Purification of a chymotrypsin-like enzyme present on adult *Schistosoma mansoni* worms from infected mice and its characterization as a host carboxylesterase

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SUMMARY

A serine protease-like enzyme found in detergent extracts of *Schistosoma mansoni* adult worms perfused from infected mice has been purified from mouse blood and further characterized. The enzyme is approximately 85 kDa and hydrolyses N-acetyl-DL-phenylalanine β-naphthyl-ester, a chromogenic substrate for chymotrypsin-like enzymes. The enzyme from *S. mansoni* worms appears to be antigenically and enzymatically similar to a molecule that is present in normal mouse blood and so is seemingly host-derived. The enzyme was partially purified by depleting normal mouse serum of albumin using sodium chloride and cold ethanol, followed by repeated rounds of purification by one-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis. The purified material was subjected to tandem mass spectrometry and its derived peptides found to belong to mouse carboxylesterase 1C. Its ability to hydrolyse α- or β-naphthyl acetates, which are general esterase substrates, has been confirmed. A similar carboxylesterase was purified and characterized from rat blood. Additional evidence to support identification of the enzyme as a carboxylesterase has been provided. Possible roles of the enzyme in the mouse host–parasite relationship could be to ease the passage of worms through the host’s blood vessels and/or in immune evasion.

Key words: *Schistosoma mansoni*, host-derived enzyme, mouse, carboxylesterase 1C, immune evasion.

INTRODUCTION

Schistosomes are long-lived parasitic flat worms inhabiting host vasculature. Five species out of 20 are able to infect man, establishing a chronic infection (Standley *et al.* 2012). One immune evasive strategy employed by the parasite and presumed to prolong its survival is the acquisition of host molecules. Several host molecules have been reported on the surface of *Schistosoma mansoni* adult worms, including blood group antigens (Smithers *et al.* 1969), major histocompatibility complex antigens (Simpson *et al.* 1986), immunoglobulins (Kemp *et al.* 1976), host lipids (Furlong *et al.* 1992), lipoproteins (Dinguirard and Yoshino, 2006), α-2-macroglobulin (Damian *et al.* 1973), contrapsin (Modha *et al.* 1988) and components of the complement system (Skelly, 2004).

A relationship between a host-derived chymotrypsin-like serine protease present in detergent extracts of *S. mansoni* worms that had been perfused from infected mice and also in mouse blood was previously reported by Darani and Doenhoff (2008). The enzyme was found in a detergent (deoxycholic acid – DOC) extract of worms with relatively little being found in non-detergent extracts and it was seemingly identical antigenically and enzymatically to the molecule in mouse serum. The antigen in the worm extract and normal mouse serum were both immunoprecipitated in immunoelectrophoresis by a rabbit antiserum raised against whole mouse serum [anti-normal mouse serum (anti-NMS)] (Darani and Doenhoff, 2008). The enzyme activity was visualized using N-acetyl-DL-phenylalanine β-naphthyl-ester (NAPBNE), a chromogenic substrate of chymotrypsin-like enzymes. The molecule in both the DOC worm extracts and NMS is of interest because it is unusual for an enzymatically active form of a protease to be present in blood – most are there as inactive pro-enzymes or in zymogen form. Furthermore, while the active enzyme has been found in mouse and rat blood, it was not found in the blood of several other mammalian species including sheep, cattle and humans (Darani and Doenhoff, 2008).

This study reports the purification and characterization of the mouse plasma-derived chymotrypsin-like enzyme, which we believe to be identical to that found on the surface of *S. mansoni* worms recovered from the infected mice. Mass spectrometry (MS) helped determine the amino acid sequence of the enzyme which in turn enabled it to be identified.
and further characterized. Information so derived gave insights into its possible role in the host–parasite relationship.

MATERIALS AND METHODS

All chemicals and buffers were of analytical grade and bought from Sigma-Aldrich Company limited, UK, except when otherwise stated. These included absolute ethanol, sodium acetate, the salt constituents of phosphate buffered saline, Tris, triton X-100, NAPBNE, fast blue B salt (FBB), β-naphthyl acetate, α-naphthyl acetate, fast red TR, dimethyl sulfoxide (DMSO) and glycine.

