Molecular characterization of a partial sequence encoding a novel *Schistosoma mansoni* serine protease

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SUMMARY

A PCR strategy using degenerate oligonucleotide primers based upon consensus sequences of the active site of serine proteases yielded a 467 bp fragment from genomic DNA from *Schistosoma mansoni* cercariae. The sequence presented a continuous open reading frame and the deduced amino acid sequence (156 aa) presented homologies with various serine proteases, in particular the highest percentage identity was observed with a mammalian plasma kallikrein. The expression of this serine protease was studied first at the mRNA level and it was only detected by RT-PCR in cercariae and in adult worms. At the protein level we were able to detect it by Western blotting and by using antigen extracts from metabolically radio-isotope labelled worms. The absence of any positive signal in Northern blot and the detection of the protein suggest that the mRNA has a very short half-life, however the protein may be accumulated in the parasite. The significance of identity with mammalian kallikrein was confirmed by cross-immunoreactivity with a native porcine pancreatic kallikrein. However, no cross-reactivity was observed with *S. mansoni* elastase, another serine protease. Thus, we suggest that the serine protease described in this paper is a kallikrein-like protease.

Key words: *Schistosoma mansoni*, serine protease, kallikrein-like protease.

INTRODUCTION

The life-cycle of *Schistosoma mansoni* consists of larval, intermediate and adult forms which have different morphologies. Asexual multiplication occurs in the snail intermediate host and released cercariae can penetrate the skin of man or laboratory animals. Early studies showed that this process was mediated by proteases. They facilitate penetration by digestion of host skin (Lansperger, Stirewalt & Dresden, 1982; McKerrow *et al*. 1983; Newport *et al*. 1988; Marikovsky, Arnon & Fishelson, 1990), which results in the formation of small 'tunnels' in the epidermis. Proteolytic enzymes are also involved in a wide range of biological processes such as nutrition (Senft, Goldberg & Byram, 1981; Lindquist *et al*. 1986; Chappell & Dresden, 1987) and play a key role in the survival of *S. mansoni* (McKerrow & Doenhoff, 1988). Aside from these activities, proteases are involved in immune evasion by destruction of antibody bound to the Fc receptor on the surface of schistosomula (Auriault *et al*. 1981), or by cleavage of complement, promoting the resistance of the larvae to complement-mediated killing (Marikovsky, Arnon & Fishelson, 1988). Moreover, proteases have been implicated in the modulation of the host immune response. Indeed, the role of proteases, particularly serine proteases, in the regulation of *in vitro* and *in vivo* IgE synthesis has been demonstrated (Verwaerde *et al*. 1986, 1988).

Since serine proteases play critical roles in *S. mansoni* infection and may be targets of anti-parasitic vaccines or chemotherapy, we undertook this study in order to identify novel serine protease gene fragments by using degenerate oligonucleotide primers based on the active site of these enzymes.

MATERIALS AND METHODS

Parasite

A Puerto Rican strain of *Schistosoma mansoni* was maintained in *Biomphalaria glabrata* snails and golden hamsters. Cercariae were released from infected snails and concentrated on ice. Adult worms were prepared by hepatoportal perfusion of infected hamsters 40 days after cercarial infection. They were immediately washed in MEM containing 0·02% gentalline–gentamycine (GIBCO).

PCR procedure

Primers were designed based upon serine protease consensus sequences flanking the histidine-5′ (CAC) and serine-3′ (AGT/c or TCi) of the active site (Sakanari *et al*. 1989). We used different degenerate oligonucleotides to amplify genomic DNA from *S. mansoni* cercariae, single-stranded cDNA reverse

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transcribed from adult worm RNA, extracted according to standard methods (Sambrook, Fritsch & Maniatis, 1989), or a DNA preparation from a cDNA library. The sequence of primers used were: primer 5′ (sense): **1**: 5′- TGG GTi GTi ACi GCi GCi CAC TG -3′, primers 3′ (antisense): **2**: 5′- AG iGG iCC iCC iCT ATC iCC -3′, **3**: 5′- AG iGG iCC iCC iGA ATC iCC -3′, **4**: 5′- AG iGG iCC iCC iCT GTC iCC -3′, **5**: 5′- AG iGG iCC iCC iGA GTC iCC -3′.

