-MS		MSSTTALAGSTEEAQFKTIT----	ASSDDEEILVLTAPLNTGDEKILTPDALRFIKDLNL	
N. americanus	ICL-MS		QSSTTALSGSTEEAQF--FT-ATAS-SEDEE	ILTLTAPTLAGDEKILTPDALRFIKDLNK	
C. elegans	ICL-MS		LSSTTALSGSTEEAQF--Q---	TAVASQDEEILSLTAQNVAGDEKILTPDALRFLHDLNT	

<i>D. viviparus</i>	ICL-MS	LSSTTALSGSTEEAQF--Q---TAVASQDEEILSLTAQNVAGDEKILTPDALRFLHDLNT
<i>C. briggsae</i>	ICL-MS	LSSTTALSGSTEEAQF--QTA-VASASEDEEILSLTAQTVAGDEKILTPDALRFLHDLNT
<i>S. stercoralis</i>	ICL-MS	SSSTTALTGSTEEAQF--KT--TAV--EDDEVMTVSKNATV-----
<i>T. canis</i>	ICL-MS	QASTVALSGSTEEAQFSDKPHTTTAPPDEDEILMMTAPEVSGDEKILTPNALRFLKELHQ

540

	<i>Tc</i>	ICL-MS	KFDGKRRELLKKRQQVQIEINDGVYFPDFSSETAHLREDMGWKGSEIPQDLQDRRVEITG
<i>A. ceylanicum</i>	ICL-MS	KFDEKRRKLLKKRVVLQKDINEGAWFPDFASDTANIRDDMGWKGAEIPDDLQNNRVEITG	
<i>A. caninum</i>	ICL-MS	KFEEKRRKLLKKRVVMQKDINEGAWFPDFASDTAQIRDDKGWKGAEIPEDLQNNRVEITG	
<i>H. placei</i>	ICL-MS	KFDGRRQELLKKRVQKQKEINDGVYFPDFSPDTANLREDLSWKGAIEPEDLMDRRVEITG	
<i>H. contortus</i>	ICL-MS	KFDGRRQELLKKRVQKQKEINDGVYFPDFSPDTANLREDLSWKGAIEPEDLMDRRVEITG	
<i>N. americanus</i>	ICL-MS	KFDDKRRKLLKKRTYLQKDINEGAWFPDFSPDTADIRNDKGWKGAEIPDLQDRRVEITG	
<i>C. elegans</i>	ICL-MS	EFNPRRLRLLSKRNQVQADINNSLWFPDFNKETEVLRSQQGWKGAEIPRDLQDRRVEITG	
<i>D. viviparus</i>	ICL-MS	EFNPRRLRLLSKRNQVQADINNSLWFPDFNKETEVLRSQQGWKGAEIPRDLQDRRVEITG	
<i>C. briggsae</i>	ICL-MS	EFNPRRLRLLSKRRQVQDDINSSLWFPDFNKETEVLRSQQGWKGAEIPRDLQDRRVEITG	
<i>S. stercoralis</i>	ICL-MS	-----	
<i>T. canis</i>	ICL-MS	KFDARRLQLLAQRKLVQVSIDDSNYFPDFDPATKGLREDHAWFGAKIPDDMLDRRVEITG	

600

	<i>Tc</i>	ICL-MS	PTDRKMVINALNSGANVFMAFEDSNTPSWRNQLEGQVNLDAVRDNISYMHPTTKKEYT
<i>A. ceylanicum</i>	ICL-MS	PTDRKMIINALNSGANVFMAFEDSNTPSWRNQLDGQINLYDAVRNNISYMHPTTKKEYT	
<i>A. caninum</i>	ICL-MS	PTDRKMIINALNSGANVFMAFEDSNTPSWRNQLDGQINLYDAVRNNISYVHPTTKKEYT	
<i>H. placei</i>	ICL-MS	PTDRKMIINALNSGANVFMAFEDSNTPSWRNQLDGQINLYDAVRNNISYEHPTTKKEYT	
<i>H. contortus</i>	ICL-MS	PTDRKMIINALNSGANVFMAFEDSNTPSWRNQLDGQINLYDAVRNNISYEHPTTKKEYT	
<i>N. americanus</i>	ICL-MS	PTDRKMIINALNSGASIFMAFEDSNTPSWRNQLDGQINLYDAVRNNISYVHPSTKKEYS	
<i>C. elegans</i>	ICL-MS	PTDRKMVINAMNSGANVFMAFEDSNSPTWRNQLEGQINLYDAVRNNISYTHPTTKKEYT	
<i>D. viviparus</i>	ICL-MS	PTDRKMVINAMNSGANVFMAFEDSNSPTWRNQLEGQINLYDAVRNNISYTHPTTKKEYT	
<i>C. briggsae</i>	ICL-MS	PTDRKMVINAMNSGANVFMAFEDSNSPTWRNQLEGQINLYDAVRNNISYVHPTTKKEYT	
<i>S. stercoralis</i>	ICL-MS	-----	
<i>T. canis</i>	ICL-MS	PTDRKMVINALNSGAKVFMAFEDSNTPSWRNQVEGQMNLYDAVRGDISYTHPTTKRDYT	