Parasite extract and antigens

A Puerto Rican isolate of *S. mansoni* was routinely used for infection of mice for experimental work and production of adult worm antigens. The isolate has been maintained by continuous passage in random-bred mice and *Biomphalaria glabrata* snails, the intermediate host for the generation of cercaria. Adult schistosomes were recovered by portal perfusion of infected mice 42 days after infection, as described originally by Smithers and Terry (1965) and modified by Doenhoff et al. (1978).

Detergent extracts of *S. mansoni* worms (WM) were prepared by re-suspending freshly perfused, gravity-sedimented worms which had had all visible traces of erythrocytes removed from the suspending fluid, in twice their volume of 2% DOC (detergent) solution in isotonic saline as previously described by Doenhoff et al. (1988). The suspension was gently agitated for 4 h at room temperature and then centrifuged. The supernatant was removed and stored at −80 °C until used for rabbit immunization or in immunoprecipitation studies.

*NMS and normal rat serum (NRS)*

NMS and NRS were prepared by exsanguinating healthy uninfected animals of the respective species. Collected blood was put into universal tubes and stored in a fridge at 4 °C for 4 h to clot. The blood clots were ringed with a pipette to aid separation of the clot from serum and the serum removed, centrifuged at 2500 × g for 6 min at 4 °C and the clear supernatant removed and stored at −80 °C until use.

*Rabbit anti-NMS*

A polyclonal rabbit anti-NMS antiserum was prepared as described by Darani and Doenhoff (2008) by repeated weekly injections of 1 mL emulsion containing equal volumes of normal mouse serum and Freund’s adjuvant. The response was assessed qualitatively in terms of the intensity of immuno-precipitation lines yielded by the serum and the homologous antigen extract. The rabbit was serially bled weekly from alternate ears until enough serum was collected. The serum pool was divided into 5 mL aliquots and stored at −20 °C.

*Rabbit anti-complete Freund’s adjuvant (anti-CFA)*

Rabbit anti-CFA was prepared as described for anti-NMS except that rabbits were injected only with repeated weekly doses of 1 mL emulsion of CFA.

*Rabbit anti-mouse serum albumin (anti-MSA)*

A rabbit anti-MSA antiserum was raised by immunization with replicate immunoprecipitin arcs produced by immunoelectrophoresis of mouse serum albumin and anti-NMS as described by Goudie et al. (1966), adapted as in Dunne et al. (1986). Immunoprecipitin arcs were excised, homogenized and injected into rabbits at weekly intervals. Antibody responses of Immunized rabbits were monitored and finally bled as described for the rabbit anti-NMS.

*Rabbit anti-worm protease antiserum*

A rabbit antiserum specific for the chymotrypsin-like enzyme in NMS was prepared as described by Darani and Doenhoff (2008).

Reduction of albumin in NMS and NRS

Serum albumin was depleted from both NMS and NRS as described by Colantonio et al. (2005). NaCl was added to a known volume of each serum in a micro-tube to give a final concentration of 0·1 M and the mixture incubated with gentle rotation for 60 min at 4 °C. Cold ethanol was added to the mixture to yield a final concentration of 42% v/v and incubated for a further 60 min at 4 °C. The mixture was centrifuged at 16 000 × g for 45 min at 4 °C and the resultant supernatant transferred into a sterile micro tube ‘B’ for further processing while retaining the pellet precipitate in the first tube ‘A’.

The pH of the supernatant was lowered to 5·7 by adjusting with cold 0·8 M sodium acetate, pH 4·0 and incubated at 4 °C for 60 min. The mixture was centrifuged as above and supernatant containing mainly albumin transferred into a sterile micro tube ‘C’ while retaining the pelleted precipitate. The first and second precipitates were separately reconstituted with 0·1 M PBS, pH 7·4 (w/v) and stored at −20 °C. The protein concentration in NMS was 23·70 mg mL−1, while concentrations in solutions of the two precipitates A and B and the supernatant were 5·50, 5·90 and 10·90 mg mL−1, respectively.

