

Expression and partial characterization of a cathepsin B-like enzyme (Sm31) and a proposed 'haemoglobinase' (Sm32) from *Schistosoma mansoni*

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Schistosoma mansoni protein Sm31 is a cysteine proteinase similar to mammalian lysosomal cathepsin B, proposed to be a key enzyme in schistosome metabolism. Protein Sm32 has been identified as a putative cysteine proteinase termed a 'haemoglobinase'. Since neither Sm31 nor Sm32 have been completely purified, some controversy of the nature of the 'true' digestive enzyme still exists. By incubating a radiolabelled cysteine-proteinase active-site-directed synthetic inhibitor with total *S. mansoni* proteins, the target of inhibition was Sm31 and not Sm32. The selectivity and irreversibility of inactivation make affinity labelling an invaluable tool for exploring key differences among closely related enzymes and also for studying proteinase

activity in a cellular environment. In order to confirm these results, we expressed the complete cDNA sequences of Sm31 and Sm32 in insect cells and analysed the recombinant gene products for proteolytic activities. Cell extracts containing *S. mansoni* cathepsin B, but not those expressing 'haemoglobinase', were demonstrated to cleave a synthetic substrate benzyloxycarbonyl-arginylarginylaminomethylcoumarin in fluorescence assays. Our findings confirm previous assertions that a cysteine proteinase resembling cathepsin B is the haemoglobinase involved in digestion of host proteins. Thus, the original proposal that Sm32 is a cysteine proteinase has not been verified, and its function remains unknown.

INTRODUCTION

Human schistosomiasis is a parasitic disease caused by the trematode *Schistosoma*, affecting well over 200 million people in tropical countries. In all life-cycle stages interacting with the intermediate and human hosts, proteolytic enzymes are believed to play critical roles (McKerrow and Doenhoff, 1988). Thus understanding the targets of proteolysis in the parasite life cycle is likely to increase our understanding of pathogenesis, and the characterization of the enzymes involved may be an important step in identifying them as potential targets for immuno- or chemo-therapy.

Early studies showed the existence of a proteolytic enzyme in the gut of the adult parasite (Timms and Bueding, 1959). The proteinase had an acidic pH optimum (Grant and Senft, 1971) and a molecular mass of 27 (Sauer and Senft, 1972) or of 32 kDa (Deelder et al., 1977). It hydrolysed haemoglobin, but not other natural blood proteins, which led to the suggestion that the proteinase was responsible for haemoglobin degradation in the schistosome digestive tract (Timms and Bueding, 1959). It was characterized further as a proteinase resembling cathepsin B via inactivation by thiol-proteinase inhibitors, but not by inhibitors specific for serine, metallo- and aspartic proteinases (Dresden and Deelder, 1979). Experiments demonstrating the incorporation of radioactivity from red blood cells labelled with [³H]leucine into schistosome proteins (Zussman et al., 1970), as well as the ingestion of red blood cells by male and female worms (Lawrence, 1973), suggested that host haemoglobin was utilized as a substrate for the nutrition of the parasite. Using partially purified material, the schistosome enzyme was shown to be capable of degrading haemoglobin and synthetic peptides containing arginine (Dresden et al., 1981). Until now, its complete

purification from the regurgitant has not been possible because of the presence of contaminating proteins with similar physical properties (Chappell and Dresden, 1986; Lindquist et al., 1986).

Interestingly, the schistosome proteinase elicits a strong immune response in infected humans and experimental animals (Senft and Maddison, 1975; Deelder et al., 1977; Senft et al., 1980; Ruppel et al., 1985). Taking advantage of the immunogenic nature of this enzyme, we used human and mouse infection sera to screen a cDNA library prepared from adult *Schistosoma mansoni* RNA. This led to the isolation of a full-length cDNA clone encoding a 31 kDa protein (Sm31), and nucleotide and deduced amino acid sequences confirmed Sm31 as the schistosome counterpart of mammalian lysosomal cathepsin B (Klinkert et al., 1989). During the course of these studies, we isolated a second cDNA sequence encoding a closely migrating protein with an apparent molecular mass of 32 kDa (Sm32). Davis et al. (1987) independently isolated a cDNA clone encoding the same protein, which they identified as a cysteine proteinase and proposed to call it a 'haemoglobinase'.