660

	<i>Tc</i>	ICL-MS	LNQKVAVL-----
<i>A. ceylanicum</i>	ICL-MS	LNKETSVL-----	

<i>A. caninum</i>	ICL-MS	LNKETSVL-----
<i>H. placei</i>	ICL-MS	LDKKVSVL-----
<i>H. contortus</i>	ICL-MS	LDKK-----
<i>N. americanus</i>	ICL-MS	LNKQTSVL-----
<i>C. elegans</i>	ICL-MS	LNEKHAVL-----
<i>D. viviparus</i>	ICL-MS	LNEKHAVL-----
<i>C. briggsae</i>	ICL-MS	LNEKHAVL-----
<i>S. stercoralis</i>	ICL-MS	-----
<i>T. canis</i>	ICL-MS	LKENHAGMTSPFLRAYTLSSIKVCHSRNIAAMGGMAAQIPIKHDQTANRKALALVRQDKE

720

		Δ	
<i>Tc</i>	ICL-MS	--N--V---RPRGWHLPEKHVLIHNKPTSGSLFDFGLFVYHNAKALKDKGSGPYFYLPKL	
<i>A. ceylanicum</i>	ICL-MS	K--V-----RPRGWHLPEKHVLIHNKPTSGSLFDFGLFVYHNARALMDKSGPYFYLPKL	
<i>A. caninum</i>	ICL-MS	-----KVRPRGWHLPEKHVLIHNKPTSGSLFDFGLFVYHNARALMDKSGPYFYLPKL	
<i>H. placei</i>	ICL-MS	K--V--R--RPRGWHLPEKHVLIHNKPTSGSLFDFGLFLFHNAKVLKDKGTGPYFYLPKL	
<i>H. contortus</i>	ICL-MS	---VSVLKVRPRGWHLPEKHVLIHNKPTSGSLFDFGLFLFHNAKVLKDKGTGPYFYLPKL	
<i>N. americanus</i>	ICL-MS	-----KVRPRGWHLPEKHVLIHNKATSGSLFDFGLFIYHNAKALLDKGSGPYFYLPKL	
<i>C. elegans</i>	ICL-MS	K--V--R---PRGWHLPEKHVLIHNQPTSGSLFDFGLFVFHNAKALIAQGSGPYFYLPKL	
<i>D. viviparus</i>	ICL-MS	K--V--R---PRGWHLPEKHVLIHNQPTSGSLFDFGLFVFHNAKALIAQGSGPYFYLPKL	
<i>C. briggsae</i>	ICL-MS	-K--V----RPRGWHLPEKHVLIHNQPTSGSLFDFGLFVFHNAKALIAQGSGPYFYLPKL	
<i>S. stercoralis</i>	ICL-MS	-----	
<i>T. canis</i>	ICL-MS	REATDVLNVRPRGWHLPEKHVLVNGQPMGALFDFGLFLFHNAKALLDKGSGPYFYLPKL	

780

		Δ	$\Delta\Delta$
<i>Tc</i>	ICL-MS	QNAEEAKLWAEVFAYSEDRLDLPRGTIKCTVLIIEHLLATFQMNEIIYALKDHIIVGLNCGR	
<i>A. ceylanicum</i>	ICL-MS	QSAEEAKLWAEVFQYTEERLDLPKGTIKCTVLIIEHLLASFQMNEIIYALKDYIVGLNCGR	
<i>A. caninum</i>	ICL-MS	QSAEEAKLWAEVFQYTEQQNLNLPKGTIKCTVLIIEHLLASFQMNEIIYELKDYIVGLNCGR	
<i>H. placei</i>	ICL-MS	QNAEEAQLWADVFDYAEHTLRPQGTIKCTVLIIEHLLASFQMNEIIYALKDYIVGLNCGR	
<i>H. contortus</i>	ICL-MS	QNAEEAQLWADVFDYAEHTLRPQGTIKCTVLIIEHLLASFQMNEIIYALKDYIVGLNCGR	
<i>N. americanus</i>	ICL-MS	QSAEEAKLWAEVFQYAEQRLELPKGTIKCTVLIIEHLLASFQMNEIIYALKDYIVGLNCGR	
<i>C. elegans</i>	ICL-MS	QSAEEAQLWADVFKYTEDKLGLARGTIKCTVLIIEHLLASFQLHEIIHALKDNIVGLNCGR	
<i>D. viviparus</i>	ICL-MS	QSAEEAQLWADVFKYTEDKLGLARGTIKCTVLIIEHLLASFQLHEIIHALKDNIVGLNCGR	
<i>C. briggsae</i>	ICL-MS	QSAEEAQLWADVFKYTEEKLGARGTIKCTVLIIEHLLASFQLHEIIHALKDNIVGLNCGR	
<i>S. stercoralis</i>	ICL-MS	-----	
<i>T. canis</i>	ICL-MS	QNAEEAKLWADVFAFAEQSVGIPRGSIKCTVLIIEHLLATFQ-----G-----	