Immunochemistry for the detection and purification of the chymotrypsin-like enzyme

Single radial immunodiffusion (RID) (Mancini et al. 1965) was carried out as described by Darani et al.
Primary incubation of the blocked adult worms was done in a 1:40 dilution of a rabbit antiseraum raised against the host-derived protease/CES) in 1 mL PBST and incubated overnight at 4 °C. Worms were washed three times in PBST as above and incubated in 1 mL 1:80 diluted FITC-labelled secondary goat anti-rabbit IgG (Abcam, Cambridge, UK) in PBST for 30 min at room temperature in the dark. The labelled worms were washed three times in PBST and examined with the aid of a GX fluorescence microscope (GMXL3201LED) using the 10 × objective lens under blue light attached to a GXCAMFLUOMAX camera (GT Vision Ltd www.gxoptical.com).

**MS analysis of purified enzymes**

Analyses of purified gel bands were carried out using tandem MS (Papayannopoulos, 1995; Steen and Mann, 2004). Purified protein was digested by trypsin and fragmented peptides ionized and accelerated in a mass analyzer where ion fragments were separated on the basis of mass-to-charge to produce spectra. Data from the resulting mass spectra were searched using the MASCOT software for peptide matching and protein identification. Amino acid sequence searches used the protein basic local alignment search tool (pBLAST) at the National Centre for Biotechnology Information (NCBI) against the non-redundant protein sequences database (nr) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify homologous proteins, while the protein sequence alignment was achieved using ClustalW software (http://www.ebi.ac.uk/Tools/msa/clustalw/).

**Detection of chymotrypsin-like enzyme activity**

Zymography for the detection of chymotrypsin-like enzyme activity in agarose films in RID and SDS-PAGE gels was performed as described by Pearse (1968), adapted as in Darani and Doenhoff (2008), using 5 mg NAPBNE as the chromogenic substrate and 5 mg FBB as the coupling agent. The substrate mixture was dissolved in 2 mL DMSO and diluted to 40 mL by adding 10 mL, 0·1 M PBS solution, pH 7·4 and 30 mL deionized water. Prior to zymography in SDS-PAGE, the gel was incubated for an hour in 2·5% triton X-100 solution.

**Detection of esterase activity**

Esterase activity of purified mouse and rat enzymes was assayed using two substrates: β-naphthyl acetate and α-naphthyl acetate. The chromogenic substrate solution for β-naphthyl acetate was adapted from Bahar et al. (2012), using FBB as coupling agent dissolved in DMSO and diluted in 50 mM sodium acetate buffer, pH 7·4. The substrate for α-naphthyl acetate was adapted from Duysen

(1997), on microscopic glass slides using 1% molten agarose in 0·06 M barbitone buffer, pH 8·6. The immuno-precipitate was washed in several changes of 0·9% saline to remove non-immunoprecipitated material.

Purification of the chymotrypsin-like enzyme in mouse and rat sera was achieved in one-dimensional sodium dodecyl 12% polyacrylamide gel electrophoresis (1-D SDS-PAGE) (Laemmli, 1970) as modified by Studier (1973). 18 μg of each serum (mouse or rat) were loaded into separate gels with broad wells (6·2 cm long) in replicates and electrophoresed. Bands with enzymatic activity were excised from replicate thin strips of the polyacrylamide gel, put into a 1·5 mL Eppendorf container and covered with a minimum volume of elution buffer (0·06 M Tris–HCl, 10% SDS, pH 7·0) (Beyer et al. 2008). The tube was incubated at 37 °C for 24 h, centrifuged at 14 000 × g for 30 min at the same temperature and the resultant eluate was removed. Further purification of the enzyme was achieved by re-electrophoresing the eluate in a fresh SDS-PAGE gel. The whole sequence of (i) SDS-PAGE, (ii) elution of enzyme activity from gel strips, (iii) re-electrophoresing in PAGE was repeated three times in an effort to obtain a sufficiently pure sample of the enzyme suitable for analysis by MS. PAGE gels carrying proteins for analysis in MS were stained using SimplyBlue SafeStain (Invitrogen, Carlsbad, CA).

