Characterization of a novel glycosylated glutathione transferase of Onchocerca ochengi, closest relative of the human river blindness parasite

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Abstract

Filarial nematodes possess glutathione transferases (GSTs), ubiquitous enzymes with the potential to detoxify xenobiotic and endogenous substrates, and modulate the host immune system, which may aid worm infection establishment, maintenance and survival in the host. Here we have identified and characterized a σ class glycosylated GST (OoGST1b), from the cattle-infective filarial nematode Onchocerca ochengi, which is homologous (99% amino acid identity) with an immunodominant GST and potential vaccine candidate from the human parasite, O. volvulus, (OvGST1b). Onchocerca ochengi native GSTs were purified using a two-step affinity chromatography approach, resolved by 2D and 1D SDS-PAGE and subjected to enzymic deglycosylation revealing the existence of at least four glycoforms. A combination of lectin-blotting and mass spectrometry (MS) analyses of the released N-glycans indicated that OoGST1 contained mainly oligomannose Man5GlcNAc2 structure, but also hybrid- and larger oligomannose-type glycans in a lower proportion. Furthermore, purified OoGST1 showed prostaglandin synthase activity as confirmed by Liquid Chromatography (LC)/MS following a coupled-enzyme assay. This is only the second reported and characterized glycosylated GST and our study highlights its potential role in host-parasite interactions and use in the study of human onchocerciasis.

Introduction

The cattle filarial nematode Onchocerca ochengi is a well-established model natural system for the study of human onchocerciasis, the causative agent of which is O. volvulus (Trees, 1992; Makepeace and Tanya, 2016). Onchocerciasis is a devastating, vector borne, neglected tropical disease, affecting over 15 million people, 99% of whom live in Africa. Symptoms range from severe itching and disfiguring skin conditions (for the majority of sufferers), to being the second-leading infectious cause of blindness in Africa at over 1 million afflicted with vision loss (WHO, 2018). In efforts to discover new ways to control onchocerciasis, much research has been focused on the molecules which may allow Onchocerca spp. to establish and maintain infection. One such protein family is the glutathione transferases (GSTs) which may aid worm survival through detoxification of drugs and evasion of host-derived immunological attack, and with the potential to play roles in immunomodulation (Chasseaud, 1979; Jakoby and Habig, 1980; Brophy and Barrett, 1990; Sheehan et al., 2001; Sommer et al., 2003; Hayes et al., 2005). Initial explorations of the Onchocerca spp genomes reveal a glutathione transferase (OoGST1 – Accession, nOo.2.0.1.09064) from O. ochengi (Armstrong et al., 2016), displaying 99% amino acid identity with an immunodominant GST (OvGST1b – Accession AAG44696.1) (Liebau et al., 1994; Alhassan et al., 2014) and potential vaccine candidate from the closely related human parasite O. volvulus (Graham et al., 1999). OvGST1b and its paralogous gene product OvGST1a (AAG44695.1) are exceptional within the GST superfamily in being glycosylated, having a cleavable signal peptide and N-terminal extension not found in other GSTs, though the roles of these novel features and glycosylation are yet to be fully elucidated (Sommer et al., 2003; Perbandt et al., 2008). The potential of these inherent glycans to function in, as yet undefined, roles at the host-parasite interface are of particular interest given OvGST1b is shown to have prostaglandin synthase activity and so may possess the ability to modulate the host immune response to filarial infection (Sommer et al., 2003; Perbandt et al., 2008). Furthermore, a deeper knowledge of protein glycosylation has important implications for future vaccine development and an understanding of host-parasite interactions.

Whilst most screening of parasite products for immunoreactive vaccine candidate antigens is predominantly protein focused (Diermet et al., 2018), antibody responses to glycosylated proteins demonstrates the high immunogenicity of glycan extensions, highlighting the clear rationale for a greater attention (Jaurigue and Seeberger, 2017).
Extending such focus on OvGST1b in the human-infecting O. volvulus filarial worm is however significantly limited through obvious logistical aspects in access to worm samples and necessary ethical constraints. Many onchocerciasis studies thus employ the closely related O. ochengi species in cattle as a valuable and accessible model for research into the genes and proteins which may play roles in this disease (Trees et al., 2000). Onchocerca ochengi GST homologue OoGST1 however, has yet to be isolated and studied to validate its status in terms of comparable structure, glycosylation state and enzymic activity with that of OvGST1b.