The present study was undertaken to characterize biochemically Sm31 and Sm32 and to define whether Sm32 is a proteinase and, if so, whether it is a cysteine proteinase. Using a radiolabelled synthetic inhibitor, we first demonstrated the presence of a functional cysteine-proteinase active site on native Sm31 and the lack of a site possessing cathepsin B-like specificity on Sm32. We have also expressed both proteins in recombinant forms in insect cells via the baculovirus expression vector system, providing us with the unique opportunity to examine them individually for enzymic activities. We further present evidence establishing experimentally that recombinant Sm31 has all the characteristics of a cysteine proteinase. The identification of Sm31 as a true haemoglobinolytic enzyme allows us to examine

Abbreviations used: Cbz, benzyloxycarbonyl; AMC, aminomethylcoumarin; WT, wild-type; AcNPV, *Autographa californica* nuclear polyhedrosis virus; -CHN₂, diazomethane; DMSO, dimethyl sulphoxide; NEPHGE, non-equilibrium pH-gradient electrophoresis; DTT, dithiothreitol.

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in greater detail its role in the schistosome digestive tract and provides a basis for understanding the mechanism of haemoglobin degradation.

MATERIALS AND METHODS

Materials

Benzyloxycarbonyl (Cbz)-Tyr-Ala-diazomethane (CHN_2) was a kind gift of Dr. E. Shaw (Friedrich Miescher-Institute, Basel, Switzerland). A synthetic peptide Cbz-Arg-Arg coupled to fluorogenic aminomethylcoumarin (AMC), Cbz-Arg-Arg-AMC, was purchased from Bachem. ^{14}C -labelled haemoglobin was obtained from Sigma.

Iodination of cysteine-proteinase inactivator

Cbz-Tyr-Ala- CHN_2 was iodinated by using the Iodogen method (Markwell, 1982). A 25 μl portion of the inhibitor [1 mM in 25% (v/v) dimethyl sulphoxide (DMSO)] was added to an iodogen-coated glass tube (Pierce) in the presence of 10 μl of 50 mM sodium phosphate buffer, pH 7.5, and 10 μl of Na^{125}I (200 μCi). Incubation was at 0 °C for 10 min, after which 455 μl of sodium phosphate buffer were added and the reaction stopped by removing the mixture from the tube.

Two-dimensional gel electrophoresis and autoradiography

Adult *S. mansoni* (25 pairs) were homogenized in 50 μl of sonication buffer [10 mM-Tris/HCl (pH 7.5)/5 mM MgCl_2 /DNAase and RNAase (20 μg each)] and solubilized in 7 M urea. Total *S. mansoni* proteins were incubated for 1 h at 37 °C with the labelled inhibitor at a final concentration of 10^{-7} M. Proteins were subsequently fractionated on two-dimensional gels. First-dimensional fractionation was by non-equilibrium pH-gradient electrophoresis (NEPHGE) in the presence of Ampholines with pH ranging from 3.5 to 10 in the gel system, whereby acidic and basic proteins are separated (O'Farrell et al., 1977). For fractionation in the second dimension, SDS/PAGE in 6–20% gradient gels was carried out. Thereafter, proteins were transferred to nitrocellulose membranes and probed with either anti-MS2-Sm31 or anti-Sm32 antisera; the details of their production were described previously (Klinkert et al., 1988). Subsequently the filters were subjected to autoradiography on Kodak X-OMAT film exposed at –70 °C for 2–20 h.

