Affinity purification and biochemical characterization of histolysin, the major cysteine proteinase of *Entamoeba histolytica*

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We report a one-step method for the purification to homogeneity of a cysteine proteinase of *Entamoeba histolytica* (histolysin) by affinity chromatography of the soluble extract of the parasite on immobilized phenylalanyl(2-phenyl)aminoacetaldehyde semicarbazone. The enzyme has an apparent M_r of 26000 by SDS/polyacrylamide-gel electrophoresis and 29000 by gel chromatography. Its pH optimum varies widely, from 5.5 with azocasein to approx. 7 with other protein substrates and benzyloxycarbonylphenylalanyl-L-citrullylaminomethylcoumarin (Z-Phe-Cit-NHMec), and to 9.5 with benzyloxycarbonylphenylalanyl-arginylaminomethylcoumarin (Z-Phe-Arg-NHMec) and benzyloxycarbonylarginylarginylaminomethylcoumarin (Z-Arg-Arg-NHMec). Values of K_m , k_{cat} , and k_{cat}/K_m are $1.5 \mu M$, $130 s^{-1}$ and $87 \times 10^6 M^{-1} \cdot s^{-1}$ for Z-Arg-Arg-NHMec, and $32 \mu M$, $0.4 s^{-1}$ and $0.012 \times 10^6 M^{-1} \cdot s^{-1}$ for Z-Phe-Arg-NHMec, respectively, at pH 7.5 and 37 °C. The enzyme is inhibited by leupeptin and such inhibitors of cysteine proteinases as L-trans-epoxysuccinyl-L-leucylamido-(4-guanidino)butane, peptidylidiazomethanes, iodoacetic acid and chicken cystatin. The tentative N-terminal amino acid sequence of the enzyme closely resembles that of papain. Histolysin does not degrade type I collagen or elastin, but it is active against cartilage proteoglycan and kidney glomerular basement-membrane collagen. It also detaches cells from their substratum *in vitro*, and could well play a role in tissue invasion.

INTRODUCTION

Proteinases of *Entamoeba histolytica* may be implicated in the infectiveness and invasiveness of this parasitic protozoan. The organism causes ulceration of the intestinal wall, and can invade the circulation and set up foci of infection in the liver. This type of pathogenic activity involves the tissue-dissolving or histolytic activity for which the organism is named. Histolytic activity of a pathogen is normally attributable to its production of extracellular proteolytic enzymes, such as collagenase by *Colstridium histolyticum* or elastase by *Pseudomonas aeruginosa*. The histolytic proteinase of *E. histolytica* has not been positively identified, but a strong candidate is a cysteine (thiol-dependent) proteinase. Studies, with partially purified enzyme preparations and inhibitors of cysteine proteinases have indicated that this enzyme has the property of degrading native collagen and other components of extracellular matrix, and also causing rounding and detachment of cells in culture (Lushbaugh *et al.*, 1979, 1984a,b, 1985; Bos & Van Den Eijk, 1980; Gadasi & Kessler, 1983; Gadasi & Kobiler, 1983; Avila *et al.*, 1985; Feingold *et al.*, 1985; Keene & McKerrow, 1985; Keene *et al.*, 1986). Circumstantial evidence for the involvement of the cysteine proteinase has come from the report that there is a correlation of invasive activity with the amount of cysteine proteinase present in strains of *E. histolytica* exhibiting various degrees of virulence (Gadasi & Kessler, 1983; Gadasi & Kobiler, 1983; Lushbaugh *et al.*, 1984a, 1985).

McLaughlin & Faubert (1977) partially purified a

cysteine proteinase from amoebal lysates by ion-exchange and gel chromatography. It was active against azocasein at pH 6, and its M_r was estimated to be 27000. Lushbaugh *et al.* (1985), using ion-exchange chromatography, organomercurial-agarose affinity chromatography and gel chromatography, purified a cysteine proteinase of M_r 16000 described as cathepsin B which was capable of rounding and detaching of HeLa cells. Keene *et al.* (1986) used anion-exchange followed by chromatofocusing for the purification of a cysteine proteinase that was secreted by the parasite and also present in the trophozoite lysate. They report an M_r of 56000 determined by SDS/polyacrylamide-gel electrophoresis. It seems probable that all these reports relate to a single enzyme, but if so, there is little agreement as to its properties.