Ouchterlony double immunodiffusion was adapted from Bailey (1996). Glass microscope slides were sterilized by spraying 70% alcohol on both sides and wiping with a sterile tissue. They were placed on a levelled table and 4 mL, 1·2% molten agarose dissolved in sodium barbitone solution (pH 8·6) was spread on the glass slide and allowed to set. Circular wells were cut in the gel and these were loaded with desired antigen solutions and antisera. The slides were incubated in a humid chamber for 16 h to allow immunoprecipitation and then washed in 0·9% saline solution for 48 h. Enzymatic activity was assayed using both chymotrypsin-like (NAPBNE+p FBB) and carboxylesterase (CES) (α-naphthyl acetate+fast red TR) substrates as described below.

**Immunofluorescence on the surface of adult worms**

Immunofluorescence on adult worms was done as described in Doenhoff et al. (1988). Briefly, adult worms perfused from infected mice were washed several times in perfusion fluid and fixed in 4% paraformaldehyde in isotonic PBS solution pH 7·4, overnight. Thereafter, the worms were washed thrice in isotonic PBS solution. The fixed worms were incubated in blocking buffer [1% BSA in PBST (PBS + 0·2% Tween-20] for an hour at room temperature and washed in PBST solution.
et al. (2011); Otto et al. (1981) using fast red TR (BDH chemicals Ltd., Poole, England), as coupling agent, dissolved in DMSO and diluted in PBS at 37 °C. A and anti-NMS in RID for the presence and concentration of albumin and the chymotrypsin-like enzyme (Fig. 1).

The presence of the chymotrypsin-like enzyme was indicated by a purplish colour after chromogenic staining using NAPBNE + FBB (Fig. 1b). The results indicated that precipitate 'A' (well 2) had relatively less albumin (Fig. 1a), while retaining more of the enzyme activity than the other fractions and it was therefore used for further purification of the enzyme by means of SDS–PAGE.

**Inhibition of enzymatic activities**

The inhibition of the purified chymotrypsin-like enzyme from the sera of mice and rats was carried out using phenylmethanesulphonyl fluoride (PMSF) (Darani and Doenhoft, 2008). The inhibition of the carboxylesterase activity of the purified enzyme from the sera of mouse and rat using bis-p-nitrophenyl phosphate (BNPP) was adapted from Xie et al. (2002). Briefly, the purified mouse and rat enzymes were electrophoresed in replicate SDS–PAGE gels. After electrophoresis, the gels were first incubated at room temperature in 2·5% triton X-100 solution for an hour (to allow the enzymes to refold), rinsed thrice in deionized water, followed by a second incubation in 0·1 M PBS solution, pH 7·4 for 10 min. Thereafter, the gels were divided into three groups with each of the groups containing three replicates of each of the purified mouse and rat enzymes. The first group was treated for 3 h by incubation in 10 mM PMSF dissolved in DMSO and diluted in PBS at 37 °C. A second was incubated at 37 °C in a solution containing 5 mM BNPP dissolved in PBS for 2 h, while the third group was incubated only in PBS under the same conditions. The reactions were stopped by washing five times in PBS and the gels immersed in chromogenic substrate mixtures for detection of enzymatic activity. A gel piece was taken from each of the three groups and incubated in three substrate solutions containing the NAPBNE (for the detection of chymotrypsin-like enzyme activity), β-naphthyl acetate and α-naphthyl acetate (both esterase substrates).

**RESULTS**

**Partial purification of the chymotrypsin-like enzyme from NMS**

Each of the three fractions from albumin-depleted mouse serum (precipitates A and B and the supernatant) was investigated using rabbit anti-MSA and anti-NMS in RID for the presence and...
sera were loaded into adjacent wells and electrophoresed in SDS–PAGE. The result of zymography to detect enzymatic activity in NMS and NRS in SDS–PAGE is shown in Fig. 3. The result revealed that the enzyme in NRS has a slightly smaller size in SDS–PAGE compared with that in NMS.