Degradation of radiolabelled haemoglobin

Proteolytic activity was assayed as described by Healer et al. (1991). [^{14}C]Haemoglobin was used to coat microtitre plates (Nunc) at 10000 c.p.m./well in 50 μl of 0.06 M carbonate buffer, pH 9.6, overnight. The plates were washed extensively and dried over a 2 h period. Samples (100 μl) diluted in 200 mM acetate buffer, pH 5.5, were added, and the plate was left overnight at 37 °C. The radioactivity of the supernatant fluids and that remaining in each well were measured in an LKB liquid-scintillation counter. Radioactivity released was calculated as a percentage of total radioactivity, and background radioactivity obtained by incubating buffer alone was subtracted.

Determination of proteolytic activity with a fluorogenic substrate

Enzyme assays using a fluorogenic peptide substrate, Cbz-Arg-Arg-AMC, were performed as described by Barrett (1980). The

reaction was performed by incubating 10–100 μl of insect-cell extracts in 1 ml of 0.2 M acetate buffer, pH 5.5, and 2 mM dithiothreitol (DTT). The mixture was incubated for 5 min at 37 °C, and the reaction was started by the addition of the substrate at a final concentration of 10 μM . The amount of AMC released was measured fluorimetrically (excitation wavelength at 360 nm and emission wavelength at 433 nm). The absorbance values were read off the calibration curve set up using AMC (concentrations ranging from 0.1 to 100 μM) and expressed as μmol of AMC/minute per mg of protein.

$(\text{NH}_4)_2\text{SO}_4$ fractionation

Insect-cell lysates were precipitated with increasing concentrations of 10–70% satd. ammonium sulphate in order to enrich for cathepsin B. Fractionated proteins were revealed after separation on SDS/polyacrylamide gels and staining with Coomassie Blue, immunoblot analysis using specific antisera and in direct fluorescence assays.

RESULTS

Labelling of cathepsin B with radioactive inhibitor

Chappell and Rege (1991) postulated that Sm32 is similar to a *Streptococcus pyogenes* cysteine proteinase (Tai et al., 1976), a very distant relative of papain. This observation was based on the locations of Cys²⁷ and His¹⁴⁶ or His¹⁴⁸ on Sm32, which readily align with the conserved cysteine and histidine at the catalytic regions of the *Streptococcus* proteinase. However, no further amino acid sequence similarity exists.

To give a definite conclusion on the possible relationship of Sm32 to the class of papain-like cysteine proteinases, we undertook to detect proteolytic activity which may be related to Sm32 in parasite material. We used a diazomethane, which is a highly selective inactivator of cysteine proteinases, capable of establishing stable covalent linkages at the functional active site (Rich, 1986). The active site-directed inhibitor Cbz-Tyr-Ala- CHN_2 (Green and Shaw, 1981) was iodinated before incubation with the parasite extract. Total proteins were subjected to separation by two-dimensional NEPHGE before transfer to nitrocellulose membrane. The labelling patterns of parasite proteins after Western blotting with specific antisera and subsequent autoradiography were compared (Figure 1). Two protein spots which presumably correspond to phosphorylated forms, were found to react with anti-Sm31 antiserum (Figure 1a). The immunoreactive spots were distinct from those recognized by anti-Sm32 antibodies in a duplicate blot (Figure 1b). A third filter was incubated simultaneously with both antibodies in order to verify unambiguously the positions of Sm31 and Sm32 on the blot (Figure 1c). Upon autoradiography, radiolabel coincided only with the immunoreactive spot corresponding to mature Sm31, and not to Sm32 (Figure 1d). We therefore conclude that active-site amino acid residues of the type belonging to cathepsin B do not exist on Sm32. Taken together with the fact that the Sm32 cDNA sequence shows no similarity to that of known proteinases, we propose a misidentification of Sm32 as a proteinase.