A definite answer to the question of whether the cysteine proteinase of *E. histolytica* is important in the histolytic activity of the organism requires complete isolation of the enzyme, and the demonstration that it has, or has not, got activity against protein substrates that are components of the extracellular matrix of tissues. Furthermore, it should be demonstrated that specific inhibitors of the proteinase do, or do not, inhibit the histolytic activity in an appropriate model system.

As a first step, we now describe a method for the purification to homogeneity of the major cysteine proteinase of *E. histolytica* (which we name histolysin) by affinity chromatography on immobilized Phe-PheSc, and report the properties of the enzyme.

Abbreviations used: Z, benzyloxycarbonyl; NHMec, 7-(4-methyl)coumarylamide; PheSc, (2-phenyl)aminoacetaldehyde semicarbazone; Cit, citrullyl; E-64, L-3-carboxy-trans-2,3-epoxypropionyl-leucylamido-(4-guanidino)butane; Bz-Arg-NPhNO₂, benzoyl-D,L-arginine p-nitroanilide; NH₂Mec, 7-amino-4-methylcoumarin.

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MATERIALS AND METHODS

Materials

Bz-Arg-NPhNO₂, Z-Arg-Arg-NHMec, Z-Phe-Arg-NHMec and NH₂Mec were obtained from Bachem, Saffron Walden, Essex, U.K. Azocasein was prepared as described by Barrett & Kirschke (1981). Azocoll and hide powder azure were from Calbiochem, 2-hydroxyethyl-disulphide from Aldrich, Milwaukee, WI, U.S.A. and leupeptin from Sigma, Poole, Dorset, U.K. Chicken cystatin, form 2, was prepared from egg-white as described by Nicklin & Barrett (1984). Acid-soluble rabbit skin collagen was a gift of Dr. G. Murphy, and ³H-labelled elastin was a gift of Dr. R. W. Mason. Human glomerular basement membrane prepared as described by Davies *et al.* (1978) was a gift of Dr. M. Davies (K.R.U.F. Institute of Renal Disease, Cardiff, U.K.). Pro-Phe-Arg-CH₂Cl, Z-Leu-Leu-Phe-CH₂Cl, Z-Leu-Lys-CHN₂ and Z-Leu-Met-CHN₂ were given by Dr. Elliott Shaw (Friedrich-Miescher Institute, Basel CH-4002, Switzerland).

Sephacrose-aminohexanoyl-Phe-PheSc

Phe-PheSc, given by Dr. Daniel H. Rich (School of Pharmacy, University of Wisconsin-Madison, 425 North Charter Street, Madison, WI 53706, U.S.A.), was coupled to CH-Sephacrose 4B (Pharmacia) as described for analogous compounds (Rich *et al.*, 1986).

Culture of *Entamoeba histolytica*

The axenic HM 1 strain was used. Trophozoites provided by Dr. F. E. Pittman (Veterans Administration Medical Center, Charleston, SC, U.S.A.) were grown in Diamond's TYI-S-33 medium (Diamond *et al.*, 1978). Organisms were harvested in the logarithmic phase of growth, washed with cold phosphate-buffered saline and stored at -20 °C until use.

Protein assay

Protein was determined by the method of Bradford (1976), bovine serum albumin being used as standard. Molar concentrations of histolysin solutions were calculated from the protein concentration and the *M_r* of 26000.

