Purification and properties of a proteolytic enzyme from the cercariae of the human trematode parasite *Schistosoma mansoni*

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Skin penetration by the cercarial stage of the human trematode parasite *Schistosoma mansoni* is mediated by the secretion of proteolytic enzymes able to digest components of mammalian connective tissues. In the present study the purification of these proteinases from cercarial homogenates is reported. The major proteinase species has a mol.wt. of approx. 25 000 and exists in monomeric form as determined by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. This proteinase has an isoelectric point of 6.0. Studies presented here, with a variety of substrates and inhibitors, confirm previous claims that these proteinases belong to the serine class, and, in addition, suggest that they resemble the vertebrate chymotrypsins rather than trypsins or elastases. However, the amino acid composition of the cercarial proteinase differs significantly from bovine chymotrypsin and from the human leucocyte chymotrypsin-like cathepsin G. The amino-acid-composition differences between these proteinases are consistent with their differences in isoelectric point. In order to obtain an insight into the role of the proteinase in skin penetration, its activity on cartilage proteoglycan monomers and on the isolated peptide backbone of proteoglycan was studied. The results of the present study indicate that the cercarial enzyme catalyses a limited specific digestion of the peptide core.

Infection by the human blood fluke *Schistosoma mansoni* occurs on penetration of the epithelial and dermal barriers (Lewert & Lee, 1954; Stirewalt, 1966; Stirewalt & Walters, 1973) by the cercarial stage of this organism. The penetration process appears to be mediated by proteolytic enzymes (Lewert & Lee, 1956; Stirewalt & Kruidenier, 1961; Stirewalt & Fregeau, 1966; Gazzinelli *et al.*, 1966; Dresden & Asch, 1972), which are localized in the preacetabular glands (Stirewalt, 1973; Campbell *et al.*, 1976). Both homogenates of cercariae and secretions from the preacetabular glands appear to

have a single major, and several minor, proteinase activities (Dresden & Asch, 1972; Campbell *et al.*, 1976; Baba *et al.*, 1977). Early studies showed that cercarial proteinases could hydrolyse a variety of substrates, including Azocoll, gelatin and denatured haemoglobin (Lewert & Lee, 1956; Dresden & Asch, 1972).

In order to obtain insight into their role in skin penetration, these proteinases were tested on connective-tissue proteins and were shown to be able to hydrolyse keratin and the non-collagenous components of basement-membrane protein preparations (Dresden *et al.*, 1977); in addition, they have been reported to possess elastolytic activity (Gazzinelli & Pellegrino, 1964; Dresden & Asch, 1972). Although no activity could be demonstrated against either soluble or fibrillar collagen, these enzymes were very active against cartilage proteoglycan (Dresden & Asch, 1972).

Because this proteolytic enzyme appears to play a major role in the mechanism of skin penetration and infection by the cercariae of *S. mansoni*, purification of this enzyme was performed. In addition, in order

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to obtain insight into the way in which this enzyme might function during penetration, the effect of the enzyme on proteoglycan structure was examined.

Experimental procedures

Materials

Cercariae of *Schistosoma mansoni* (Puerto Rican strain) were collected from infected *Biomphalaria glabrata* as previously described (Dresden & Asch, 1972), frozen and stored at −20°C, or freeze-dried.

The following reagents were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.): soya-bean trypsin inhibitor, N-α-Tos-Lys-CH$_2$Cl, L-TosNH-2-PhEt-CH$_2$Cl, bovine pancreatic α-chymotrypsin, and bovine pancreatic trypsin. Triton X-100 was from Beckman Instruments Co. (Fullerton, CA, U.S.A.).

Chymostatin was from Peninsula Laboratories (San Carlos, CA, U.S.A.). Bio-Rad Laboratories (Richmond, CA, U.S.A.) supplied low-molecular-weight protein standards, precast 10%-polyacrylamide/SDS gels, Bio-Beads (styrene divinylbenzene beads) and Coomassie Brilliant Blue R-250. Aquacide and Azocoll were from Calbiochem–Behring Corp. (San Diego, CA, U.S.A.). Ampholytes, Ultrodex and Ultrogel AcA54 were obtained from LKB Instruments (Bromma, Sweden).