Expression of Sm31 and Sm32 in insect cells

In order to ascertain the above results, we expressed both proteins in recombinant forms in insect cells. The cloning of complete Sm31 and Sm32 coding cDNA sequences in Sf9 insect cells has been described previously [Götz et al., 1992; Felleisen et al., 1990 (respectively)]. Total proteins synthesized in infected

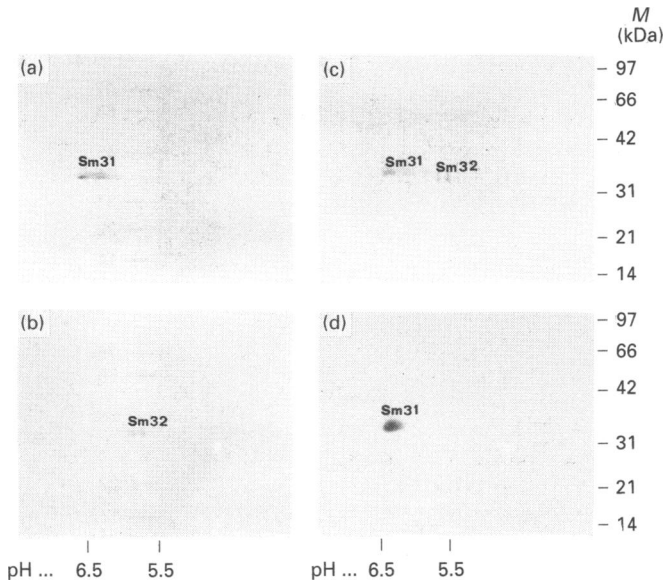


Figure 1 Immunoblots and autoradiograph of inhibitor-labelled *S. mansoni* proteins

Total parasite proteins were incubated in the presence of radioiodinated Cbz-Tyr-Ala-CHN₂ and separated by two-dimensional NEPHGE. After transfer to nitrocellulose membrane, proteins were analysed for immunoreactivity to anti-Sm31 (a), anti-Sm32 (b) and anti-Sm31 and anti-Sm32 (c) antibodies. Thereafter the filters (shown only for one) were subjected to autoradiography (d). The radiolabel consistently coincided with one of the immunoreactive spots recognized by anti-Sm31 antibodies. It was estimated that 10^{-7} M radiolabelled inhibitor was incubated with approx. 10^{-9} M cathepsin B. Abbreviation: *M*, molecular mass.

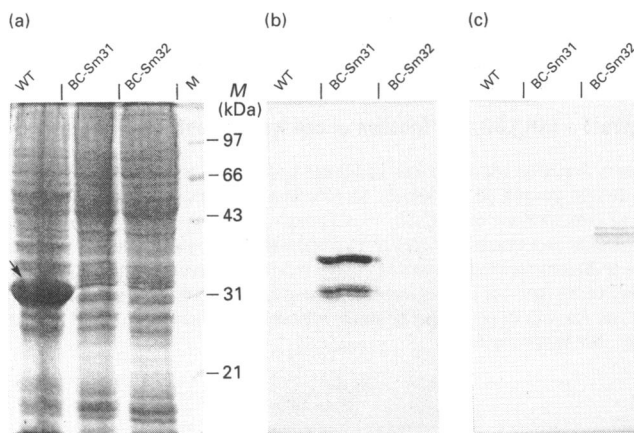


Figure 2 Analysis of total proteins synthesized by infected insect cells

(a) Coomassie Blue-stained gel showing total proteins of cells infected with WT AcNPV (WT), recombinant virus carrying Sm31 cDNA (BC-Sm31) and recombinant virus carrying Sm32 cDNA (BC-Sm32). Each sample was prepared from 0.5 ml of insect-cell cultures harvested 4 days after infection. The polyhedrin protein is present only in cells of the WT infection (arrow). Molecular-mass (*M*) markers are shown. (b) Immunoblot of a duplicate gel of (a), probed with rabbit antiserum raised against *S. mansoni* cathepsin B. The preprocathepsin B with a molecular mass of 37.5 kDa and the mature protein of 31 kDa are observed only in extracts of BC-Sm31 infected cells. (c) Immunoblot of a duplicate gel of (a) probed with rabbit antiserum raised against Sm32. Three protein bands with estimated molecular masses of 40, 41.5 and 43 kDa are recognized in extracts of BC-Sm32 infected cells.