The cycling parameters were as follows: 94 °C for 7 min as an initial denaturing step, followed by 94 °C for 1 min, 45 °C for 1 min, 1·5 min at 72 °C for 40 cycles and a final extension at 72 °C for 10 min.

Cloning and sequencing

All PCR products were subcloned into the PCR™ II vector using the TA Cloning system (Invitrogen), and were sequenced. The sequencing reaction by the dideoxy chain termination method was performed using fluorescein labelled primers (Autoread™ Sequencing kit, Pharmacia, Sweden), and analysed on an ALF sequencer (Pharmacia).

Specific primers

We have synthesized specific primers based upon the fragment of interest (SmSP1). The sequence of primers applied were: P1 (sense): 5′ TGT ATT CAA CCA ATG CCA 3′ and P2 (antisense): 5′ CTT GGC AAG CAT CAA TTC 3′. The expected fragment size of the fragment amplified with P1 and P2 is 427 bp long.

mRNA preparations and RT-PCR

Total RNA from adult worms and cercariae was prepared using the 4 M guanidine thiocyanate lysis solution and the caesium chloride gradient purification method (Chirgwin *et al*. 1978). The mRNA was isolated by passage of total RNA through an oligo (dT)-cellulose column (mRNA Purification Kit, Pharmacia).

Single-stranded cDNA was synthesized from 5 μg of mRNA in the presence of Mu-MLV reverse transcriptase and oligo (dT) for 1 h at 37 °C. PCR experiments were then performed on 1 μl of these matrices with synthetic primers (P1 and P2) derived from the sequence of interest. Forty cycles were performed, each composed of a denaturation step for 1 min at 94 °C, primer annealing for 1 min at 50 °C and extension for 1 min at 72 °C.

Expression of recombinant protein

The sequenced PCR product, cloned into the PCR™ II vector was digested by *Sac* I and *Xho* I and was subcloned into the *Sac* I/*Sal* I-digested pQE30 vector (Qiagen) which produces a fusion protein with 6 histidines at the N-terminus. $(His)_6$-tagged protein produced in this system has the advantage of needing only 1 affinity-chromatography step for purification on a Ni-agarose column. The expression and purification of the recombinant protein were done using the QIAexpress system (Qiagen). The concentration of protein content was measured by the BCA method (Pierce, USA).

Antisera production

LOU M rats were immunized by injection (50 μg/rat) of recombinant protein as described by Vaitukaitis *et al*. (1971) in the presence of complete Freund's adjuvant. A secondary boost was performed 4 weeks later with incomplete Freund's adjuvant. The sera were collected 3 weeks later. Serum specificity was tested by Western blotting.

Western blotting

Purified recombinant protein or kallikrein from porcine pancreas (Sigma), were loaded independently on a 13% SDS–PAGE and then electrophoretically transferred onto nitrocellulose filters (Towbin, Staehelin & Gordon, 1979). The nitrocellulose was washed in PBS and pre-incubated, to block non-specific binding, in PBS containing 5% non-fat dried milk for 2 h at room temperature. Filters were incubated with the primary serum diluted in PBS containing 5% non-fat dried milk overnight at 4 °C. After 3 washes in PBS containing 0·4% Tween-20, detection with goat anti-rat IgG conjugated to peroxidase (1/500) (Sigma, USA) was done by using the HRP colour development reagent (BioRad).