The same method that had been adopted for the purification of the enzyme in NMS was applied to NRS; i.e. albumin depletion followed by SDS–PAGE, elution from the gel and re-electrophoresing the contents of the eluate. The result is shown in Fig. 4.

The partially purified Coomassie Brilliant blue-stained band indicated by the arrow in Fig. 4b was subjected to tandem MS and derived peptides were searched in MASCOT for protein identification. Significant matches for the peptides identified by MS were given by two protein entries in the

| Peptide match       | Peptide ion score | Expect  |
|---------------------|-------------------|---------|
| MNEETASLLLRR        | 75                | 0-0001  |
| EGASEEETNLSK        | 70                | 0-00032 |
| APEEILAEK           | 52                | 0-019   |
| QKTESELLGSK         | 51                | 0-023   |
| FAPPQAEPSFVK        | 22                | 18      |
| FWANFAR             | 22                | 17      |
| TESELLGSK           | 18                | 56      |
| ISEDCILNIYSPADLTK   | 9                 | 2.8e + 02 |

Mascot Protein score: the sum of all the peptide ion scores matching a protein; Mass, predicted protein mass in Daltons (Da); Coverage, percentage of sequence covered by MS-matched peptides; Peptide Ion score, a score assigned to individual matching peptide by Mascot based on the probability of best match; Expect, frequency of chance of obtaining an equal or higher score for a peptide.

Table 1. MASCOT search output of tandem MS data from the purified ~85 kDa gel band from mouse serum. Peptides with significant scores are shown in italics.
Swiss-Prot database, namely rat carboxylesterase 1C (RCES 1C) (Table 2) and rat α1B-glycoprotein (RA1BG) (result not shown). The same proteins with identical peptide sequences are present in the NCBI database (RCES 1C: GI: 2506388 and NP: 10959·3; RA1BG: GI: 25453392 and NP: 071594·2).

The MS result for RCES 1C indicated it was marginally smaller than MCES 1C, as had been indicated by the results of zymography after PAGE (Fig. 3).

Investigation of the purified chymotrypsin-like enzyme for esterase activity

Following the indications from MS that the purified host-derived chymotrypsin-like enzyme from mouse and rat plasmas may be carboxylesterases, samples of the enzymes purified from mouse and rat sera were subjected to zymography with two esterase substrates: β-naphthyl acetate and α-naphthyl acetate and NAPBNE as a positive control. The purified enzymes from both mouse and rat were observed to hydrolyse both esterase substrates at the same position in the gel as the activity against NAPBNE, providing additional evidence for the enzyme in question to be an esterase (Fig. 5).

Inhibition of the chymotrypsin-like enzyme and esterase activities in samples purified from NMS and NRS

The enzyme activities purified respectively from mouse and rat sera and visualized by zymography with the two esterase substrates were further characterized using a carboxylesterase substrate inhibitor: BNPP and the protease inhibitor PMSF. Both inhibitors were observed to have inhibited the ability of both the purified enzymes to hydrolyse the two esterase substrates as well as the substrate of chymotrypsin-like enzymes (Fig. 6b, c, e, f, h and i), as compared with the control groups which were incubated in the absence of any of the two inhibitors (Fig. 6a, d and g).

Enzyme detection in adult worm membrane and purified CES from NMS

The purified CES was investigated in Ouchterlony double immunodiffusion to ascertain its immunological identity with an enzyme in a detergent extract of mouse-derived adult worms and the enzyme in NMS. A rabbit anti-NMS antiserum was loaded in one well while the purified CES and...
unfractionated WM or NMS were loaded in wells adjacent to each other and opposite the well containing the antiserum. A pattern of identity of precipitin lines formed between the purified CES enzyme and an antigen in WM and NMS indicated that antibodies in the anti-NMS were immunoprecipitating the same molecule in all three antigen solutions (Fig. 7).