insect cells were analysed on SDS/polyacrylamide gels stained with Coomassie Blue (Figure 2a). Polyhedrin protein (arrow) is observed only in cells infected with wild-type (WT) *Autographa californica* nuclear polyhedrosis virus (AcNPV) and not in cells

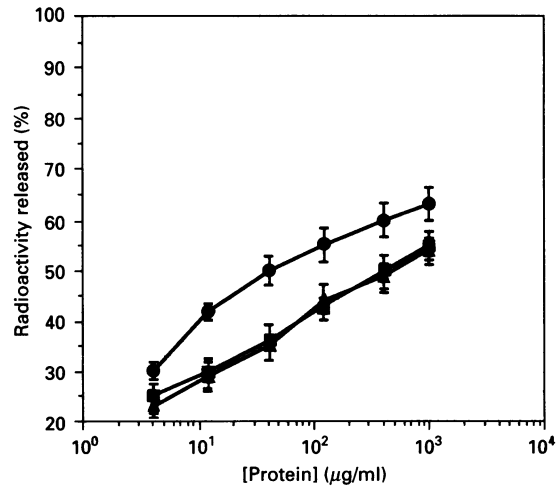


Figure 3 Degradation of haemoglobin

Insect-cell extracts were assayed in microtitre plates for their ability to degrade ¹⁴C-radiolabelled haemoglobin, monitored by the release of radioactivity into the supernatants. BC-Sm31 virus-infected cells (●) exhibited an overall higher activity than WT AcNPV (▲)- and BC-Sm32 (■)-infected cells. Each value is the mean (± 2 S.D.) for two or three experiments.

infected with recombinant viruses carrying Sm31 cDNA (BC-Sm31) or Sm32 cDNA (BC-Sm32). Since we were unable to reveal the recombinant products, the identities of insect-cell-derived Sm31 and Sm32 were confirmed by immunoblotting. Using Sm31-specific antibodies, two proteins with apparent molecular masses of 37.5 and 31 kDa were detected in cells infected with recombinant virus BC-Sm31 (Figure 2b). No reaction was found with lysates from cells of the WT or BC-Sm32 infections. The total coding capacity of Sm31 cDNA was calculated to be 38.5 kDa. Thus, like the proteins expressed in rabbit reticulocyte lysates (Felleisen and Klinkert, 1990), the larger product synthesized in insect cells is interpreted as the precursor molecule of cathepsin B and the smaller product the mature protein.

Anti-Sm32 antibodies reacted with products synthesized specifically in BC-Sm32-infected insect cells, namely three polypeptides with estimated molecular masses of 40, 41.5 and 43 kDa (Figure 2c). The triplet was previously shown to represent differentially glycosylated intermediates (Felleisen et al., 1990). In a previous experiment we obtained an *in vitro* translation product of 47 kDa (Klinkert et al., 1992) that was consistent in size with a protein inferred from the complete coding sequence of Sm32 cDNA. Thus the processing into a smaller molecule with an apparent molecular mass of 32 kDa, compatible in size with the natural product, does not appear to occur in rabbit reticulocyte lysates nor in insect cells.

Haemoglobin cleavage

Using [¹⁴C]haemoglobin we analysed insect-cell cultures expressing recombinant *S. mansoni* gene products for proteolytic activity. Cell extracts were incubated in the wells of microtitre plates coated with the radiolabelled substrate, and radioactivity released into the supernatant was measured. Although other proteinases capable of degrading haemoglobin may be present in crude homogenates, we observed significantly higher activities in extracts prepared from BC-Sm31 virus-infected insect cells as compared with WT AcNPV-infected cells and BC-Sm32-infected cells (Figure 3). This led us to conclude that the schistosomal precursor protein synthesized in insect cells undergoes processing and activation mechanisms to generate a proteolytically active