Substituted benzamidines (*m*-phenyloxypropyloxy- and benzoxyethyl-benzamidines) were a gift from Dr. M. Cory (Stanford Research Institute). Dr. J. Powers (Georgia Institute of Technology) generously supplied Ac-Ala-Pro-Ala-Ala-CH$_2$Cl, Ac-Ala-Ala-Pro-Ala-CH$_2$Cl, Ac-Phe-Gly-Ala-Leu-CH$_2$Cl, Z-Gly-Leu-Phe-CH$_2$Cl and Z-Phe-CH$_2$Cl. Purified bovine nasal-cartilage proteoglycan monomers (A$_1$D$_1$) were generously given by Dr. Bruce Caterson (University of Alabama, Birmingham). Purified proteoglycan core protein, obtained by treatment of cartilage proteoglycan with chondroitinase ABC, was supplied by Dr. Jim Christener (University of Alabama, Birmingham).

Methods

Purification of cercarial enzyme. Frozen cercariae (approx. 6×10^6 per experiment) were thawed and centrifuged for 20 min at 9000 g. The supernatant containing lysed cercarial contents was set aside while the pelleted cercariae were resuspended in 10 ml of buffer consisting of 20 mM-Tris/HCl, pH 8.0, 10 mM-CaCl$_2$, 0.02% NaN$_3$ and 0.1% Triton X-100. The suspended cercariae were passed through a French pressure cell (Aminco, Silver Springs, MD, U.S.A.) twice at 27.6–34.5 MPa (4000–5000 lbf/in^2); approx. 90–95% of the cercariae were ruptured as determined by optical microscopy. Extracts were centrifuged for 15 min at 15000 g and the pellets discarded. A small amount of enzymic activity, representing 5% or less of the

total, could be measured in these pellets. This supernatant was pooled with the original cercarial lysate and concentrated by ultrafiltration (Amicon PM-10 membrane). During this concentration step, 60–65% of the initial protein (as measured by the method of Lowry *et al.*, 1951) was lost in the form of a precipitate, which was removed by centrifugation. The concentrate obtained from ultrafiltration contained 85% of the homogenate activity. The volume was adjusted to 3 ml and dialysed overnight against 1% glycine/NaOH buffer, pH 8.8, containing 10 mM-CaCl$_2$.

Preparative isoelectric focusing was performed on an LKB Multiphor apparatus with 2% (w/v) carrier Ampholines, pH range 5–8. Electrofocusing of 2.0–2.5 ml of the dialysed cercarial extract was done on a 100 ml bed of Ultrodex containing 0.1% Triton X-100 for 15 h at a constant power of 8 W. After electrofocusing, the gel bed was divided into 30 fractions with an LKB template. A portion of each gel fraction (100 μl) was placed in 1 ml of deionized water and refrigerated overnight for measurement of pH. The remainder of each fraction was eluted on LKB–PEGG elution columns with 8 ml of 20 mM-Tris/HCl (pH 8.0)/0.02% NaN$_3$/10 mM-CaCl$_2$.

Each fraction was assayed for protein content at 280 nm and for proteinase activity by using Azocoll as a substrate. The fractions containing proteinase activity were passed through a 1 cm × 4 cm column bed of Bio-Beads previously prepared as described by Holloway (1973) to remove Triton X-100. Pooled active fractions were concentrated with Aquacide, followed by dialysis against the elution buffer containing 0.2 M-NaCl. Final purification of the proteinase and removal of carrier Ampholines was done by applying the extract (0.5 ml) to an Ultrogel AcA54 column previously equilibrated with 20 mM-Tris/HCl (pH 8.0)/10 mM-CaCl$_2$/0.2 M-NaCl. Fractions (1 ml) were eluted with this buffer at a flow rate of 8 ml/h and assayed for proteinase activity. Active fractions were pooled, concentrated and stored at −20°C.