840

		$\Delta\Delta$	Δ	
	TcICL-MS	RDYIFSYIKTFQNHRKFLLPDRFQIGMTSPFMRAYSLLCIKTCHQRGIHAMGGMAAQIPI		
<i>A. ceylanicum</i>	ICL-MS	WDYIFSYIKTFQNHRKFLLPDRFQIGMTSPFMRAYSLLCIKTCHQRGIHAMGGMAAQIPI		
<i>A. caninum</i>	ICL-MS	WDYIFSYIKTFQNHRKFLLPDRFQIGMTSPFMRAYSLLCIKTCHQRGIHAMGGMAAQIPI		
<i>H. placei</i>	ICL-MS	WDYIFSYIKTFQNHRKYLPLDRFQIGMTSPFMRAYSLLCIKTCHQRGIHAMGGMAAQIPI		
<i>H. contortus</i>	ICL-MS	WDYIFSYIKTFQNHRKYLPLDRFQIGMTSPFMRAYSLLCIKTCHQRGIHAMGGMAAQIPI		
<i>N. americanus</i>	ICL-MS	WDYIFSYIKTFQNHRRLPLDRFQIGMTSPFMRAYSLLCIKTCHLRGIHAMGGMAAQIPI		
<i>C. elegans</i>	ICL-MS	WDYIFSYIKTFQNHRKFLLPDRFQIGMTAPFMRNYSLEVIKACHLRGIHAMGGMAAQIPI		
<i>D. viviparus</i>	ICL-MS	WDYIFSYIKTFQNHRKFLLPDRFQIGMTAPFMRNYSLEVIKACHLRGIHAMGGMAAQIPI		
<i>C. briggsae</i>	ICL-MS	WDYIFSYIKTFQSHRKFLLPDRFQIGMTAPFMRNYSLEVIKACHQRGIHAMGGMAAQIPI		
<i>S. stercoralis</i>	ICL-MS	-----		
<i>T. canis</i>	ICL-MS	-----MTSPFLRAYTLSSIKVCHSRNIAAMGGMAAQIPI		

900

Δ

	TcICL-MS	KNDD-----V-----ANSKALALVHQDKEREATDGHGDTWVAHPGLVPI
<i>A. ceylanicum</i>	ICL-MS	KNDEV-----
<i>A. caninum</i>	ICL-MS	KNDERWQVCRYGNAIFYHMSREQCEVANSKALS LVRQDKEREATDGHGDTWVAHPGLVSI
<i>H. placei</i>	ICL-MS	KNDE----V-----ANSKALALVHQDKDREVIDGHGDTWVAHPGLVQI
<i>H. contortus</i>	ICL-MS	KND-----EVANSKALALVHQDKDREVIDGHGDTWVAHPGLVQI
<i>N. americanus</i>	ICL-MS	KNDE-----V-----ANGKALALVRQDKEREATDGHGDTWVAHPGLVHV
<i>C. elegans</i>	ICL-MS	KHDQ----V-----ANDKAFALVRADKEREATDGHGDTWVAHPGLVPL
<i>D. viviparus</i>	ICL-MS	KHDQ----V-----ANDKAFALVRADKEREATDGHGDTWVAHPGLVPL
<i>C. briggsae</i>	ICL-MS	KHDP----A-----ANDKAFALVRADKEREVT DGHGDTWVAHPGLVPL
<i>S. stercoralis</i>	ICL-MS	-----
<i>T. canis</i>	ICL-MS	KHDOT-----ANRKALALVRODKEREATDGHGDTWVAHPDLVS V