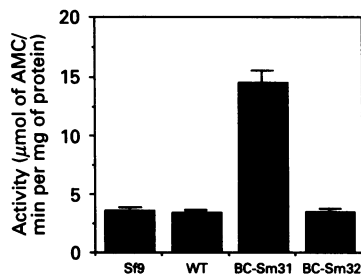


Figure 4 Comparison of extracts prepared from uninfected cells (Sf9), WT-infected cells (WT) and recombinant virus (BC-Sm31 or BC-Sm32)-infected cells for hydrolysis of synthetic substrate Cbz-Arg-Arg-AMC

Activity was measured as an increase in fluorescence and calculated from a calibration curve set up using a concentration range (0.1–100 µM) of AMC. Activity of BC-Sm31-infected cells was determined to be 14.516 ± 0.933 µmol of AMC/min per mg of protein, 4–5 times higher activity than uninfected Sf9 cells (3.562 ± 0.091 µmol/min per mg), WT-infected cells (3.375 ± 0.083 µmol/min per mg) as well as BC-Sm32-infected cells (3.425 ± 0.078). Each value is the mean (± 2 S.D.) for four experiments.

cathepsin B, in agreement with the observed 37.5 and 31 kDa bands.

Hydrolysis of Cbz-Arg-Arg-AMC as substrate

The ability of the recombinant proteins to cleave a specific dipeptide substrate was examined in fluorescence assays. Figure 4 shows the results of proteinase activity tests with extracts of uninfected and infected insect cells measured with Cbz-Arg-Arg-AMC, a synthetic substrate designed for cathepsin B. The values obtained represent the averages for extracts from four independent infections. Extracts containing recombinant cathepsin B revealed 4–5 times higher activities than control extracts prepared from uninfected or WT infected cells. In contrast with BC-Sm31-infected cells, activity measured in BC-Sm32-infected cells was not higher than the controls.

Next we investigated the effects of proteinase inhibitors on the proteolytic activity of the cell extracts using Cbz-Arg-Arg-CHN₂ as substrate. The activity of the extract measured in the absence of inhibitor was regarded as 100%. No activity was observed when cell extracts were incubated with 2 µM E64, a specific cysteine-proteinase inhibitor (0.5%). On the other hand, very little effect on enzymic activity was measured with 2 mM PMSF (serine-proteinase inhibitor) (99%), 0.1 mM pepstatin (aspartic-proteinase inhibitor) (101%) and 1 mM 1,10-phenanthroline (metalloproteinase inhibitor) (94%).

Identification of cathepsin B in (NH₄)₂SO₄ fractions

To enrich for cathepsin B, cell lysates were subjected to fractionation using increasing concentrations of (NH₄)₂SO₄. The degree of purification after the (NH₄)₂SO₄ precipitation at 60–70% saturation was estimated to be approx. 25-fold after separation of total proteins on SDS/polyacrylamide gels and staining with Coomassie Blue (Figure 5a). An analysis of the precipitated proteins in Western blotting using anti-Sm31 antibodies revealed that the preprocathepsin B of 37.5 kDa and the mature cathepsin B molecule of 31 kDa (arrows) were present in the 60–70%-satd.-(NH₄)₂SO₄ fraction. Whereas weaker reactivity was also observed in the 50–60%-satd. fraction, no signal was detected in the other fractions (Figure 5b). The precipitated fractions were also tested for proteolytic activity. Only those