Therefore, investigations to resolve O. ochengi GSTs, characterize the enzymic activity and unravel the structure of N-glycan modifications of the homologous OoGST1 protein, are presented here to allow comparative exploration of potential roles in host-parasite interactions. Our findings reveal subtle differences in glycosylation state between OvGST1s and OoGST1.

Materials and methods

Parasite material

Onchocerca ochengi adult female gravid worms were collected from nodules in hides of infected Gudali cattle from the Ngaoundéré abattoir in the Adamawa region of Cameroon. Worm masses were dissected from collateral tissue within nodules and male worms were removed. The females were washed in PBS and separated into 2 mL cryovial tubes before freezing and storage at −80°C. Isolated female worms were transported to the UK on dry ice.

Glutathione transferase purification

Cytosolic extracts from O. ochengi were obtained by homogenization of frozen worms in an ice-cooled glass grinder in buffer containing 20 mM potassium phosphate, pH 7.0, 0.2% Triton X-100, 5 mM DTT and a cocktail of protease inhibitors (Roche, Mini-Complete, EDTA-free). Following homogenization, samples were centrifuged at 100,000 × g for 1 h at 4°C and the supernatant, termed the cytosolic fraction, was retained for purification of GSTs.

GSTs were partially purified and further resolved to isolate glycosylated forms from the cytosolic fraction in two steps; 'Step 1' employed S-hexylglutathione-affinity (S-hexylGSH-affinity) chromatography according to the adapted method of (Simons and Vander Jagt, 1977). In brief, the O. ochengi cytosolic fraction was passed at 0.5 mL min⁻¹ through Econo-columns (1.0 × 5 cm, 4 mL Bio-Rad, U.K.), containing 1 mL of S-hexylGSH-agarose (Sigma Aldrich), re-hydrated according to manufacturer's instructions and equilibrated with 20 mL of 20 mM potassium phosphate buffer pH 7.0, 50 mM NaCl (equilibration buffer). Non-S-hexylGSH-affinity proteins were washed from the column with 20 mL equilibration buffer at 0.5 mL min⁻¹. Affinity-bound proteins were eluted in 3 mL 50 mM Tris–HCl pH 8.0 buffer, containing 2 mM S-hexylGSH, and concentrated via centrifugal filtration in 10 kDa molecular weight cut off filters (Amicon Ultra-4, Millipore). GST samples were reduced to a final volume of 300 µL through three successive cycles of ten-fold dilutions/centrifugal reductions in 50 mM Tris–HCl pH 8.0 to remove proteins, free glutathione and low molecular mass substances of a native weight below 10 kDa.

The partially purified pool of GSTs obtained in ‘Step 1’ was incubated with a range of different lectins to determine optimum lectin selection for ‘Step 2’ isolation of glycosylated GST from the GST pool. Glycosylated GSTs were isolated from the partially purified pool of GSTs obtained in ‘Step 1’ via lectin-affinity chromatography using concanavalin A-agarose (Sigma Aldrich) according to the manufacturer's instructions.

Glutathione transferase enzyme activity

Establishment of GST presence within O. ochengi cytosolic extracts and S-hexylglutathione-binding protein samples was assayed via enzyme activity at 25°C over 3 min at 340 nm using 1 mM 1-chloro-2, 4-dinitrobenzene (CDNB) as standard second substrate in 100 mM potassium phosphate pH 6.5, containing 1 mM reduced glutathione in accordance with the adapted method of Habig et al. (1974). Assays were undertaken in triplicate in a Cary Varian spectrophotometer with specific activity expressed as nmol GSH/CDNB conjugated min⁻¹ mg⁻¹ protein (± standard deviation), and calculated as described by (Barrett, 1997) (see equation (1) below):

$$\Delta OD = \frac{V \times L}{\epsilon \times t} \times \frac{1}{Pr} \times \frac{S}{10^9}$$

Key to equation (1). ΔOD = change in optical density over time (t) in min; ϵ = extinction coefficient; V = total volume of assay mixture in the cuvette (mL); L = path length of the cuvette in cm; Pr = protein concentration of enzyme extract (mg mL⁻¹); S = volume of enzyme extract added to a cuvette (mL); The value of n is dependent on the extinction coefficient (ϵ): If ϵ is in cm⁻¹ M⁻¹, then n = 9, If ϵ is in M⁻¹ cm⁻¹, then n = 6, If ϵ is in cm⁻¹ M⁻¹, then n = 3

Protein concentrations were estimated via the adapted method of Bradford (Bradford, 1976) using the Sigma (UK) Bradford Reagent protocol according to the manufacturer’s instructions.