Metabolic labelling and immunoprecipitation

Adult worms (2 pairs/ml) were maintained first in MEM containing 0·02% gentalline-gentamycine and 10% foetal calf serum (FCS) without methionine (GIBCO) for 1 h at 37 °C in a 5% CO_2 gassed incubator. The parasites were then incubated overnight with ^{35}S-Met (50 μCi/ml) (Amersham). After washes with MEM, adult worms (about 15 pairs) were homogenized in lysis buffer (Tris 10 mM, NaCl 0·15 M, NP40 0·5%, EDTA 1 mM, PMSF 0·5 mM, pH 7·4), sonicated using a Labsonic sonicator (B. Braun, USA) and centrifuged at 12000 ***g*** for 5 min. The supernatant fraction was recovered and the labelling was estimated after precipitation by TCA (25%) for 1 h on ice. Before immunoprecipitation, antigens were depleted on protein G–Sepharose (Sigma) with normal rat serum in TNTE buffer (Tris 5 mM, NaCl 0·15 M, NP40 0·5%, EDTA 2 mM, PMSF 0·5 mM, pH 7·4) for 2 h at 4 °C. The sample of antigens was then mixed with the

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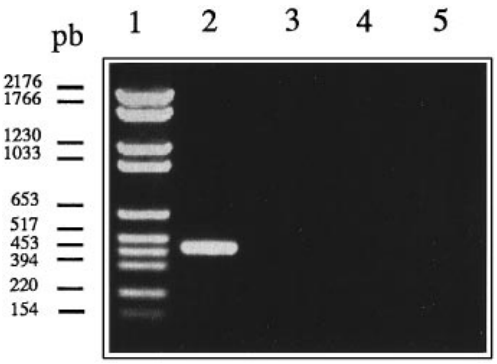

Fig. 1. PCR on genomic DNA with degenerate primers based upon consensus sequences of the active site of serine proteases. Lane 1, λ-digested by *Hin*d III- *Eco*R I (Appligen); Lane 2, primer 1 and 2; Lane 3, primer 1 alone; Lane 4, primer 2 alone; Lane 5, primers without DNA.

protein G–Sepharose pre-incubated with normal rat serum or rat antiserum directed against recombinant protein, in TNTE overnight at 4 °C. After several washes, the immune complexes were recovered from beads by boiling in sample buffer (62·5 mM Tris–HCl, pH 6·8, 2 % SDS, 10 % sucrose and 0·005 % bromophenol blue) and analysed on 15 % SDS–PAGE. After treatment with an 'Amplify' solution (Amersham), the dried gel was exposed to Kodak Biomax™ MR film.

RESULTS

Amplification of serine protease gene fragments

Genomic DNA, a cDNA library and reverse transcription products obtained from adult worm mRNA were used in PCR for amplification of serine protease genes. After several PCR rounds, a specific fragment of 467 bp with degenerate primers 1 and 2 was obtained in the presence of genomic DNA. Controls using only the 5′ and 3′ oligonucleotide primers and the two primers without DNA were negative (Fig. 1).

Identification of clones and sequencing

Following purification of the PCR product, the fragment was subcloned and sequenced. Different clones that had inserts of the right size gave identical sequences, the nucleotide and derived amino acid sequences of a clone named SmSP1 are shown in Fig. 2. The nucleotide sequence has an open reading frame (ORF) of 467 bp. This ORF encoded 156 amino acids and contained an Asp residue (D) which constitutes 1 of the 3 amino acids involved in the active site of serine proteases along with the His (5′) and the Ser (3′) residues. However, due to the fact that these two amino acids were predicted by using degenerate primers, we screened an *S. mansoni* genomic library in order to confirm the presence of the histidine and serine amino acids. This screening led us to the isolation of 2 genomic clones which hybridized with the SmSP1 fragment. Partial sequencing of these 2 genomic clones without prior amplification confirmed the nucleotide sequence of SmSP1 and the presence of the histidine and the serine residues in this serine protease. The complete gene sequence will be further characterized by sequencing of these genomic clones.