Immunoﬂuorescent detection of the host-derived enzyme on adult worms

The presence of the enzyme on the surface of mouse-derived adult worms was investigated. An immunoﬂuorescent test was done by probing the surface of freshly perfused adult worms with rabbit antibodies raised against the chymotrypsin-like enzyme derived from a detergent extract of adult worms (WM), while control worms were probed with rabbit antibodies raised against complete Freund’s adjuvant (anti-CFA). Results showed that the surfaces of worms probed with antibodies raised against the chymotrypsin-like enzyme present in a detergent extract of adult worms were immunoﬂuorescent while control worms were not (Fig. 8).

Investigating peptide homology between mouse and rat CES 1C

The homology of the host-derived mouse CES 1C (GI: 247269929, NP: 031980·2) to other mammalian proteins was investigated by a pBlast search of its amino acid sequence on the NCBI database. The search identiﬁed RCES 1C (GI: 2506388, NP: 10959·3) with a score of 913, an E value of 00 and identity of 83%, as the mammalian protein most closely similar to that of mouse CES 1C.
A host-derived enzyme found on the surface of S. mansoni worms

A host-derived enzyme found on the surface of S. mansoni worms was identified as chymotrypsin-like enzyme (CES) using immunodiffusion and zymography techniques. The enzyme was purified from infected mouse serum using albumin depletion and further characterized using tandem mass spectrometry (MS). The sequence identity between mouse CES 1C and human CES was 71-22%, with a score of 759 and an E value of 0.000. The host-derived peptides also matched that of mouse α-1B-glycoprotein (result not shown). The host-derived enzyme was considered most likely to be carboxylesterase as the MS-derived peptides gave a lower molecular weight than that observed in rat CES 1C and human CES 1. MS-derived peptides matching each of the mouse and rat sequences were highlighted by underlining in Figure 9.

Further evidence of the identity of the chymotrypsin-like enzyme was obtained from examination of a seemingly analogous enzyme in normal rat serum (NRS) which in SDS-PAGE and zymography had a stronger staining intensity and a slightly lower molecular weight than the enzyme in mouse serum (Figure 3). Tandem MS analysis of the partially purified enzyme from rat serum revealed peptides, the sequence of which significantly matched those of rat CES 1C and RA1BG (Table 2). Interestingly, the MS-derived peptides matching the aligned amino acid sequences of the mouse and rat enzyme were present in only one or the other sequence, with the exception of two (Figure 9). A study of the properties and characteristics of the two MS-identified molecules showed similar characteristics/properties between mouse CES 1C and the chymotrypsin-like enzyme. Thus CESs, similarly to determine its identity and perhaps thus provide insight into its role(s) in the parasite-host relationship.

The enzyme in DOC extracts of the parasite (WM) was observed to be similar antigenically and enzymatically to that in NMS and little or none was present in aqueous extracts of adult worms without DOC (Darani and Doenhoff, 2008). It has been confirmed (Figure 7) that the enzyme in mouse blood was identical to that in the detergent extracts of worms and purification from NMS was necessitated because of the limited availability of worm-derived material. The enzyme was previously reported to have a molecular weight of ~70 KDa (Darani and Doenhoff, 2008), and thus quite similar to that of serum albumin. The high concentration of albumin in serum made it difficult to isolate the enzyme and methods to reduce the concentration of the former were therefore employed (Chen et al. 2005; Colantonio et al. 2005).

The method of albumin depletion resulted in fractionating NMS into two precipitates (first and second) after two successive centrifugations and a supernatant containing mainly albumin (Figure 1). The first precipitate, which had a reduced concentration of albumin, but had contained more of the enzyme, was purified further (Figure 2). Tandem MS-derived peptides showed a significant match for mouse CES 1C (Table 1), although some MS-derived peptides also matched that of mouse α-1B-glycoprotein (result not shown). The host-derived enzyme was considered most likely to be carboxylesterase as the MS-derived peptides gave a lower molecular weight than that observed in rat CES 1C and human CES 1. MS-derived peptides matching each of the mouse and rat sequences were highlighted by underlining in Figure 9.