Enzyme assays. Measurement of general proteolytic activity was done as previously described, with Azocoll as substrate (Dresden & Asch, 1972). Unless otherwise indicated, the assay mixture consisted of 3 mg of Azocoll, 100 μmol of glycine/NaOH, pH 8.8, 3 μmol of NaN$_3$ and 1 μmol of CaCl$_2$ (incubation buffer) in a volume of 1 ml. Incubation was at 35°C and release of azo dye was measured at 540 nm. Units of enzyme activity were defined as mg of Azocoll solubilized/h per mg of protein.

Gel electrophoresis of the cercarial proteinase. SDS/polyacrylamide-gel electrophoresis was done on precast 10% (w/v) acrylamide gels in 0.05 M-Tris/0.38 M-glycine, pH 8.3. A portion (50 μg) of cercarial enzyme was heated for 5 min in a boiling-

water bath in the presence of 1% SDS. The sample was dialysed overnight against 500 ml of 10 mM-sodium phosphate (pH 7.0)/1% SDS/1% 2-mercaptoethanol/10% glycerol/0.02% Bromophenol Blue. Volumes up to 100 μl were applied for electrophoresis. Protein was stained with Coomassie Brilliant Blue. SDS/polyacrylamide-gel electrophoresis of the digestion products of the proteoglycan core protein was done in a similar manner.

Gel electrophoresis of proteoglycan-monomer digestion products. After incubations at 35°C of cartilage proteoglycan A_1D_1 monomers (10 mg in 0.7 ml of incubation buffer) with cercarial enzyme (20 μg) or heat-inactivated enzyme, reactions were terminated by boiling samples for 10 min in the presence of 1% SDS. Samples were dialysed for 6 h in 1% SDS/1% 2-mercaptoethanol/20% glycerol/40 mM-Tris/acetate (pH 6.8)/1 mM-Na$_2$SO$_4$ (gel buffer). Portions of each sample (100 μl) were applied to composite 0.6%-agarose/1.8%-acrylamide gels prepared essentially as described by McDevitt & Muir (1971) that had been prerun at 4 mA/gel for 1 h, after which fresh electrode buffer was added. Electrode buffer was a 4-fold dilution of gel buffer plus 1 mM-EDTA. The gels were run at 5 mA/gel.

Viscosity measurements. Proteoglycan A_1D_1 monomers (2 mg) were dissolved in 0.1 M-glycine/NaOH (pH 8.8)/1 mM-CaCl$_2$. Cercarial enzyme (5 μg) was added for a final mixture volume of 1 ml. A control was run with heat-inactivated enzyme (20 min in a boiling-water bath). The change in specific viscosity was monitored at 35°C by using Ostwald semi-micro viscometers (Cannon Instrument Co., State College, PA, U.S.A.).

Amino acid analyses. Amino acid analyses were performed on a Durrum D-502 amino acid analyser (Dionex Corp., Sunnyvale, CA, U.S.A.) equipped with the DOS-2 operating system using ninhydrin detection. Before analyses, samples were hydrolysed *in vacuo* with 6 M-HCl at 110°C for 20 h, dried over NaOH, dissolved in loading buffer and centrifuged at 13 000 g for 10 min.

Results

Purification of the proteinase

Isoelectric focusing. Preliminary experiments to purify the cercarial proteinase activity by flat-bed preparative isoelectric focusing resolved Azocollytic activity into three peaks, with isoelectric points of 6.0, 6.4 and 7.0 respectively.

With the addition of the non-ionic detergent Triton X-100 to cercarial suspensions during the preparation of the extracts, the activity of extractable proteinase increased by approx. 30–40%. When these extracts were electrofocused between pH 5 and 8 in the presence of detergent, the enzyme-activity profile was obtained seen in Fig. 1. Two peaks with
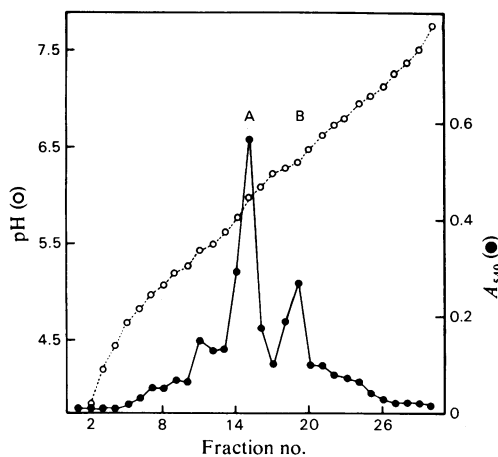