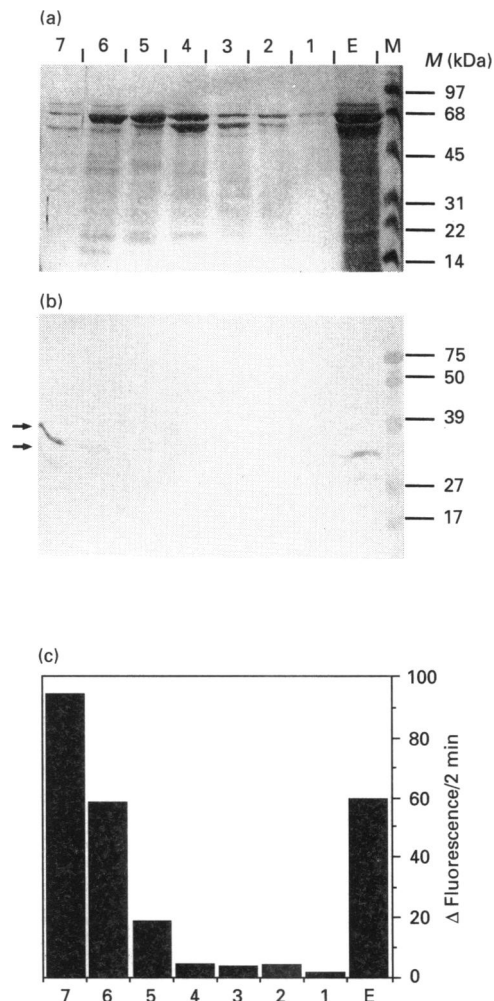


Figure 5 (NH₄)₂SO₄ fractionation of cell lysates containing cathepsin B

Proteins of a 30 ml cell culture were fractionated by (NH₄)₂SO₄ at 0–10% (1), 10–20% (2), 20–30% (3), 30–40% (4), 40–50% (5), 50–60% (6) and 60–70% (7) saturation. The starting extract before addition of (NH₄)₂SO₄ is designated E; 0.02 vol. of each fraction (a) was subjected to electrophoresis on SDS/12.5%-polyacrylamide gels and stained with Coomassie Blue or (b) immunoblotted with anti-cathepsin B antiserum. Molecular-mass (M) markers are shown on the right. The samples were also assayed for their ability to degrade Cbz-Arg-Arg-AMC (c). Fractions showing proteolytic activity with cathepsin B specificity correlated with those which were immunoreactive.

fractions with positive signals in Western-blot analysis were shown to contain cathepsin B, as monitored by their ability to degrade Cbz-Arg-Arg-AMC substrate (Figure 5c).

DISCUSSION

The widely proposed concept that the major digestive proteinase from adult *S. mansoni* worms is a thiol proteinase resembling cathepsin B has eluded experimental demonstration so far, mainly because complete purification of the enzyme from the gut has not been possible. Biochemical evidence suggested that more than one enzyme is present in the crude acidic extract of adult *S. mansoni* [reviewed by McKerrow and Doenhoff (1988)]. Thus it has never been clear whether the proteolytic activity determined in semi-purified fractions of the regurgitant was from a single or different enzymes.

Two candidate proteins Sm31 and Sm32 have been the focus of this study. We had considered it most likely that Sm31 is a

(a)

Papain	W R Q K G A V T - P V K N Q G S C G S C W A F S A V V T
Cathepsin B	W P Q C P T I K - E I R D Q G S C G S C W A F G A V E A
Sm31	W P G C K S I A - T I R D Q S R C G S C W S F G A V E A
Calpain	F I V G G A T R T D I C - Q G A L G D C W L L A A I G S
<i>Strep.</i> proteinase	E K V K P G E Q S - F V G Q A A T G H C V A T A T A Q I
Sm32	A V L V A G S N G Y P N Y R H Q A D V C H A Y H V L R S
Clostripain	T L T E X Q S V D L L A F D A C M
Poliovirus 3C	G G R Q T A K T L M Y N F P T R A G Q C G G V I T C T G

(b)

Papain	D H A V
Cathepsin B	G H A I
Sm31	G H A I
Calpain	G H A Y
<i>Strep.</i> proteinase	G H A F
Sm32	L H S H (His ¹⁴⁶)
Sm32	S H K R (His ¹⁴⁸)
Poliovirus 3C	M H V G

Figure 6 Cysteine-proteinase catalytic sites

Sequences around the (underlined) catalytically active residues (a) cysteine and (b) histidine of representative enzymes of the papain superfamily are shown. The bold letters are amino acids that are identical with those in papain. The *Streptococcus* (*Strep.*) proteinase sequence shows no sequence identity with that of papain. The sequences of Sm32, clostripain and poliovirus 3C proteinase are similarly unrelated to papain. Sources of the sequences are given in the text.

digestive enzyme, since the cDNA clone encoding the 31 kDa protein showed extensive sequence identity with mammalian cathepsin B. As it was associated with the schistosome digestive tract, Sm32 was also proposed to participate in haemoglobin degradation and was therefore termed a 'haemoglobinase' (Davis et al., 1987; El Meanawy et al., 1990). The deduced amino acid sequence of Sm32 shows no similarity to that of any known proteinases. The observation that the vast papain superfamily accommodates proteinases of very different kinds probably prompted Chappell and Rege (1991) to propose Sm32 to be a novel cysteine proteinase.