Electrophoresis

Two-dimensional gel electrophoresis (2DE)

20 µg of native purified GSTs (S-hexylglutathione-binding proteins) was resuspended into immobilized pH gradient (IPG) rehydration buffer [6 M urea, 1.5 M thiourea, 3% w/v CHAPS, 66 mM DTT, 0.5% v/v amphotolines pH 3–10 (Pharmalytes, Amersham BioSciences, UK)] to a final volume of 300 µL. In-gel passive rehydration and isoelectric focusing of IPG gel strips with protein samples was at 20°C with mineral oil overlay according to IPG strip manufacturer’s instructions (Bio-Rad, UK). Isoelectric focused strips were equilibrated, in two stages: a ‘reducing stage’ for 15 min in ‘equilibration buffer’ (50 mM Tris–HCl pH 8.8, 6 M urea, 30% glycerol, 2% SDS) containing 1% (w/v) DTT, followed by a 15 min ‘alkylating stage’ in ‘equilibration buffer’ containing 2.5% (w/v) iodoacetamide replacing 1% DTT (LaCourse et al., 2009). Gels were then fixed overnight in 40% methanol/10% acetic acid, stained in colloidal Coomassie Blue G-250 overnight and then de-stained in 1% acetic acid.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Protein samples were resolved by SDS-PAGE according to methods adapted from Laemmli (1970) on 12.5% polyacrylamide gels as previously described (LaCourse et al., 2009). Gels were Coomassie or Periodic-acid Schiff (PAS) stained (Sigma) and scanned upon a GS-800 densitometer (Bio-Rad).
Glycan structural analysis

N-glycans from OoGST were released by PNGase F and purified by gel filtration chromatography as indicated in Kozak et al., 2015. Hydrophilic Interaction Liquid Chromatography – Ultra high-performance liquid chromatography (HILIC-UHPLC) analysis was performed using a Dionex Ultimate 3000 UHPLC instrument. The conditions included using a BEH-Glycan 1.7 mm, 2.1 × 150 mm column at 40 °C, with a fluorescence detector (λdαex = 310 nm and λdαem = 370 nm). These conditions were controlled by Bruker HyStar 3.2 buffer A (50 mM ammonium formate pH 4.4) and Buffer B (acetonitrile). Sample volume for injection was 25 µL, at a ratio of 24 and 76% acetonitrile. Glucose unit (GU) values of peaks were assigned by chemoCleaver 7.2 data software with a cubic spline fit. The system standard and the GU calibration standard was a glucose homopolymer labelled with procainamide. The mass spectra were collected in a Bruker AmaZon Speed ETD electrospray mass spectrometer, performed immediately after the UHPLC fluorescence detector without splitting. Samples were scanned in maximum resolution mode, positive ion settings, MS scan + three MS/MS scans. The MS/MS scans were done on three ions in each scan sweep with a mixing time of 40 ms at a nebulizer pressure of 14.5 psi, a nitrogen flow of 10 litres min⁻¹ and using 4500 V capillary voltage.

Prostaglandin-synthase assay

Prostaglandin synthase activity was assessed via the composite method of LaCourse et al. (2012) based upon aspects adapted from the original methods of Sommer et al. (2003), Meyer and Thomas (1995) and Meyer et al. (1996), with extraction modifications based upon Schmidt et al. (2005).