Purification of histolysin

The pellet of frozen trophozoites [approx. (50–100) × 10⁶] was thawed and resuspended in an equal volume of 20 mM-Tris/HCl buffer, pH 7.5, (buffer A) containing 4 mM-dithiothreitol/4 mM-EDTA. The suspension was frozen and thawed three times before centrifuging (10000 *g*, 5 min). The supernatant (soluble lysate, typically 1.5–3 ml containing 10–20 mg of protein), was run on a column (80 mm × 5 mm) of Sepharose-aminohexanoyl-Phe-PheSc equilibrated with buffer A containing 2 mM-dithiothreitol/2 mM-EDTA. The flow was stopped for 1 h to allow the enzyme to bind to the matrix, and unbound material was washed out with 2 ml of buffer A containing dithiothreitol/EDTA and 2 ml of buffer A. Buffer A containing 50 mM-hydroxyethyl-disulphide was then run into the column, left for 1 h, and run again to elute the bound proteinase.

Gel chromatography

An LKB 2135 Ultropac TSK-G 2000 SW column (600 mm × 7.5 mm) was run in 500 mM-NaCl/50 mM-

Tris/HCl buffer (pH 7.5) with a 200 μl sample at 0.25 ml/min.

Electrophoresis

Samples were run with reduction in the SDS/polyacrylamide-gel electrophoresis system of Bury (1981) [gels (10% total polyacrylamide) being calibrated with the same *M_r* standard proteins].

Enzyme assays

Enzyme assays at fixed incubation times, with Z-Phe-Arg-NHMec and Z-Arg-Arg-NHMec as substrates, were as described previously (Barrett, 1980; Barrett & Kirschke, 1981) except as follows. The incubation mixture (1 ml) contained 35 mM-glycine/NaOH buffer (pH 9.5)/2 mM-EDTA/2 mM-dithiothreitol/0.05% Brij 35 and 5 μM substrate. The reaction was allowed to proceed at 40 °C for 10 min, and was then stopped by addition of 1 ml of 100 mM-sodium monochloroacetate in 100 mM-sodium acetate buffer, pH 4.3. The release of NH₂Mec was quantified fluorimetrically with excitation at 360 nm and emission at 460 nm (one unit of activity corresponding to release of 1 μmol of product/min).

To measure activity against the protein substrates azocasein, azocoll and hide powder azure, the enzyme was diluted in 0.5 ml of 72 mM buffer (see below) containing also 0.05% Brij 35/2 mM-dithiothreitol/2 mM-EDTA and 0.6% of the protein substrate. The mixture was incubated at 40 °C for an appropriate time, and the reaction was stopped by addition of 0.5 ml of 20% (w/v) trichloroacetic acid. The tubes were centrifuged at 10000 *g* for 5 min, and the absorbance of supernatants was determined at 366 nm for azocasein, at 520 nm for azocoll and at 595 nm for hide powder azure.

The pH dependences of activity against protein and synthetic substrates were determined by use of the above assay procedures, but with substitution of other buffers in the pH range 4.0–10.6; these were sodium phosphate/citric acid, Tris/HCl, Ammediol (2-amino-2-methyl-1,3-propanediol)/HCl, and glycine/NaOH.

Active-site titration

A solution of commercial papain (Sigma) was titrated with E-64 as described previously (Barrett *et al.*, 1982), except that Bz-Arg-NPhNO₂ was used as substrate in the assays. The papain solution was then used to standardize a solution of cystatin (a high-affinity protein inhibitor of cysteine proteinases; Nicklin & Barrett, 1984) in a further titration. The standard cystatin solution was used for titration of histolysin solutions, with Z-Phe-Arg-NHMec as substrate.

Kinetic studies

Determination of *K_m* and *k_{cat}* values for the synthetic substrates was at 37 °C in reaction mixtures (3 ml) containing 100 mM-sodium phosphate buffer (pH 7.5)/2 mM-dithiothreitol/2 mM-EDTA/0.01% Brij 35 [enzyme concentrations being 0.4 μg/ml (15.4 nM) for Z-Phe-Arg-NHMec and 0.4 ng/ml (15.4 pM) for Z-Arg-Arg-NHMec]. Rates of product formation were monitored by continuous fluorimetry (excitation wavelength 360 nm, emission wavelength 460 nm), and results were calculated as described by Wilkinson (1961).