In order to examine this more closely, the sequences of *Carica papaya* (papaya) papain (Barrett et al., 1984), human lysosomal cathepsin B (Chan et al., 1986), *S. mansoni* Sm31 (Klinkert et al., 1989), chicken calpain (Ohno et al., 1984), *Streptococcus pyogenes* proteinase (Tai et al., 1976) and *S. mansoni* Sm32 (Klinkert et al., 1989; El Meanawy et al., 1990), *Clostridium histolyticum* clostripain (Gilles et al., 1983) and poliovirus 3C proteinase (Argos et al., 1984) are aligned so as to achieve maximal identity (Figure 6). Cysteine-proteinase-related enzymes found in bacteria are represented by the proteinase of *S. pyogenes* and clostripain from *C. histolyticum*. In both these enzymes the positions of the cysteine and histidine residues involved in catalytic activity are not very different from the papain-like cysteine proteinases, even though the amino acids around them exhibit virtually no sequence similarity. Poliovirus proteinase 3C is a viral cysteine proteinase responsible for cleavage of the polyprotein precursor, but is also unusual in that it reveals no similarity to known cysteine proteinases. However, despite the lack of identical amino acids, these enzymes show substrate specificities similar to papain and many of its relatives (Kortt and Liu, 1973; Siffert et al., 1976). On the other hand, the only resemblance of Sm32 to these enzymes resides in their cysteine and histidine residues. Evidence

to justify the proposal that Sm32 belongs to this class of cysteine proteinases came from the probably erroneous assertion that the β -galactosidase fusion protein was proteolytically active (Davis et al., 1987).

In the present study we used a potent inactivator directed at the active site of cathepsin B, but found that parasite-derived Sm32 was not labelled, indicating that a functional active site with cathepsin B specificity is not present in this protein. This result argues against Sm32 being a cysteine proteinase, and analysis of the deduced amino acid sequence showing lack of identity with known proteinases is consistent with this conclusion. On the other hand, we have succeeded in identifying an active form of Sm31 in parasite material without extracting and purifying the protein, and used specific antibodies to confirm its identity.

By expressing both genes in insect cells we were able to extend these findings. Our results provide the first experimental proof that Sm31 has characteristic properties of the cysteine proteinase cathepsin B, in its ability to degrade haemoglobin and specific substrates as well as in its sensitivity to naturally occurring and synthetic inhibitors. The finding that insect-cell-derived Sm32 lacks proteolytic activity does not provide conclusive evidence that the molecule is not a proteinase, as we cannot discount the possibility that, in our system, the protein is not post-translationally processed to an active form. Moreover, a larger number of substrates and inhibitors have to be tested, and it is still possible that Sm32 represents a separate class of cysteine proteinases or a novel fifth type of catalytic mechanism for proteolysis.

Overall our results support the notion that cathepsin B as represented by Sm31 is an enzyme involved in haemoglobin degradation in the schistosome digestive tract. Although important questions concerning haemoglobin degradation in the

schistosome metabolism still remain, we can now begin a thorough study of Sm31 regarding its structural and functional characteristics. One important step is to express and purify the protein in quantities allowing crystallographic studies. This will enable us to explore fundamental differences amongst known cathepsin B species with respect to their catalytic mechanisms. Additionally, these studies might be helpful in the design of selective therapeutic agents. If inhibition of cathepsin B activity can be shown to interfere with viability of the worm, then its key role in parasite metabolism will be confirmed.

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