Fig. 1. *Preparative isoelectric focusing of cercarial homogenates over the pH range 5–8*
Cercarial protein (76 mg) was applied and electrofocused for 15 h at 5°C as described in the Experimental procedures section. Fractions were assayed for Azocollytic activity (●) by absorbance at 540 nm. The pH gradient (○) was determined as described in the Experimental procedures section.

isoelectric points at pH 6.0 (A) and pH 6.4 (B) constitute 80–85% of the recovered activity.

Fraction 15 of peak A (Fig. 1) was purified further by Bio-Bead affinity column chromatography to remove Triton X-100. All enzymic activity was recovered in this step.

Gel filtration. Final purification and removal of carrier Ampholines was done by gel filtration on Ultrogel AcA54, as shown in Fig. 2. The enzyme was eluted in a single peak before the contaminating Ampholines.

The results of the purification procedure are summarized in Table 1. An overall yield of about 7% of the initial activity was obtained with an overall increase in specific activity of 54–55-fold. This yield is for fraction 15 (from preparative isoelectric focusing) only. When all fractions from these two peaks are combined and carried through the Bio-Bead and AcA54 steps, recovery of activity (five experiments) ranges from 18 to 27% of the initial activity, with a 25–40-fold increase in specific activity. The activity peaks obtained by electrofocusing in the absence of Triton X-100 are also eluted from AcA54 gel columns (results not shown) with the same elution volume as fraction 15.

Assessment of purity. The enzyme fraction obtained from gel filtration was analysed by SDS/polyacrylamide-gel electrophoresis and analytical isoelectric focusing. Electrophoresis in the presence

Table 1. *Purification of the major proteinase species from cercariae of S. mansoni*
Details of the individual purification steps are described in the Experimental procedures section.

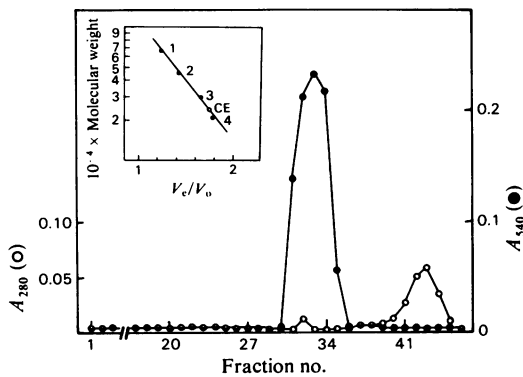| Purification step	Protein (mg)	Proteinase activity (towards Azocoll)		Yield (%)
		Units	Units/mg of protein	
French-press homogenate	216	1200	5.6	100
Concentrated supernatant	75.9	1020	13.4	85
Isoelectric focusing	0.3	92	307	7.7
Bio-Bead column	—	91	—	7.6
AcA54 gel filtration	0.27	83	307	6.9

Fig. 2. *Gel filtration of cercarial proteinase*
The contents of fraction 15 (Fig. 1) were subjected to gel filtration on a column (1.5 cm × 60 cm) of AcA54 Ultrogel, equilibrated and eluted with 20 mM-Tris/HCl (pH 8.0)/10 mM-$CaCl_2$/0.2 M-NaCl. Fractions (1 ml) were analysed for protein content (O) at 280 nm and proteinase activity (●) with Azocoll. Inset: this column was calibrated with: 1, bovine serum albumin; 2, ovalbumin; 3, carbonic anhydrase; and 4, soya-bean trypsin inhibitor. The elution position of the cercarial proteinase (CE) is indicated.
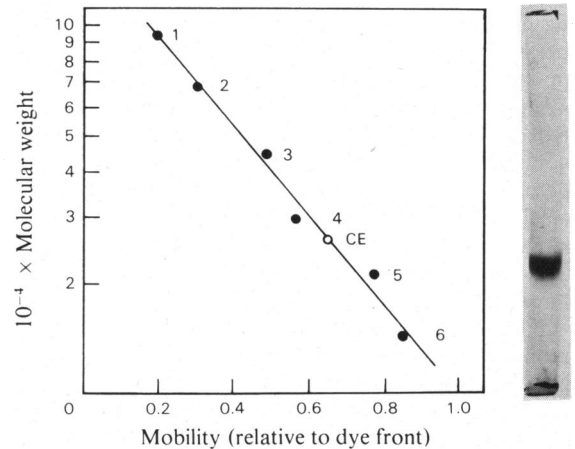