Kinetics of inhibition by reversible and irreversible inhibitors were determined with reaction mixtures as

Table 1. Summary of results of a typical purification of histolysin

Activity was measured against Z-Phe-Arg-NHMec. See text for details.

Purification step	Protein (mg)	Activity (units)	Specific activity (munits/mg)	Purification factor	Yield (%)
Lysate supernatant	14.800	0.0324	2.19	(1)	(100)
Affinity column	0.141	0.0352	249.6	114	109

described for the kinetic studies and Z-Phe-Arg-NHMec (or Z-Arg-Arg-NHMec for cystatin) as substrate. Once a stable uninhibited reaction rate (v_0) had been recorded, the inhibitor was added, and the progress of inhibition was followed. For the tight-binding reversible inhibitors, K_i was calculated according to Henderson (1972). Second-order rate constants for the irreversible inhibitors were calculated as $k_{app}/[I]$, k_{app} being the apparent first-order rate constant for the exponential decline of enzyme activity in mixtures of enzyme, substrate and inhibitor.

Determination of collagenolytic activity

Tubes containing 40 μ g of soluble type I collagen in 150 μ l of 100 mM-Tris/HCl buffer (pH 7.5)/2 mM-dithiothreitol/2 mM-EDTA were incubated at 30 °C. The reaction was started by the addition of enzyme and stopped with 10 μ l of a 1 mM solution of iodoacetic acid. Samples were then boiled with an equal volume of the sample buffer for the SDS procedure, and run in 7% polyacrylamide gels. In some cases, samples were boiled without previous inactivation with iodoacetic acid.

Degradation of bovine nasal cartilage proteoglycan

The assay was essentially as described by Buttle *et al.* (1984), but a buffer of pH 7.5 was used (20 mM-Tris/HCl/2 mM-dithiothreitol/2 mM-EDTA) at 37 °C. Samples were taken periodically for measurement of released sulphated glycosaminoglycans (Farndale *et al.*, 1986). At the end of the experiment, the residual cartilage was completely digested with papain. The results are reported as percent of total glycosaminoglycan released in each tube.

Degradation of glomerular basement-membrane collagen

The assay was essentially as described by Davies *et al.* (1978), except that the human glomerular basement-membrane preparation was incubated with the enzyme in 20 mM-Tris/HCl (pH 7.5)/2 mM-dithiothreitol/2 mM-EDTA buffer, for 16 h at 37 °C with gentle agitation. After centrifugation the supernatants and precipitates were hydrolysed in 6 M-HCl, and the hydroxyproline contents determined as described by Bergman & Loxley (1963).

RESULTS

Purification of the enzyme

The results of a typical purification procedure are summarized in Table 1. The enzyme showed high affinity for the immobilized Phe-PheSc ligand. When inactive protein had been washed out with buffer, the enzyme was blocked with the reversible inhibitor hydroxy-

Fig. 1. Gel chromatography of purified histolysin

Affinity-purified histolysin was run on an LKB 2135 Ultropac TSK SW column equilibrated with 50 mM-Tris/HCl buffer (pH 7.5)/500 mM-NaCl. The column was calibrated with bovine serum albumin, egg albumin, chymotrypsinogen and cytochrome *c*, and their elution volumes are indicated by the corresponding M_r values.

ethylsulphide, and eluted as a single peak. This resulted in approximately 100-fold purification with high yield.

The molar concentration of a solution of the proteinase determined by titration with chicken cystatin was found to be identical to that calculated from the protein concentration (within the limits of experimental error), indicating that the enzyme had been completely isolated from inactive material.

Physicochemical properties

Run on the TSK gel chromatography column the purified enzyme appeared as a single sharp peak of both activity and protein that had an elution volume corresponding to an M_r of 29000 (Fig. 1). This may be compared with previous values of 27000 (McLaughlin & Faubert, 1976), 16000 (Lushbaugh *et al.*, 1985) and 21000 (Scholze & Werries, 1984) obtained by gel chromatography.