**DISCUSSION**

The uptake of host molecules by schistosomes is considered to play a crucial role in immune evasion and enhancement of parasite survival (Smithers et al. 1969; McLaren et al. 1975; Skelly, 2004; Dingirard and Yoshino, 2006). Here, the purification and characterization of an enzyme, apparently with chymotrypsin-like activity, and present in a DOC detergent extract of S. mansoni adult worms (WM) recovered from infected mice, was undertaken to determine its identity and perhaps thus provide insight into its role(s) in the parasite-host relationship.

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to the serine protease chymotrypsin, possess a serine residue in their catalytic triad (Fig. 9) (Stoops et al. 1969; Satoh and Hosokawa, 2006).

The chromogenic substrate for chymotrypsin-like enzymes (NAPBNE) has previously been reported to be hydrolysed by rat plasma CES (Choudhury, 1974). The ability of the purified enzymes from both mouse and rat plasma to hydrolyse two esterase substrates (α- and β-naphthyl acetate) (Bahar et al. 2012), is an indication of their esterolytic capability. Moreover, the protein bands with esterolytic activity were observed at the same molecular weight as the band which hydrolysed the chymotrypsin-like substrate (NAPBNE + FBB), a result consistent with the same molecule being active on the three different substrates.

In terms of mode of action of the chymotrypsin-like enzyme and CES, both share very similar active sites in possessing a catalytic triad composed of serine, histidine and either glutamic or aspartic acid (indicated in bold and italicized font in Fig. 9) (Bahar et al. 2012; Brayer et al. 1979; Satoh and Hosokawa, 2006; Stoops et al. 1969). Moreover, the observation that the esterase and chymotrypsin-like activities were both inhibited by PMSF, a chymotrypsin (serine protease) inhibitor and BNPP, a CES inhibitor, indicated similarities of hydrolytic action of the enzymes (Fig. 6). Previous findings on the inhibition of the chymotrypsin-like enzyme and CES in NMS using PMSF and BNPP respectively, further buttress this point (Xie et al. 2002; Darani and Doenhoff, 2008).

The immunoprecipitation of the host-derived enzyme in detergent extracts of the parasite (WM), purified CES and NMS by Ouchterlony double immunodiffusion using a rabbit antiserum raised against adult worm-derived mouse chymotrypsin-like enzyme; (b) was probed with a rabbit anti-complete Freund’s adjuvant antiserum (anti-CFA).

Fig. 8. Immunofluorescence probing the surface of mouse-derived S. mansoni adult worms. (a) Worm was probed with a rabbit antiserum raised against adult worm-derived mouse chymotrypsin-like enzyme; (b) was probed with a rabbit anti-complete Freund’s adjuvant antiserum (anti-CFA).
secretion of the CES from the liver into the blood of these animals (Satoh and Hosokawa, 2006; Hosokawa, 2008). Twenty families of carboxylesterase are encoded for in the mouse genome and only one encoded by the ES-1 gene exhibits the disrupted retention signal, meaning that most CES activity in mouse serum is generated in the liver by expression of the ES-1 gene (Duysen et al. 2011).

A BLAST analysis of sequences in the NCBI database indicated the most similar homologue of mouse CES 1C was rat CES 1C and that human brain CES was the closest human CES to mouse CES 1C (results not shown). However, a human serine carboxylesterase expressed at high levels in the liver and less in lungs and heart was of particular interest as it has been shown to possess convertase activity in human alveolar lavages and to function as a lung detoxification enzyme (Munger et al. 1991). Similarly to mouse CES 1C, inhibition of the human serine carboxylesterase by PMSF and BNPP has also been reported (Munger et al. 1991).

An alignment of amino acid sequences of the mouse, rat and human CESs using ClustalW software revealed an identity of 83·06% between mouse and rat CES 1C, 65·52% identity between mouse CES 1C and human CES and 71·22% identity between the rat and human molecules, reflecting homology between all three enzymes (Fig. 9).