Sequence analysis of O. ochengi glycosylated glutathione transferase OoGST1

Onchocerca ochengi glycosylated σ-class GST (OoGST1) amino acid sequence (accession number nOo.2.0.1.t09064) was obtained from the University of Edinburgh’s O. ochengi genome assembly v. nOo.2.0.1 hosted by WormBase ParaSite (Howe et al., 2017; Consortium, 2019). Onchocerca ochengi GST Oo_GST_Ib was aligned to highlight key residues involved in prostaglandin H₂ binding using ClustalX Version 2.1 (Thompson et al., 1997; Larkin et al., 2007) with homologues Ov_GST_Ia (AAG44965.1) and Ov_GST_Ib (AAG44966.1) from O. volvulus along with the two mammalian haematopoietic prostaglandin D synthases (PGDS), highlighted in Perbandt et al., 2008.
Structural analysis of OoGST1

Initial protein tertiary models of O. ochengi σ class GST were produced in silico using SwissModel (Arnold et al., 2006) and Phyre2 (Kelley et al., 2015) with prostaglandin D synthase of O. volvulus (Protein Data Bank (PDB) 2HNL) used as a template structure. Homology prediction was also carried out by RaptorX (Kallberg et al., 2012) to predict disordered amino acid regions and I-Tasser (Yang et al., 2015) for increased confidence before refinement of the predicted model via ModRefiner (Xu and Zhang, 2011). Final structures were modified in PyMol (DeLano, 2002).

Results

Sequence analyses and homology prediction

OoGST1 shares 99 and 96% identity to O. volvulus GST1b and 1a respectively (Fig. 1a). All three nematode GSTs are σ class, have a 25-amino acid signal peptide that is cleaved prior to maturation and possess a 25-amino acid N-terminal extension not found in any other GST to date. Sequence similarity to other σ class GSTs commences after this extension and, like OvGST1b, OoGST1 shows 32 and 35% identity to the human and rat haematopoietic prostaglandin D synthase (PGDS), respectively (accession numbers gi:30749302 and gi:6435744). Similarly, the proposed prostaglandin H2 binding pocket differs significantly between the mammalian and Onchocerca GSTs and may suggest a potentially different binding mode for OoGST1 and OvGST1b than for the rat and Homo PGDS (Perbandt et al., 2008).

There are also 6 potential N-glycosylation sites in OoGST1: one in the signal peptide (Asn5), one in the N-terminal extension (Asn7), two in the N-terminus (Asn50, Asn79), and two in the C-terminus (Asn134, Asn144) of the mature protein. Homology modelling of OoGST1 predicts, in accordance with other σ class

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Fig. 1. In silico analyses of O. ochengi σ class GST OoGST1. (A) Alignment of amino acid sequences of OoGST1 with homologues from its sister species O. volvulus, and PGDS from rat and human. Blue boxes show the regions that are predicted to form the PDH2 binding pocket across rat, human and O. volvulus σ class GST (information adapted from (Perbandt et al., 2008)). Yellow boxes highlight the predicted N-glycosylation sites in the mature Onchocerca spp. GSTs, whilst green box highlights a predicted N-glycosylation site in the cleavable signal peptide. Red boxes indicate the GSH binding regions. Global alignment was produced using ClustalX Version 2.1 (Thompson et al., 1997; Larkin et al., 2007). Accession numbers for the proteins used in the alignment are as follows; 1PD2_2_Rat_PGS – gi:6435744 (1PD2_2) from Rattus norvegicus; 1IY1_A_Homo_PGS – gi:30749302 (1IY1_A) from Homo sapiens; Ov_GST_Ia – gi:12005978 (AAG44695.1) from Onchocerca volvulus; Ov_GST_Ib – gi:12005978 (AAG44695.1) from Onchocerca volvulus; nOo.2.0.1.t09064– WormBase ParaSite (Armstrong et al., 2016). (B) This initial model produced in silico using SwissModel (Arnold et al., 2006) is based upon the alignment of the O. ochengi sequence with the Protein Databank template pdb.2HNL from the closely related Onchocerca volvulus σ GST. The dimeric protein model is shown here with the 25 disordered amino acids N-terminal extension. Blue, yellow and red are used to highlight the PDH2 binding pocket, predicted N-glycosylation sites and GSH binding sites respectively. Rotating the protein 90° shows the wide PDH2 binding pockets, revealing bound GSH (red ball and stick).
GSTs, that this enzyme forms a dimeric protein (Fig. 1b). However, each homodimer possesses a 'lock and key' mechanism, typically observed only in other GST classes (α, μ, π) and vertebrate PGDS, but not normally observed in other nematodes aside from OvGST1b previously (Inoue et al., 2003; Line et al., 2019). Additionally, although topologically similar to π class GSTs, the structural differences in the substrate binding pocket of OoGST1 causes significant conformity changes resulting in a wider, shallower cleft.