SDS/polyacrylamide-gel electrophoresis of the purified enzyme under reducing conditions (Fig. 2) showed one major band of protein with apparent M_r 26000 (lane *b*). This protein did not appear as a major component of the crude lysate (lane *a*). Heavily loaded gels showed minor bands at M_r values of approx. 50000 and 70000, but since these minor bands were seen even with material

Fig. 2. SDS/polyacrylamide-gel electrophoresis of reduced samples of *E. histolytica* cysteine proteinase at different stages of purification

S, Standards; (a) soluble lysate; (b) affinity-purified enzyme; (c) peak of activity from gel chromatography.

Fig. 3. Tentative *N*-terminal amino acid sequence of histolysin aligned with sequences of other cysteine proteinases

The residues identified in a single automated sequencing run with histolysin show a high degree of homology with those of papain. (Identical residues in the other sequences are represented by shaded blocks). It is notable that the four-residue-insertion characteristic of all cathepsins B sequenced so far does not appear in the histolysin sequence. References to the original sequence data for other cysteine proteinases are: papain (Cohen *et al.*, 1986); actinidin, aleurain, cathepsin B (rat, human), cathepsin H (rat), cathepsin L (mouse) (Portnoy *et al.*, 1986); cathepsin B (mouse) (Chan *et al.*, 1986); cathepsin B (pig) (Takahashi *et al.*, 1984); cathepsin B (pig) (Pohl *et al.*, 1982); cathepsin L (human) (Mason *et al.*, 1986b); cathepsin L (chicken) (Wada & Tanabe, 1986); *Dictyostelium* proteinases (Pears *et al.*, 1985). Data for cathepsin H (human) are from Machleidt *et al.* (1986b).

from the 29000 M_r peak by gel chromatography (lane c), we suggest that they represent an artefact of the SDS-electrophoresis procedure.

Keene *et al.* (1986) found proteinase activity of apparent M_r 50000–70000 when unreduced samples were run in gels of acrylamide co-polymerized with

Table 2. pH optima of histolysin with various substrates

See text for details.

Substrate	pH
Z-Phe-Cit-NHMec	7.5
Z-Arg-Arg-NHMec	9.5
Z-Phe-Arg-NHMec	9.5
Azocasein	5.5
Hide powder azure	6.7
Azocoll	7.5

gelatin. Similarly, high apparent M_r values were obtained by us (results not shown) using this technique with both purified enzyme and the peak of activity from gel chromatography. We conclude that the active enzyme (not fully denatured and unfolded) runs with lower mobility than a denatured protein of 26000 M_r , at least in the gelatin-containing gels.

Activity was completely stable during overnight incubation at 4 °C in the pH range 5.5–9.5, but declined at lower pH values.

Tentative *N*-terminal amino acid sequence

In a single experiment, 100 μ g of purified histolysin was covalently coupled to porous glass support (Machleidt *et al.*, 1986a), and subjected to automated solid-phase Edman degradation in a non-commercial sequencer. The amino acid phenylthiohydantoin derivatives were identified and quantified by reverse-phase h.p.l.c. operating on-line to the sequencer (Machleidt, 1983). The results are shown in Fig. 3. A single amino acid sequence was obtained, which showed very strong homology with that of papain. The signal was weaker than expected for the amount of material used, however, suggesting that the *N*-terminal was partially blocked.

Characteristics of substrate hydrolysis

When activity was tested against the two arginine-containing synthetic substrates, Z-Arg-Arg-NHMec and Z-Phe-Arg-NHMec, maximal activity was found at pH 9.5 (Table 2). The substrate Z-Phe-Cit-NHMec, however, showed a lower pH optimum, as did the protein substrates azocasein, azocoll and hide powder azure (Table 2). Acidic pH optima were reported for protein substrates by Scholze & Werries (1984) and McLaughlin & Faubert (1976).