Homology with other proteins

The combined Swiss-Prot and NBRF-PIR™-protein databases (DNA Star, Madison, WI, USA) were screened for sequences related to the predicted polypeptide sequence of the SmSP1 clone. The SmSP1 clone encodes a protein sequence which is 42 % identical to the vampire bat tissue plasminogen activator from the N terminal side (positions 1–59) and 36 % identical to the human protein C precursor from the C terminal side (positions 44–99). Comparison of the amino acid sequence derived from the sequence of SmSP1 with mouse plasma kallikrein shows an overall 35 % identity (Fig. 3). It is interesting to note that the SmSP1 encoded protein has a conserved sequence NDIALL which is found in all compared serine proteases. Moreover, the comparison with another *S. mansoni* serine protease, the elastase, revealed an overall 26 % identity (data not shown).

Expression of mRNA corresponding to SmSP1 clone at different life-cycle stages

The stage-specific expression of mRNA corresponding to SmSP1 was detected by RT-PCR experiments on mRNA from cercariae and adult worms in the presence of specific primers derived from the SmSP1 nucleotide sequence. As expected, we obtained a fragment of 427 bp at both stages (Fig. 4). The sequencing of these fragments confirmed the presence of the mRNA. In order to rule out any genomic DNA contamination of the RT-PCR products, we amplified the same products used above with the primers derived from the elastase gene of *S. mansoni* which contains an intron between these two primers. The absence of amplification of this intron confirmed that the SmSP1 fragment gene was amplified from the mRNA. Controls using only the 5′ and 3′ oligonucleotide primers and the 2 primers without DNA were negative. In addition, RT-PCR control containing all components without reverse transcriptase did not give any amplification product.

Study of SmSP1 protein in S. mansoni

Fig. 5A shows SDS–PAGE of recombinant protein (rSmSP1) expressed in *E. coli*, that migrates at about 21 kDa (lane 3). The recombinant protein was

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TGGGTAATGACAGCTGCACACTGTATTCAACCAATGCCAGATCCAAAGCGATGGTTTGTA      60
 W  V  M  T  A  A  H  C  I  Q  P  M  P  D  P  K  R  W  F  V

GACGTTGGAAGATACTATAGAAACTTTGGTGGTCCTGAAGTTCAAAGAATAAAACTTTCA     120
 D  V  G  R  Y  Y  R  N  F  G  G  P  E  V  Q  R  I  K  L  S

CAAATTGTTATACACCCATCTTACAATAAAAAAATTTACGCCAATGACATAGCACTGTTA     180
 Q  I  V  I  H  P  S  Y  N  K  K  I  Y  A  N  D  I  A  L  L

CGTCTACAAACTCCAGCTAATTTAGATAATCGTCAGGTACGACTTTCTCCAGTTCCACGT     240
 R  L  Q  T  P  A  N  L  D  N  R  Q  V  R  L  S  P  V  P  R

AATCCTCATTTATCCGATTTATTAACAGATAATGTTCAGTGCATGGTCGCTGGTTGGGGA     300
 N  P  H  L  S  D  L  L  T  D  N  V  Q  C  M  V  A  G  W  G

GATACACATAATACAGGTTCAAATGATGTTCTAAGACAAGCAGTTCTTCCTGTCATTAAT     360
 D  T  H  N  T  G  S  N  D  V  L  R  Q  A  V  L  P  V  I  N

TATGATCTTTGCAAATCATGGTATCAATATCTTAATAAAGCAAGCTTTTGTGCTGGATAC     420
 Y  D  L  C  K  S  W  Y  Q  Y  L  N  K  A  S  F  C  A  G  Y

AAACAACGAGGAATTGATGCTTGCCAAGGTGATAGTGGGGGTCCTCT                  467
 K  Q  R  G  I  D  A  C  Q  G  D  S  G  G  P  L
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Fig. 2. Nucleotide and amino acid sequences from SmSP1. H, D and S which constitute the catalytic site are bolded. Sequences predicted by degenerate primers and confirmed by 2 genomic sequences are underlined with broken lines.