Fig. 3. *SDS/polyacrylamide-gel electrophoresis of the cercarial proteinase*
The cercarial enzyme (50 μg) was electrophoresed as described in the Experimental procedures section and stained with Coomassie Blue (inset right). Protein standards used to derive a molecular-weight estimate for the enzyme were: 1, phosphorylase *b*; 2, bovine serum albumin; 3, ovalbumin; 4, carbonic anhydrase; 5, soya-bean trypsin inhibitor; and 6, lysozyme. Abbreviation used: CE, cercarial proteinase.

of SDS and 2-mercaptoethanol (10% polyacrylamide gels at pH 8.3) shows a single band after staining with Coomassie Brilliant Blue (Fig. 3). Analytical isoelectric focusing on polyacrylamide gels (pH 3.5–9.5) of the purified cercarial enzyme also showed a single protein band, which contained Azocollytic activity.

Properties of the cercarial proteinase

Molecular weight and isoelectric point. By calibration of the AcA54 gel-filtration column with appropriate protein standards (Fig. 2, inset), the molecular weight corresponding to the peak of Azocollytic activity was shown to be 24 000–25 000. SDS/polyacrylamide-gel electrophoresis (Fig. 3)

gave an estimate of 24 000–26 000. The isoelectric point determined both by preparative and analytical isoelectric focusing was pI = 6.0 ± 0.1.

Amino acid analysis. Table 2 shows the amino acid composition of the cercarial enzyme (peak A) and compares it with bovine chymotrypsin and cathepsin G, a chymotrypsin-like enzyme from human leucocytes. The acidic amino acids glutamate and aspartate and their amides (glutamine and asparagine) together account for approx. 25% of the total amino acid residues, and the basic amino acids arginine, lysine and histidine account for 14%.

Effect of specific inhibitors. To obtain information on the relationship between the cercarial proteinases

and certain vertebrate serine proteinases, a series of inhibitors were used. The results are summarized in Table 3. No inhibition was observed with typical trypsin and elastase inhibitors at concentrations at which these inhibitors inhibited the appropriate bovine pancreatic proteinases. However, chymotrypsin inhibitors were effective against the cercarial proteinase. The most potent inhibitor, Ac-Phe-Gly-

Ala-Leu-CH$_2$Cl, had an apparent K_i of approx. 5×10^{-6}–10×10^{-6} M.

Effect of cercarial proteinase on proteoglycan components. Activity in cercarial homogenates against cartilage proteoglycan has been previously reported (Dresden & Asch, 1972). In order to obtain additional information on the mechanism by which cartilage proteoglycan is degraded, the effect of the cercarial enzyme was tested on purified proteoglycan monomers, i.e. core protein subunits retaining their chondroitin sulphate and keratan sulphate side chains (Hascall & Sajdera, 1970), and on purified core protein from bovine nasal cartilage.

The results of incubation of purified A$_1$D$_1$ monomers with purified cercarial enzyme is shown in Fig. 4. After 2 h the specific viscosity ($\eta_{sp.}$) was decreased to 20% of the initial viscosity and did not change appreciably with continued incubation. The reaction products were analysed by electrophoresis on composite agarose/acrylamide gels (Fig. 4). The intact monomer, of mol.wt. 2.2×10^6 (Hascall & Sajdera, 1970) is too large to enter these composite gels, but the products of the action of the cercarial enzyme clearly can.