The enzyme showed a high degree of specificity for the Z-Arg-Arg-NHMec substrate, k_{cat}/K_m being 7250-fold greater than for Z-Phe-Arg-NHMec at pH 7.5 (Table 3).

Kinetics of inhibition

Chicken cystatin inhibited histolysin with a K_i of approx. 8×10^{-12} M. This value is at the lower limit of measurement, even with the very sensitive Z-Arg-Arg-NHMec substrate, so the true value may be lower. The K_i for leupeptin was 4.3×10^{-8} M.

The active-site-directed inhibitors characteristic for cysteine proteinases, E-64 and iodoacetic acid, inactivated histolysin with second-order rate constants of 1100 and 180 $M^{-1} \cdot s^{-1}$, respectively. The value for E-64 is much

Table 3. Kinetics of histolysin action against synthetic substrates

See text for details.

Substrate tested	K_m (μM)	k_{cat} (s^{-1})	$10^{-6} \times k_{\text{cat}}/K_m$ ($\text{M}^{-1} \cdot \text{s}^{-1}$)
Z-Arg-Arg-NHMec	1.5 ± 0.2	130	87
Z-Phe-Arg-NHMec	31.8 ± 2.8	0.4	0.012

lower than that found for cathepsin B (89400) or other cysteine proteinases of the papain superfamily previously examined (Barrett *et al.*, 1982). Z-Leu-Met-CHN₂ was the most effective irreversible inhibitor found, with a second-order rate constant of $1380 \text{ M}^{-1} \cdot \text{s}^{-1}$, whereas that of Z-Leu-Lys-CHN₂ was only $58 \text{ M}^{-1} \cdot \text{s}^{-1}$. Surprisingly, both Pro-Phe-Arg-CH₂Cl and Z-Leu-Leu-Phe-CH₂Cl produced reversible inhibition with K_i values of about $1.5 \mu\text{M}$, and no irreversible inhibition was detected.

Lack of effect on type I collagen

SDS/polyacrylamide-gel electrophoresis of type I collagen, incubated for up to 8 h with histolysin ($5 \mu\text{g}/\text{ml}$), showed no evidence of degradation. In contrast, a parallel incubation with papain ($20 \mu\text{g}/\text{ml}$), resulted in the characteristic conversion of β - to α -components and further degradation. A sample incubated for 8 h, and not inhibited with iodoacetic acid before heating with SDS sample buffer, gave the appearance of appreciable degradation of the collagen. Keene *et al.* (1986) and Gadasi & Kessler (1983) reported degradation of native type I collagen in solution by the cysteine proteinase of *E. histolytica*, but also in experiments in which the samples were heated in SDS buffer without previous inactivation of the enzyme. We attribute the activity reported by the previous workers to the transient degradation of gelatin formed by heat denaturation of the collagen during the preparation of samples for electrophoresis, and do not consider histolysin to degrade type I collagen.

Degradation of glomerular basement-membrane collagen

Histolysin ($5 \mu\text{g}/\text{ml}$) degraded glomerular basement-membrane collagen during overnight incubation at 37°C , values for percentage solubilization of hydroxyproline being 28, 38 and 58 (means for $n = 3$) for 0.5, 1.0 and $5.0 \mu\text{g}$ of histolysin/ml, respectively. The effect of $5 \mu\text{g}$ of histolysin/ml was completely suppressed by $25 \mu\text{g}$ of chicken cystatin/ml.

Degradation of bovine nasal cartilage proteoglycan

Histolysin ($4 \mu\text{g}/\text{ml}$) released 25% of the glycosaminoglycan from bovine nasal cartilage discs during 4 h incubation at 37°C .

Lack of effect on elastin

³H-Labelled elastin was treated with histolysin ($3 \mu\text{g}/\text{ml}$) in 20 mM-Tris/HCl buffer (pH 7.5)/2 mM-EDTA/2 mM-dithiothreitol for 16 h at 30°C , and the insoluble elastin was removed by centrifugation (Mason *et al.*, 1986a). No solubilization of radioactivity attributable to elastin degradation was detected.