The primary structure of the unusual 25 amino acid N-terminus extension is composed of a higher percentage (68%) of disorder-promoting amino acids; Ala, Arg, Gly, Gln, Ser, Glu, Lys and Pro, a low content of hydrophobic residues (12%), and no aromatic residues. This combination of amino acids suggests this part of the protein is unable to form the well-organized hydrophobic core that makes up a structured domain and thus is predicted to be an intrinsically disordered region (IDR) (Uversky, 2013). Indeed, x-ray crystallography of the OvGST1a on which this model was based revealed that this region lacked electron density and was therefore not modelled (Perbandt et al., 2008).

**Purification and enzymic characterization of OoGST**

**Onchocerca ochengi** GST proteins from gravid, adult female worms were purified by affinity chromatography as described in Materials and Methods, and the enzymic activity carried out as shown by (Habig et al., 1974; Simons and Vander Jagt, 1977). Using CDNB as a model substrate, we found significant differences in cytosolic GST activity between whole worm extract, affinity-purified and column flow through (non-affinity) of

ences in cytosolic GST activity between whole worm extract, Using CDNB as a model substrate, we found significant differ-

GSTs are shown around the 25 kDa mark, but with different iso-

molecular mass (~35 kDa), but with spots less well-resolved than the π class isofoms. This indicates that σ class GST proteins were post-translationally modified, most likely glycosylated (see below).

Proteins were then excised from gels and identified by mass spectrometry in conjunction with MS-MS ions searches of a partially revised, publicly available O. ochengi database (Armstrong et al., 2016). Proteins identified were almost exclusively of the π and σ classes of GSTs (Table 1 and Supplementary file 1). As expected, we also identified bovine proteins, including a pi-class GST in several spots (Supplementary file 1).

**Onchocerca ochengi GSTs are N-glycosylated and mainly modified by oligomannose N-glycans**

To verify that σ class GSTs were glycosylated, as predicted by the presence of several N-glycosylation sequons (5–6) and also sug-

gested by their migration on 2DE gels, S-hexylGSH-affinity-purified GSTs were incubated with either PNGase F or Endo-H. After digestion, only σ class GSTs appeared susceptible to either enzyme, as indicated by their faster migration on a Coomassie-stained gel (Fig. 3a and b). Furthermore, the lack of PAS reactivity after PNGase F treatment suggests that these pro-

teins are specifically N-glycosylated (Fig. 3a, lanes 3 and 4). Whilst PNGase F caused a shift in migration of ~10 kDa, samples treated with Endo-H yield an extra band of ~28 kDa suggesting the possible presence of fucosylated, hybrid- or complex-type gly-

cans on these proteins (Fig. 3b, lane 2). The identity of all GST proteins, before or after deglycosylation, was confirmed by mass spectrometry (not shown).

**Onchocerca ochengi GST are mainly modified by mannosylated N-glycans**

To further determine the types of N-glycans present on O. ochengi GST, lectin-blotting was carried out using ConA for the recognition of terminal α-mannose residues (Fig. 4). As expected, in the untreated sample, ConA recognized all the σ class GSTs, which migrated ~35–40 kDa, although recognition of a band with an apparent molecular mass of ~25 kDa suggests some degradation may have occurred (Fig. 4a). Following digestion with PNGaseF, most of the ConA binding was lost.

We took advantage of ConA recognition of the O. ochengi σ class GSTs for protein purification and glycan structural analyses.
<table>
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<th>Description</th>
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<th>Score</th>
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After total O. ochengi GSTs were enriched by affinity chromatography using an S-hexylGSH column, only the glycosylated, σ class GSTs bound and were eluted off from the ConA column (Fig. 5). These samples were subsequently digested with PNGase F and the released glycans tagged with procanaimide and analysed by HILIC-liquid chromatography followed by ESI-MS and ESI-MS/MS. As shown in Fig. 6, the Man$_5$GlcNAc$_2$-Proc structure represents the main glycan species (~45%) from σ class OoGST. This was confirmed by positive-ion EIS-MS analysis, which showed the presence of abundant [M + H$^+$] and [M + H$^+$]$^2$ pseudomolecular ions at m/z 1454.8 and 727.9, respectively, corresponding to a glycan of composition Hex$_5$HexNAc$_2$-Proc (Fig. S1 and Table 2). In addition, short paucimannose structures (Man$_4$GlcNAc$_2$-Proc and Man$_3$GlcNAc$_2$-Proc), oligomannoses (Man$_{n-4}$GlcNAc$_2$-Proc) and a few hybrid-type species (e.g. Fuc$_1$Man$_3$GlcNAc$_2$-Proc and Man$_3$GlcNAc$_3$-1-Proc) were found (Table 2). The identity of all glycan species, including that of the Man$_5$GlcNAc$_2$-Proc oligosaccharide, was further corroborated by MS/MS analyses, which produced the characteristic fragment ions (Fig. 7a and b).