A

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SmSP1   WTAAHCIQPMPDPKRWFVDVGRYYRNFGGPEVQRIKLSQIVIHPSYNKKIYANDIALLRLQTP   64
        :TAAHC:: :P P  W :  G    :    E    :: :::IH  Y:      DIAL::LQTP
MpKLK   VTAAHCFDGIPYPDVWRIYGGILSLSEITKETPSSRIKELIIHQEYKVSEGNYDIALIKLQTP  492

SmSP1   ANLDNRQVRLSPVPRNPHLSDLLTDNVQCMVAGWGDTHNTG-SNDVLRQAVLPVINYDLCKSW  125
         N .: Q .:. :P.::: :.: T   :C V:GWG T.: G ::::L::A.:P:: :  C:.
MpKLK   LNYTEFQKPIC-LPSKADTNTIYT---NCWVTGWGYTKEQGETQNILQKATIPLVPNEECQKK  551

SmSP1   YRDYVINKQMICAGYKEGGTDACKGDSGGPL  582
        Y :Y: NK. :CAGYK: G.DAC:GDSGGPL
MpKLK   Y-QYL-NKASFCAGYKQRGIDACQGDSGGPL  155
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B

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SmSP1   TAAHCIQPMPDPKRWFVDVGRYYRNFGGPEVQRIKLSQIVIHPSYNKKIYANDIALL    60
        TAAHC:Q    P::  V :GR YR   G E Q : : : :IH  ::   Y NDIALL
VbtPa   TAAHCFQERYPPQHLRVVLGRTYRVKPGKEEQTFEVEKCIIHEEFDDDTYNNDIALL   324
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C

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SmSP1   HPSYNKKIYANDIALLRLQTPANLDNRQVRLSPVPRNPHLSDLLTDNVQCMVAGWG     98
        HP:Y:Y    NDIALL:L  PA L :  V :          :L     : :v:GWG
HuPC    HPNYSKSTTDNDIALLHLAQPATLSQTIVPICLPDSGLAERELNQAGQETLVTGWG    301
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Fig. 3. Alignment of the amino acid sequences encoding for *Schistosoma mansoni* (SmSP1) protein with mouse plasma kallikrein (A), vampire bat tissue plasminogen activator (B) and human protein C (C). The histidine (H), aspartic acid (D) and serine (S) which constitute the catalytic site are bolded. *MpKLK*, mouse plasma kallikrein; from aa 428 to 582. *VbtPa*, vampire bat tissue plasminogen activator; from aa 269 to 324, *HuPC*, human protein C; from aa 246 to 301.

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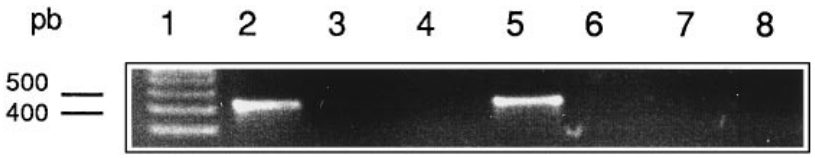

Fig. 4. Agarose gel analysis of RT-PCR products after amplification with specific primers based upon the SmSP1 fragment of RNA extracted from cercariae (Lanes 2–4) and adult worms (Lanes 6–8). In each case, PCR was performed with 5′ and 3′ primers (Lanes 2 and 5), with the 5′ primer (Lanes 3 and 6) and with the 3′ primer (Lanes 4 and 7). Lane 8, PCR control with no template; Lane 1, 100 bp DNA ladder (Pharmacia).