Since we had not been able to detect chondroitinase activity in cercarial homogenates (Dresden & Asch, 1972), the susceptibility of core protein to degradation by the cercarial proteinase was examined. After incubation the reaction products were subjected to electrophoresis on SDS/10%-polyacrylamide gels (Fig. 5). In order to determine the size of the observed products, additional samples were run concurrently with molecular-weight standards. The three core-protein bands obtained after digestion with the cercarial enzyme

Table 2. *Amino acid composition of cercarial proteinase* (%)

Amino acid	Cercarial proteinase	Bovine chymotrypsin*	Cathepsin G†
Asp/Asn	12.3	9.5	7.7
Thr	5.9	9.5	5.5
Ser	7.9	11.1	6.8
Glu/Gln	12.4	6.2	10.4
Pro	5.2	3.7	5.9
Gly	10.3	9.5	9.5
Ala	6.7	9.1	5.9
Cys	0.9	4.1	2.8
Val	5.6	9.5	6.4
Met	1.3	0.8	1.8
Ile	4.6	4.1	5.0
Leu	7.4	7.8	7.3
Tyr	2.4	1.6	2.3
Phe	3.1	2.5	4.5
Trp	N.D.‡	3.3	N.D.
His	3.3	0.8	2.8
Lys	5.6	5.8	1.4
Arg	5.3	1.2	14.1

* From Wilcox (1970).
† From Travis *et al.* (1978).
‡ Abbreviation used: N.D., not determined.

Table 3. *Effect of inhibitors on the Azocollytic activity of the cercarial proteinase*

Inhibitors, dissolved in appropriate solvents, were tested over a wide concentration range and the concentration required to obtain 50% inhibition of enzyme activity was determined; for those inhibitors not showing inhibition of the cercarial proteinase, the maximum concentrations tested are indicated; these concentrations were sufficient to inhibit the appropriate serine proteinase. Approx. 5–10 μg of partially purified cercarial proteinase was used in each assay, and enzyme and inhibitors were preincubated at 4°C for 15 min before assay. Controls to test the effect of the solvents used were run where appropriate.

| Serine–proteinase subclass	Inhibitor (M)	Concentration required for 50% inhibition (M)
Trypsin	Tos-Lys-CH$_2$Cl (10^{-3})	0
	Substituted benzamidines (4×10^{-4})	0
	Soya-bean trypsin inhibitor (10μg/ml)	0
Elastase		
Leucocyte	Ac-Ala-Pro-Ala-Ala-CH$_2$Cl (10^{-4})	0
Leucocytic and pancreatic	Ac-Ala-Ala-Pro-Ala-CH$_2$Cl (10^{-4})	0
Chymotrypsin	Tos-Phe-CH$_2$Cl	8×10^{-4}
	2-aminoacylpyridylbenzenesulphonyl fluoride	1.5×10^{-4}
	Z-Gly-Leu-Phe-CH$_2$Cl	1.1×10^{-4}
	Z-Phe-CH$_2$Cl	8×10^{-5}
	Chymostatin	7×10^{-5}
	Ac-Phe-Gly-Ala-Leu-CH$_2$Cl	5×10^{-6}
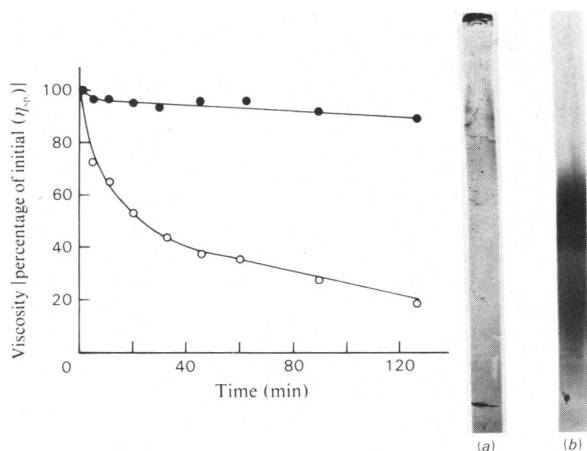
Fig. 4. *Effect of cercarial enzyme on the viscosity of proteoglycan monomers*