**OoGST1 displays prostaglandin synthase activity**

The σ class GSTs have previously been reported to synthesize prostaglandin D2 (PGD2), PGE2 and PGF2; eicosanoids that function in diverse physiological systems and pathological processes (Meyer and Thomas, 1995; Sommer et al., 2003). Using the ConA-affinity-purified O. ochengi GST fraction shown to contain only OoGST1 (Fig. 5, lane 3) we employed a coupled assay with cyclooxygenase (COX-1) and arachidonic acid which showed OoGST1 also has the ability to synthesize prostaglandins. Nano-LC/MS detected the presence of both PGD2 and PGE2 in the assay mixture with the PGD2 form being the more significantly abundant of the two eicosanoids (Fig. 8). OoGST1 appears to reflect a similar proportionality and specificity to catalyse predominantly, or only PGD2 from PGH2, in a concentration-dependent manner as described for OvGST1a (Sommer et al., 2003). The presence of PGE2, a relatively limited product observed, may, as proposed by Sommer et al. (2003) represent a by-product of rapid degradation of the highly unstable PGH2.
Discussion

The \(\sigma\) class GST of \(O. \) ochengi is of particular interest in terms of the potential involvement in host immune modulation as well as possible roles in detoxification of other endogenous host- and parasite- derived toxins. Given the presence of a signal peptide and its detection at the host-parasite interface in bovine nodule fluid (Armstrong et al., 2016), suggesting probable roles in the long-term survival of the parasite, further investigation of this GST in \(O. \) ochengi is warranted. Furthermore, the ability of GSTs

![Fig. 6. HILIC-LC separation of procainamide labelled N-glycans from \(O. \) ochengi GST1. Asterisks indicate contaminants (mainly from chitin hydrolysate ladder).](image)

Table 2. Summary of most abundant N-glycan species from OoGST1

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>GU</th>
<th>% Relative abundance</th>
<th>Detected ([M + \text{Proc} + 2\text{H}^+]^{2+})</th>
<th>Detected ([M + \text{Proc} + \text{H}^+]^{1+})</th>
<th>Theoretical ([M + \text{Proc} + \text{H}^+]^{1+})</th>
<th>Composition</th>
<th>Proposed structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>4.21</td>
<td>7.7</td>
<td>565.84</td>
<td>1130.67</td>
<td>1130.5</td>
<td>(Hex)(_3)(HexNAc)(_2)</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>17</td>
<td>4.64</td>
<td>0.7</td>
<td>637.67</td>
<td>1276.69</td>
<td>1276.6</td>
<td>(Hex)(_2)(HexNAc)(_2)Deoxyhexose)(_1)</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>18</td>
<td>4.79</td>
<td>3.2</td>
<td>ND</td>
<td>1333.71</td>
<td>1333.3</td>
<td>(Hex)(_3)(HexNAc)(_2)</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>20</td>
<td>5.03</td>
<td>19.7</td>
<td>646.87</td>
<td>1292.73</td>
<td>1292.3</td>
<td>(Hex)(_4)(HexNAc)(_2)</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>21</td>
<td>5.34</td>
<td>1.8</td>
<td>767.90</td>
<td>ND</td>
<td>1536.3</td>
<td>(Hex)(_3)(HexNAc)(_4)</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>26</td>
<td>5.99</td>
<td>45.2</td>
<td>727.89</td>
<td>1454.78</td>
<td>1454.3</td>
<td>(Hex)(_3)(HexNAc)(_2)</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>33</td>
<td>6.89</td>
<td>12.0</td>
<td>808.92</td>
<td>ND</td>
<td>1616.3</td>
<td>(Hex)(_3)(HexNAc)(_2)</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>39</td>
<td>7.81</td>
<td>6.1</td>
<td>889.83</td>
<td>ND</td>
<td>1778.3</td>
<td>(Hex)(_2)(HexNAc)(_2)</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>44</td>
<td>8.67</td>
<td>0.5</td>
<td>971.02</td>
<td>ND</td>
<td>1940.3</td>
<td>(Hex)(_3)(HexNAc)(_2)</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>46</td>
<td>9.39</td>
<td>3.1</td>
<td>1054.55</td>
<td>ND</td>
<td>2102.3</td>
<td>(Hex)(_3)(HexNAc)(_2)</td>
<td><img src="image" alt="Structure" /></td>
</tr>
</tbody>
</table>