Fig. 9. An alignment of mouse and rat CES 1C and human CES. MS-derived peptides that match the aligned sequences of mouse and rat CES 1C in MASCOT are underlined. Residues forming the catalytic triad for CES and chymotrypsin-like enzymes are shown in bold font and italicized.
A requirement for detergent (DOC) to extract the enzyme into solution suggests it is membrane-bound in the parasite, perhaps on the outer surface. If that is so, several roles could be suggested to explain the presence of CES on the surface membrane. Firstly, mouse CES 1C could be exploited by *S. mansoni* as an immunological disguise for masking surface antigens, thereby preventing the recognition and activation by antigen-presenting cells and activation of the complement system (Furlong et al. 1992).

Alternatively or additionally, the convertase potential of CES 1C (Krishnasamy et al. 1998), could be exploited by *S. mansoni* for inactivating the complement system of the host for the purpose of immune evasion. Another possible role of the CES on the surface of *S. mansoni* could be to neutralize harmful/foreign host molecules which pose a threat to the parasite’s survival, specifically those stemming from anti-parasite immune activity. Members of the CES family from the liver microsome in the endoplasmic reticulum are known to hydrolyse and inactivate foreign substances such as toxins, although their physiological role *in vivo* is as yet unclear (Krishnasamy et al. 1998).

Mouse CES 1C has been shown to be capable of exhibiting convertase activity of lung surfactant subtypes (Genetta et al. 1988, Krishnasamy et al. 1998). *Schistosoma mansoni* could thus perhaps ‘use’ the CES 1C obtained from its mouse host’s blood to ease its passage through the host blood vessels as it may help maintain blood vessel stability by inhibiting blood vessel constriction. Mouse blood CES is known to metabolize several pharmaceutical compounds such as temocapril: an angiotensin converting enzyme inhibitor (Takai et al. 1992). Mouse CES 1C has been shown to be capable of metabolizing acquired host lipids and lipoproteins, as the parasite cannot synthesize all its needed fatty acids and steroids *ab initio* (Brouwers et al. 1997; Bahar et al. 2012). Angiotensin converting enzyme plays an important physiological role, the outcome of which is a constriction of blood vessels, thereby raising blood pressure. Consistent with this pharmacological role, the enzyme has previously been found by indirect immunofluorescence to be present on the surface of mouse lung-derived schistosomula, but not on the surface of mechanically transformed larvae (Darani and Doenho, 2008).

Mammalian CESs are known to be involved in lipid metabolism (Meyer et al. 1970, Smith et al. 1970; Holmes et al. 2010). The metabolic potential of CES could be manipulated by *S. mansoni* in hydrolysing acquired host lipids and lipoproteins, as the parasite cannot synthesise all its needed fatty acids and steroids *ab initio* (Brouwers et al. 1997; Berriman et al. 2009). These are important in the parasite’s survival as they serve a crucial role in nutrition, membrane synthesis and maintenance (Furlong, 1991, Furlong et al. 1992; Dinguiraud and Yoshino, 2006). The high intensity of immunofluorescence observed herein on mouse-derived adult worms, particularly on the female, could be directly proportional to the above metabolic roles which are crucial for their survival.

Paradoxically, none of the above possible roles of blood-borne CES enable *S. mansoni* to survive well in the rat, a host considered relatively non-permissive for this schistosome species (Cioli et al. 1977), nor apparently is a presence of such an enzyme in the blood of humans a necessity for the *S. mansoni*-permissive-ness of that host species. However, the successful characterization of a carboxylesterase seemingly present in extracts of *S. mansoni* is an addition to the list of host-derived molecules acquired by this schistosome species and is the first enzyme to be recorded to have this role. The means by which the molecule adheres to the schistosome remains to be determined. Moreover, the existence of a similar serine carboxylesterase in humans, released by alveolar macrophages, is noteworthy, but investigations to ascertain if it is exploited by the parasite in any of the ways suggested here for mouse CES 1C would of course be difficult.

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