Mixtures containing 2 mg of A_1D_1 monomers, 5 μg of purified cercarial enzyme, 0.1 M-glycine/NaOH, pH 8.8, 1 mM-$CaCl_2$ in a total volume of 1 ml were incubated in Ostwald viscometers at 35°C (O). The viscosity is plotted as a percentage of initial viscosity ($\eta_{sp.}$). A control with heat-inactivated enzyme is shown (●). On the right is shown agarose/poly-acrylamide-gel electrophoresis of A_1D_1 monomers (10 mg/0.7 ml) incubated in the presence of (a) 20 μg of heat-inactivated and (b) 20 μg of active cercarial enzyme at 35°C, as described in the Experimental procedures section.

Fig. 5. *SDS/polyacrylamide-gel electrophoresis of purified core-protein digestion products*

Mixtures containing 30 μg of core protein, 2.5 μg of cercarial enzyme in 0.1 M-glycine/NaOH (pH 8.8)/1 mM-$CaCl_2$ in volumes of 0.1 ml were incubated overnight at 35°C. The digestion products as well as a control containing heat-inactivated cercarial enzyme were electrophoresed on SDS/10%-poly-acrylamide gels. The control core protein failed to enter the gel; the products of digestion with active cercarial enzyme are shown on the right. Estimates of the molecular weight of the three fragments (I, II, III) (O) were obtained by using protein standards (●). The protein standards used were: 1, phosphorylase *b*; 2, bovine serum albumin; 3, ovalbumin; 4, carbonic anhydrase; 5, soya-bean trypsin inhibitor; 6, lysozyme.

were designated I, II, and III and had approx. mol.wts. 9.6×10^4, 5.3×10^4 and 4.6×10^4 respectively. Their additive molecular size was 1.95×10^5 daltons, which corresponds closely to the molecular weight reported for the intact core protein (Hascall & Riolo, 1972).

Discussion

The present paper describes the purification of a proteolytic enzyme from the cercarial stage of the human parasite *Schistosoma mansoni*. The probable function of this enzyme is to mediate the penetration of schistosome cercariae through the skin of susceptible vertebrate hosts.

Previous work by ourselves and others suggested that there were multiple proteinase activities present in homogenates of cercariae (Gazzinelli *et al.*, 1966; Dresden & Asch, 1972). However, it is likely that these multiple activities may have been due to the omission of Triton X-100 from the extraction and isolation solutions. Since the cercarial proteinase is stored in secretory granules of the preacetabular glands (Stirewalt, 1973; Asch *et al.*, 1977), it may be
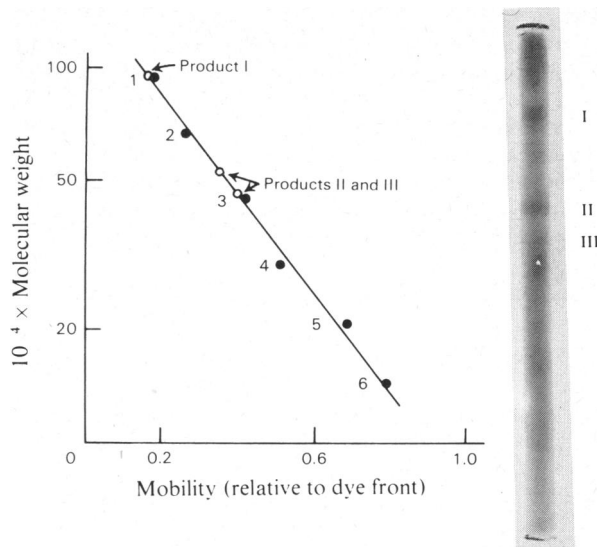
that the multiple peaks observed in previous experiments were due to membrane fragments, associated with the proteinase, that we removed by treatment with the detergent Triton X-100. Gel filtration of peaks A and B seen in Fig. 1 indicates that both have the same molecular weight, and they respond similarly to inhibitors such as chymostatin. In addition, both activities bind to 4-phenyl-butylamine–Sepharose (Stevenson & Landman, 1971), an affinity matrix for chymotrypsin-like enzymes. When the isolated A and B peaks are submitted to analytical isoelectric focusing, however, they retain their differences in isoelectric point.