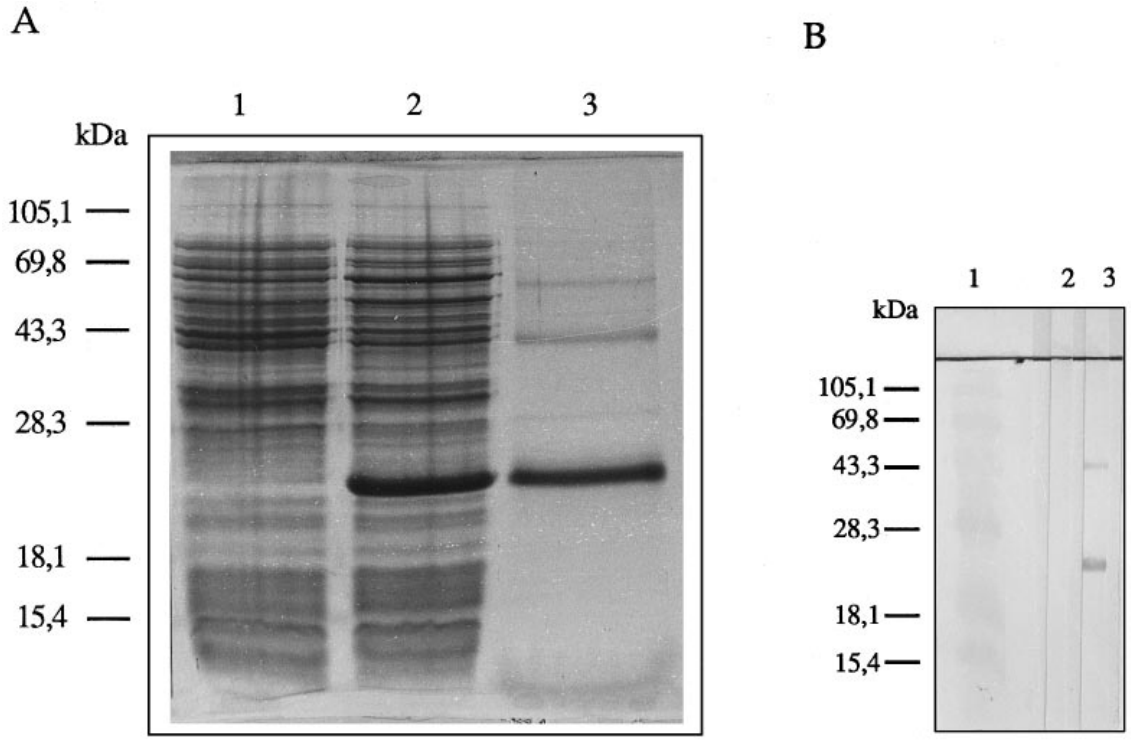

Fig. 5. (A) SDS–PAGE analysis of recombinant SmSP1 protein. Lane 1, whole extracts of non-induced bacterial culture; Lane 2, whole extracts of induced bacterial culture; Lane 3, recombinant protein after Ni column purification. (B) Western blotting of purified recombinant protein with normal rat serum (1:500) (Lane 2) and rat antiserum directed against the rSmSP1 protein (1:500) (Lane 3). Lane 1, molecular weight markers (GIBCO).

extremely pure and was used for production of antisera. The specificity of rat antiserum raised against purified recombinant protein was tested by Western blotting (Fig. 5B) which showed that the serum recognized the recombinant protein. In order to check whether the mRNA is in fact translated, we immunoprecipitated soluble antigens obtained from adult worms labelled with ^{35}S Met. Results presented in Fig. 6 showed that the antiserum directed against rSmSP1 specifically precipitated an antigen at about 30 kDa. This result was further confirmed by Western blotting (data not shown).