	TcICL-MS	ARKVFDDCMPSANQIEKQLQSFFVTNQELTAIPEGTRTDHGFRHNISVTLGYLDLSWLRGV	960
A. ceylanicum	ICL-MS	-----	
A. caninum	ICL-MS	ARQVFDECMPTPNQLHKQLESLLTTKEDLTAIPEGTRTEAGFRHNISVTLGYLDLSWLRGV	
H. placei	ICL-MS	AKRIFDEHMPTPNQIDKQLQGFFATNQELTAIPEGTRTEHGFRHNISVTLGYLDLSWLRGV	
H. contortus	ICL-MS	AKRIFDEHMPTPNQINKQLQGFFATNQELTAIPEGTRTEHGFRHNISVTLGYLDLSWLRGV	
N. americanus	ICL-MS	ARQVFDAHMPSANQIQKYENLVTTKEDLTAIPEGTRTENGFRHNISVTLGYLDLSWLRGV	
C. elegans	ICL-MS	AKRVFDQMMPKPNQISKNLTRANCTKEDLTVIPEGTRTEAGFRHNISVTLGYLDLSWLRT	
D. viviparus	ICL-MS	AKRVFDQMMPKPNQISKNLTRANCTKEDLTVIPEGTRTEAGFRHNISVTLGYLDLSWLRT	

<i>C. briggsae</i>	ICL-MS	AKGVFDQMMPKPNQINKLLSRSPCKEDLTVIPEGTRTEAGFRHNISVTLGYLDSWLRGT
<i>S. stercoralis</i>	ICL-MS	-----
<i>T. canis</i>	ICL-MS	AREVFDGLMPSKNQIEKRLDSFSVTNADLTAIPEGTRTERGFRHNISVTLGYMDSWLRGV

			1020
		Δ Δ Δ	
<i>Tc</i>	ICL-MS	GCVPLYNLMEDAATAEISRSQWLQWLRHDARLE-----D-----GRTID	
<i>A. ceylanicum</i>	ICL-MS	-----	
<i>A. caninum</i>	ICL-MS	GCVPLYNLMEDAATAEISRAQLWQWLRHDARLE-----D-G----RTVD	
<i>H. placei</i>	ICL-MS	GCVPLYNLMEDAATAEISRSQWLQWLRHDARLE-----DG-----RTID	
<i>H. contortus</i>	ICL-MS	GCVPLYNLMEDAATAEISRSQWLQWLRHDARLE-----DGRTID	
<i>N. americanus</i>	ICL-MS	GCVPLYNLMEDAATAEISRAQLWQWLRHDARLEGGKFRKGRGQGCEWLGFDDGGIPDGRTID	
<i>C. elegans</i>	ICL-MS	GCVPLYNLMEDAATAEISRAQLWQWLHHDAAKLE-----DG-----RTID	
<i>D. viviparus</i>	ICL-MS	GCVPLYNLMEDAATAEISRAQLWQWLHHDAAKLE-----DG-----RTID	
<i>C. briggsae</i>	ICL-MS	GCVPLYNLMEDAATAEISRAQLWQWLHHDAAKLE-----DG-----RTID	
<i>S. stercoralis</i>	ICL-MS	-----	
<i>T. canis</i>	ICL-MS	GCVPLYNLMEDAATAEISRAQLWQWLHHEAAKLE-----DG-----RTID	

			1021
<i>Tc</i>	ICL-MS	AQLVKQTIAAETERRLIRAGSVVSRLPEAAELLEKFAL EEHMSDFLTLDAYDKLVSEGH	% identity
<i>A. ceylanicum</i>	ICL-MS	-----	[92%]
<i>A. caninum</i>	ICL-MS	AQLVKQTIAAETERRLIRAGSVVSRLPEAAELLEKFAL EENMSDFLTLDAYDKLVSEGH	[88%]
<i>H. placei</i>	ICL-MS	AQLVKQTIAAETERRLIRAGSVVSRLPEAAELLEKFAL EEQMSDFLTLDAYDKLVSEGH	[90%]
<i>H. contortus</i>	ICL-MS	AQLVKQTIAAETERRLIRAGSVVSRLPEAAELLEKFAL EEQMSDFLTLDAYDKLVSEGH	[89%]
<i>N. americanus</i>	ICL-MS	AQLVKQTIAAETERRLIRAGSIVSRLPEAAELLEKFAL EENMSDFLTLDAYDKLVSEGH	[87%]
<i>C. elegans</i>	ICL-MS	AGLVKQTIAAETERRLIRAGSVVNRIPEAADLLEKFVTE EKMSDFLTLDAYDRLVSEGY	[83%]
<i>D. viviparus</i>	ICL-MS	AGLVKQTIAAETERRLIRAGSVVNRIPEAADLLEKFVTE EKMSDFLTLDAYDRLVSEGY	[83%]
<i>C. briggsae</i>	ICL-MS	AGLVKQTIAAESERRLIRAGSVVNRIPEAAELLEKFVTE EKLSDFLTLDAYDKLVSEGY	[83%]
<i>S. stercoralis</i>	ICL-MS	-----	[72%]
<i>T. canis</i>	ICL-MS	VEMVRQTIAAETERRMIRAGSIVNRLPEAAELLEKFAL EPELSDFLTLDAYDRLVSEGK	[71%]