GlcNAc; Mannose; Fucose.

Proposed structures and relative abundance of the most common glycans from OoGST1 were taken from HILIC-LC (Fig. 6), and EIS-MS (Fig. S1) and EIS-MS/MS (Fig. 7) analyses.
to detoxify endogenous and exogenous compounds, although well researched in other organisms, has not been fully explored in *O. ochengi*; that is, how any Phase II function may facilitate parasite establishment or survival.

We also show the predicted homology model between *OvGST1a* and *OoGST1* is almost identical, with both homodimers forming a dimeric functional protein. The significance of the 25-amino acid disordered region that both GSTs possess is yet to be determined. Intrinsically disordered proteins (IDPs) and proteins containing IDRs lack stable tertiary and/or secondary structures under physiological conditions, but are nevertheless fully functional and actively participate in diverse functions mediated by proteins (Dunker *et al.*, 2008; van der Lee *et al.*, 2014). These functions include cell signalling, cell regulation, molecular recognition and have also been shown to modulate immune responses (Wright and Dyson, 2015). Recent evidence has shown that parasites such as *Schistosoma mansoni*, *Plasmodium falciparum* and *Toxoplasma gondii* overexpress several predicted disordered protein families, with transcripts more abundant in life stages that are exposed to the mammalian host immune system (Feng *et al.*, 2006; Lopes *et al.*, 2013; Ruy *et al.*, 2014). Moreover, some of these families undergo disordered-to-ordered transitions in response to the external environmental conditions and have been shown to interact and bind with human immunomodulatory proteins (Lopes *et al.*, 2013).

Although not tested here, the disordered region of *OoGST1* may facilitate the way in which the GST is inserted at the host-parasite interface in the cuticular basal layer and the outer layer of the hypodermis. Interestingly, *OoGST1* is the only σ-class *O. ochengi* GST that is expressed in all parasite life stages (Armstrong *et al.*, 2016). Whilst several additional σ-class GSTs were previously detected in a shotgun proteomic analysis of the whole *O. ochengi* lifecycle, their absence in the current LC-MS study suggests they are expressed at a much lower level than *OoGST1*.

Here we have demonstrated that *OoGST1* is able to synthesize two prostaglandins; PGD2 and PGE2. In line with studies on the *O. volvulus* *OvGST1a* (Sommer *et al.*, 2003) PGD2 appears to predominate as the major isomerization product of the reaction. Research highlights the potential for prostaglandins to be involved in a variety of host-parasite interactions and roles including reproduction, inflammatory responses and immunomodulation, yet much still remains to be understood; specifically, how these mechanisms and roles may relate to GSTs in helminth parasites and their interactions with mammalian hosts (Daugschies and Joachim, 2000; Szkudlinski, 2000; Brattig *et al.*, 2006; Kubata *et al.*, 2007; Biserova *et al.*, 2011; Joachim and Ruttkowski, 2011; Sankari *et al.*, 2013; Kutyrev *et al.*, 2017; Laan *et al.*, 2017). Several reports highlight the potential involvement of eicosanoids in filarial worm infections. For example, Sommer *et al.* (2003) propose microfilariae of *O. volvulus* present in the skin of humans may employ PGD2 to avoid the cutaneous immune response in a similar way to that demonstrated by Angeli *et al.* (2001) in the *Schistosoma mansoni*-mouse model of human infection. Lui and Weller (1992) demonstrate prostanoids secreted by *Brugia malayi* inhibited host platelet aggregation, whilst production of PGD2 in the supernatant of *Dirofilaria immitis* indicated involvement in the relaxation of the aorta during canine heartworm disease. Further studies exploring the potential of Onchocerca-derived PGD2 to modulate the host immune system are currently underway in our laboratory.
Our lectin-binding and structural analyses demonstrated that OoGST1 is a glycosylated protein. The expression of glycosylated GSTs has previously only been observed in the closely related filarial nematode, *O. volvulus*. OvGST1a and OvGST1b share 96 and 99% identity, respectively, to *O. ochengi* OoGST1, although the latter has an additional potential N-glycosylation sequon in the N-terminus. A combination of HILIC chromatography and LC-EIS-MS/MS of procainamide-labelled glycans showed that OoGST1 is mainly modified by a Man$_5$GlcNAc$_2$ oligosaccharide and, in a lower proportion, a series of larger oligomannose.