Immunoreactivity of porcine kallikrein with rat sera directed against rSmSP1 protein

Due to the identity of amino acid sequences between the SmSP1 protein and kallikrein, we tested whether this similarity could be reflected in cross-immunoreactivity. To address this question, we performed Western blotting on kallikrein purified from porcine pancreas with the rat antiserum directed against the rSmSP1 protein. Results presented in Fig. 7 showed that the purified kallikrein is recognized by antibodies directed against the rSmSP1, suggesting that this protein could induce cross-reactive antibodies. The presence of 2 bands might be explained either by a degradation product of the porcine kallikrein or/and by the presence of the proenzyme (higher band) and the active enzyme (lower band) which lost the pro-peptide. However, when this antiserum was tested against the recombinant elastase of *S. mansoni*, another serine protease, results did not show a positive signal (data not shown) suggesting that antibodies induced by rSmSP1 are specific for kallikrein protease.

DISCUSSION

Through the evolution of the host–parasite relationship in schistosomiasis, the parasite has developed a variety of proteolytic enzymes (McKerrow *et al.*

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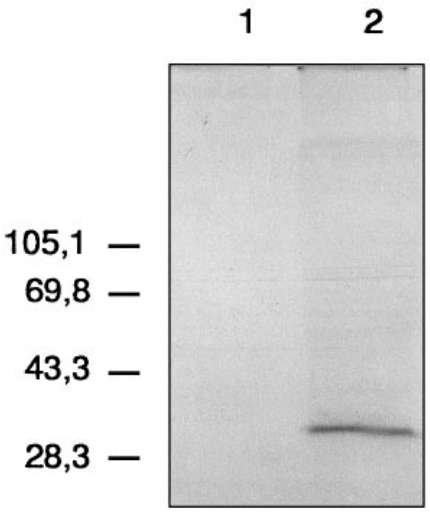

Fig. 6. Immunoprecipitation of adult worm antigens labelled with ^{35}S by normal serum (Lane 1) and rat antiserum directed against the rSmSP1 (Lane 2). Soluble extracts of labelled antigens were incubated with 20 μl of total serum. Immune complexes were adsorbed on protein G and were analysed by SDS–PAGE.

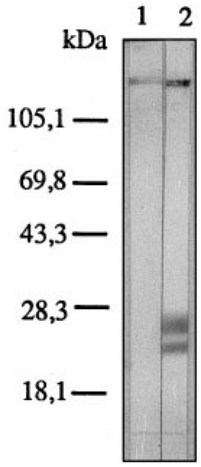

Fig. 7. Immunoblot analysis of rat antiserum anti-rSmSP1 protein against native kallikrein purified from porcine pancreas. Native kallikrein was subjected to SDS–PAGE. After transfer onto a nitrocellulose filter, membrane strips were incubated with control serum (Lane 1), or with rat anti-rSmSP1 serum (Lane 2). Rat antibodies were probed with a goat anti-rat IgG conjugated with horse-radish peroxidase.

1988) to assist in different aspects of its invasion, metabolism and exit from its host. Thus several serine proteases have been described; among them, the elastinolytic (30 kDa) preacetabular gland secretions of cercaria, soluble proteins (28 and 60 kDa) released from transforming schistosomula, 2 proteins in SRP (27·5 and 29 kDa) which are involved in the enhancement of IgE Fc receptor expression (Verwaerde *et al*. 1988), and a membrane-anchored protease (28 kDa) on schistosomula (Ghendler *et al*. 1996). In addition, several cysteine proteases have been previously described in extracts of adult worms, in eggs and in miracidia (Pino-Heiss, Brown & McKerrow, 1985; Sung & Dresden, 1986; Dalton *et al*. 1995).