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figures

Figure 1

Figure 1

Multiple protein sequence alignment of TciICL-MS, Ancylostoma ceylanicum ICL-MS (GI: AB031935), Ancylostoma caninum ICL-MS (GI: ABU68338), Haemonchus placei ICL-MS (GI: VDO27591.1), Haemonchus contortus ICL-MS (GI: CIL915172.1), Necator americanus ICL-MS (GI: XP_013301212.1),

Caenorhabditis elegans ICL-MS (GI: NP_001256387.1), Dictyocaulus viviparus ICL-MS (GI: KJH51538.1), Caenorhabditis briggsae ICL-MS (GI: CAP39673.2), Strongyloides stercoralis ICL-MS (GI: AAF00535.1) and Toxocara canis ICL-MS (GI: KHN81706.1). Active and ligand binding sites are The amino acid residues in grey represent ICL and the rest MS. The % homology of each sequence with TciICL-MS is shown at the end of the alignment.

Figure 2

The predicted tertiary structures of the TciICL (left) and TciMS (right) monomers. Locations are shown of the C- and N-terminus in the predicted tertiary structure of TciICL (A) and TciMS (D). Superposition of the predicted tertiary structures showing the location of the active sites (mesh) within TciICL (B) and TciMS (E). The active sites of TciICL are shown in green (C) and TciMS in salmon (F) within 4Å of the superimposed 3P0X and 3S9Z active sites (mesh), respectively.

Figure 3

A: Purified recombinant TciICL on a NuPageTM 4 - 12% Bis-Tris protein gel stained with SimplyBlue safe stain. Lane 1: SeeblueTM plus2 pre-stained protein standard in kDa; Lane 2: filtered soluble bacterial lysate; Lane 3: unbound material; Lane 4: elution fraction (1 µg of purified recombinant TciICL indicated by the black arrow). B: Western blot of recombinant purified TciICL. Lane 1: SeeblueTM plus 2 pre-stained protein standard in kDa; Lane 2: 1 µg of recombinant TciICL detected by a monoclonal anti-polyhistidine-peroxidase antibody. C: Purified recombinant TciMS on a 4 - 12 % Bis-Tris protein gel stained with SimplyBlue safe stain. Lane plus2 pre-stained protein standard in kDa; Lane 2: non-induced fraction; Lane 3: induced fraction; Lane 4: filtered soluble bacterial lysate; Lane 5: solubilised inclusion bodies; Lane 6: elution fraction pre-refolding of purified recombinant TciMS; Lane 7: purified recombinant TciMS. D: Western blot of recombinant purified TciMS. Lane 1: SeeblueTM plus2 pre-stained protein standard in

kDa; Lane 2: 1 μ g of recombinant TciMS detected by a monoclonal anti-polyhistidine-peroxidase antibody.

Figure 4

Effects of pH on the activity of recombinant TciICL at 30 oC (mean $\pm$ SEM, n = 3). Enzyme activity was measured in a coupled assay in which isocitrate was converted to glyoxylate, which combined with phenyldrazine; the increase in absorption was measured spectrophotometrically at 324 nm.

Figure 5

Effects of varying the substrate concentration on the activity of recombinant TciICL at 30 °C (mean $\pm$ SEM, $n = 3$). Enzyme activity was measured in a coupled assay in which isocitrate was converted to glyoxylate, which combined with phenylhydrazine; the increase in absorption was measured spectrophotometrically at 324 nm.

Figure 6

The effects of bivalent metal ions and potential inhibitors on recombinant TciICL activity (relative activity, mean $\pm$ SEM, n = 3) at 30 oC. Enzyme activity was measured in a coupled assay in which isocitrate was converted to glyoxylate, which combined with phenyldrazine; the increase in absorption was measured spectrophotometrically at 324 505 nm.

Figure 7

Recognition of recombinant TciICL by serially diluted immune serum (IgG) (top) 507 or saliva (IgA) (bottom) (■), but not by parasite-naïve serum or saliva (●).