Fig. 8. Detection of prostaglandin synthase activity of *O. ochengi* σ class GST via a mass spectrometry approach. A coupled assay with *O. ochengi* native σ class GST and COX-1 catalyses the conversion of arachidonic acid to the H2 form before the prostaglandin isomer is converted to either the D or E form. Nano-LC/MS analysis allowed detection of both PGE2 (A) and PGD2 (B) in the assay mixture with the PGD2 form being the more abundant of the two prostanoids (C). Dashed, boxed figures above peaks show the fragmentation ions specific to detection of PGE2 (i) and PGD2 (ii) according to the method used and described by LaCourse et al. (2012).

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structures (i.e. Manα1,3GlcNAcβ). This is similar to the glycan profile observed in glycopeptides from OvGST1 (Sommer et al., 2001). Interestingly, we found that OoGST1 also contains a fucosylated hybrid-type structure, with the fucose residue potentially linked as α(1–3) to innermost GlcNAc residue (based on PNGase F sensitivity). Previous studies have shown that N-glycans containing an α(1–3)fucoside residue are common in helminth glycoproteins, which are highly immunogenic and elicit TH2 immune responses (Faveeuw et al., 2003). It remains to be determined whether the presence of fucosylated glycans renders OoGST1 more immunogenic. Interestingly, OoGST1 N-glycans appear to render this protein more immunogenic to humans infected with *O. volvulus*, although no fucosylated glycans were detected by mass spectrometry (Sommer et al., 2001). Whilst the role of OoGST N-glycans remains to be determined, one potential function could be to increase its solubility in animal serum. Furthermore, the predominant presence of oligomannose structures could facilitate recognition by immune cell receptors with lectin domains, like the mannose receptor and DC-SIGN (Guo et al., 2004; Taylor et al., 2005).

Following this initial characterization of OoGST1, the opportunity exists for a wider range of studies upon this enzyme within the *O. ochengi* cattle experimental model system to inform studies of onchocerciasis and subsequent application to the human parasite *O. volvulus*. Indeed, cloning, expression and crystallographic studies, explorations of immunological aspects, as well as biochemical characterizations with a range of natural and model substrates, are underway with this glycosylated σ class GST of *O. ochengi*.

**Supplementary material.** The supplementary material for this article can be found at https://doi.org/10.1017/S0031182019000763.

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**Acknowledgements.** We thank Dr Richard Gardener and Dr Daniel Spencer (Ludger, Oxford) for performing the glycan analysis. We are grateful to the field team led by Dr Germanus Bah and Dr Vincent Tanya at the Institut de Recherche Agricole pour le Développement, Regional Centre of Wau, for co-ordinating the supply of worm material.

**Author contributions.** EJL, CR, BLM, AAS conceived and designed the experiments: EJL, CR, GP, ACS, JD, GM, CY, MP, SP, AR, ZS performed the experiments: EJL, CR, GP, SDA, JD, GM, CY, MP, SP, AR, BLM, AAS, ZS analysed the data: EJL, BLM, AAS contributed reagents/materials/analysis tools: EJL, CR, GP, SDA, GM, CY, MP, BLM, AAS wrote the paper.

**Financial support.** SDA and BLM were supported by the 7th Framework Programme of the European Commission (project identifier HEALTH-F3-2010-242131). ACS was supported by GlycoPar-EU FP7 Marie Curie Initial Training Network (GA. 608295) (Awarded to ACS and AAS; http://www.ec.europa.eu). GP, JD, ZS and AR were supported by LSTM MSc Degree research project funding.

**Conflict of interest.** None.

**Ethical standards.** Not applicable.

**References**