In the present paper, we describe the isolation and characterization of a novel *S. mansoni* serine protease with a partial cDNA sequence which encodes a 17 kDa polypeptide. The deduced amino acid sequence shows a significant identity with the vampire bat tissue plasminogen activator, the human protein C precursor but the greatest overall similarity was to the mouse plasma kallikrein. In general, kallikrein is involved in a wide variety of essential physiological functions, among which are the processing of bioactive peptides, blood coagulation and the enhancement of glycosylation of IgE binding factors (Iwata, Munoz & Ishizaka, 1983). The role of the protein encoded by SmSP1 in the cellular function of *S. mansoni* and its location are unknown. Analysis of the amino acid sequence revealed the presence of the catalytic triad site (H, D and S) surrounded by conserved amino acids. The molecular cloning and sequencing of the gene encoding *S. mansoni* elastase showed the presence of at least 2 genes, one of them EL1 presented the catalytic triad site (H, D, S). However, the second gene (EL2) appears not to encode an active enzyme due to the absence of the aspartic acid (D) residue (Pierrot, Capron & Khalife, 1995). It was proposed by Brenner (1988) that there are 2 subclasses of serine protease; one contains a TCN codon where N = A, T, C or G and the other containing an AGY codon where Y = C or T for the active site serine protease amino acid. Sequence data showed that both the elastase (EL1) and the SmSP1 genes encoded 2 serine proteases of *S. mansoni* using the AGY codon where Y = T to code for the active site serine residue.

At the nucleotide sequence level, the alignment of SmSP1 with the EL1 sequence and other serine proteases revealed the absence of an intron in the SmSP1 sequence. However, comparisons between different serine proteases often revealed that the intron–exon junctions are conserved even when intron sizes are different. These data suggest that the gene fragment described here encodes a kallikrein-like serine protease that is structurally and may be functionally different from *S. mansoni* elastase. Moreover, Southern blot analysis with *S. mansoni* DNA digested with different restriction enzymes showed a single hybridizing fragment (data not shown) suggesting the presence of a single SmSP1 copy in this haploid genome which is not the case for mammalian species. Indeed, the analysis of mouse kallikrein genes led to the identification of a multigene family (Evans, Drinkwater & Richards, 1987), among which 14 genes are potentially able to encode functional proteins and 10 are pseudogenes. In humans, there are only 3 genes and in the rat 13 genes (McDonald, Margolius & Ergos, 1988; Muray *et al*. 1990). As would be expected, some schisto-

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somes genes show developmental regulation of expression, while others do not. In the case of *S. mansoni* elastase, we showed that cercariae and schistosomula produce more elastase than do other life-cycle stages (Pierrot *et al*. 1996). Concerning SmSP1 gene expression, we were unable to detect the corresponding mRNA by Northern blot (data not shown). However, we showed by RT-PCR that the mRNA was present at both cercarial and adult worm stages. Taken together, these results led us to check whether the native protein corresponding to the SmSP1 gene could be detected using metabolically radio-isotope labelled adult worms in the presence of ^{35}S-Met. The results of immunoprecipitation experiments performed on soluble extracts using rat serum directed against the recombinant protein of SmSP1 gene showed the presence of a single band at about 30 kDa, the expected size of serine proteases. These results suggest that the adult mRNA is in fact translated. We further confirmed these results by Western blotting using total antigen extracts from adult worms. This was not the case for *S. mansoni* elastase. Indeed, the elastase mRNA was found in the adult worms but not its protein product. These data are in favour of a stage-specific expression of serine proteases in *S. mansoni* which may be related to different physiological functions.

Sequence analysis has shown that *S. mansoni* SmSP1 is more closely related to mammalian serine protease and particularly to kallikrein. The significance of these similarities and a possible immunological cross-reactivity with known kallikrein was examined using the rat antiserum directed to the recombinant protein of SmSP1 gene. The outcome of the Western blot experiments showed the reactivity with the kallikrein from porcine pancreas confirming that we isolated a partial cDNA that encodes part of a kallikrein-like molecule in *S. mansoni*.

Currently, with the rat antiserum directed against the kallikrein-like protein and with the partial cDNA probe, we intend to isolate the full length clone from a schistosome cDNA library. By producing the recombinant protein, we will be able to elucidate its function and the role of this serine protease in host–parasite interactions.

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