Fig. 4. Effect of histolysin on the morphology of human fibroblasts in culture

Human fibroblasts were cultured and treated as described in the text. (a) Control cells exposed to dithiothreitol and EDTA, but not histolysin; (b) cells exposed to $0.5 \mu\text{g}$ of histolysin/ml of culture medium.

Effect on cells in culture

Human skin fibroblasts were cultured in square plastic dishes (400 mm^2 compartments, Sterilin, Feltham, U.K.) in Dulbecco's modified Eagle medium containing 10% fetal bovine serum. Before the experiment, the cells were washed with serum-free medium. To each compartment, $500 \mu\text{l}$ of serum-free medium containing $0.5 \mu\text{g}$ of enzyme/ml was added. After 16 h incubation at 37°C , $10 \mu\text{l}$ of 100 mM-EDTA/100 mM-dithiothreitol stock solution was added to each well. After 1 h the cells were observed to be rounded and detached from the plastic, whereas controls without the enzyme continued to grow normally (Fig. 4). The Trypan Blue dye exclusion test showed no evidence of cell killing. The introduction of 2 mM-dithiothreitol and EDTA was considered not too far from biological reality, in that *E. histolytica* trophozoites grow in the intestine at low oxygen tension, and the EDTA concentration was below that of Ca^{2+} and Mg^{2+} in the culture medium.

DISCUSSION

On the basis of its activity against Z-Arg-Arg-NHMec, Lushbaugh *et al.* (1985) described the cysteine proteinase of *E. histolytica* as cathepsin B. It is true that

activity against Z-Arg-Arg-NHMec distinguished cathepsin B from the other mammalian lysosomal cysteine proteinases, cathepsins L and H (Kirschke & Barrett, 1987), but such activity is by no means sufficient to identify an enzyme as cathepsin B. The cysteine proteinase of *E. histolytica* that we propose to call histolysin differs from mammalian cathepsin B in many respects. Thus, cathepsin B has a higher k_{cat}/K_m value for Z-Phe-Arg-NHMec than it has for Z-Arg-Arg-NHMec ($1.46 \times 10^6 \text{ M}^{-1} \cdot \text{s}^{-1}$ versus $0.88 \times 10^6 \text{ M}^{-1} \cdot \text{s}^{-1}$; Kirschke & Barrett, 1987), whereas the ratio is very much reversed for histolysin ($0.012 \times 10^6 \text{ M}^{-1} \cdot \text{s}^{-1}$ versus $87 \times 10^6 \text{ M}^{-1} \cdot \text{s}^{-1}$). Cathepsin B rapidly and irreversibly loses activity at pH values above 7, whereas histolysin is stable up to pH 9.5. Histolysin is 200-fold more powerfully inhibited by chicken cystatin than cathepsin B, but 80-fold more slowly inactivated by E-64. Also, the tentative N-terminal sequence of histolysin does not show the four-residue insertion (residues 6a-d) characteristic of cathepsins B (or the extra residue 12a seen in cathepsins H). For these reasons, it is not appropriate to describe the cysteine proteinase of *E. histolytica* as cathepsin B.

It is expected that a proteinase that plays a role in tissue invasion will show activity against extracellular structural macromolecules, and it has been pointed out that the degradation of basement membrane is a crucially important step (Liotta, 1986). We therefore consider it very significant that we have found histolysin to degrade human kidney glomerular basement membrane.

Histolysin causes rounding of cells in culture, which would be consistent with the possibility that it is identical with the cytopathic factor and enterotoxin described by previous workers (see the Introduction). We did not see evidence of cytotoxicity, however, so this effect could have been due to the degradation of extracellular proteins involved in cell attachment.

The activity of histolysin against basement-membrane collagen, taken together with its property of detaching cells, would allow the enzyme to play a major role in the penetration of the epithelial lining of the gut. The proposed name, histolysin, which derived initially from the species name of *E. histolytica*, may therefore prove to be appropriate to its biological function also.

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