We (Dresden & Asch, 1972) and Gazzinelli *et al.* (1966) previously demonstrated that the cercarial proteinase activity of cercarial homogenates belonged to the class of serine proteinases by virtue of its inhibition by agents such as Dip-F (Gazzinelli *et al.*, 1966) and phenylmethanesulphonyl fluoride

(Dresden & Asch, 1972). As shown in Table 3, more detailed studies with inhibitors indicate that inhibitors of trypsin and both leucocyte and pancreatic elastases (Tuhy & Powers, 1975) are ineffective. On the other hand, inhibitors of chymotrypsin-like enzymes were clearly effective. The most effective inhibitor tested was Ac-Phe-Gly-Ala-Leu-CH$_2$Cl, previously shown to be an effective inhibitor of subtilisin (Powers *et al.*, 1977*a*) and cathepsin G (Powers *et al.*, 1977*b*). The effectiveness of these inhibitors is consistent with our previous findings with human serum anti-proteinases (Asch & Dresden, 1977). Further evidence for the chymotryptic nature of the cercarial proteinase is seen in its activity towards synthetic substrates (results not shown; and Gazzinelli *et al.*, 1972).

The cercarial proteinase, however, differs significantly from vertebrate chymotrypsin in a number of properties. Comparison of sensitivity to various inhibitors shows that the cercarial enzyme is equally as sensitive as chymotrypsin to Dip-F, 4–6-fold less sensitive to phenylmethanesulphonyl fluoride and approx. 50% more sensitive to Ac-Phe-Gly-Ala-Leu-CH$_2$Cl. The heat-stability of the cercarial enzyme is significantly less than that of chymotrypsin (results not shown).

In addition, the amino acid composition of the cercarial proteinase is significantly different from that of bovine chymotrypsin (Wilcox, 1970) (Table 2) and the chymotrypsin-like neutrophil proteinase cathepsin G (Travis *et al.*, 1978). The cercarial proteinase is relatively high in glutamate (glutamine) and aspartate (asparagine); this finding is reflected in the lower isoelectric point (6.0) of the cercarial proteinase compared with chymotrypsin (8.7). The cercarial proteinase is also considerably lower in isoelectric point from the very basic cathepsin G (Starkey & Barrett, 1976).

The precise mechanism by which these proteolytic enzymes aid the penetration of schistosome cercariae through skin is unclear. The cercarial proteinase preparations are inactive against both soluble and fibrillar collagen, although they can hydrolyse denatured collagen (Dresden & Asch, 1972). However, the proteinase appears to be very active against the peptide backbone of cartilage proteoglycan. Fig. 4 shows that the decrease in viscosity of isolated A$_1$D$_1$ monomers is accompanied by degradation of the monomers. The bands seen on composite acrylamide/agarose gels cover a relatively limited portion of the gel, suggesting that the enzyme catalyses a limited number of cleavages of the peptide backbone. The pattern of cleavages is different from that we have observed for bovine trypsin and α-chymotrypsin (results not shown). Similar observations on the specificity of various proteolytic enzymes on proteoglycan were made by Roughley (1978), who tested a variety of commer-

cially available proteinases. The possibility that the enzyme is rather specific in its activity towards the core protein is also substantiated by the results of Fig. 5.

A further factor of importance in regard to the role of the cercarial enzyme in host skin penetration is the presence of large amounts of CaCO$_3$ in the preacetabular glands of these organisms (Dresden & Asch, 1977). Ca^{2+} released in the extracellular matrix during penetration would undoubtedly affect the anionic proteoglycan. Thus a 'melting' or disaggregation of the proteoglycan matrix of the dermis by the proteolytic and cationic secretions of cercariae might be of physiological importance in the penetration of *S. mansoni* through the skin.

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