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MULTIPLE ACID PROTEINASES IN THE CELLULAR SLIME MOULD *DICTYOSTELIUM DISCOIDEUM*

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Summary

Proteinase activity in the cellular slime mould *Dictyostelium discoideum* has been analyzed by electrophoresis on polyacrylamide gels containing denatured heamoglobin. At least eight bands due to acid proteinases have been defined using extracts of myxamoebae, four bands A—D which move faster than the fifth and major band E, a minor band E′ which moves just behind E and two slow bands G and H. Fruiting body formation was accompanied by the appearance of one new proteinase band F. The proteinases were present in extracts of both axenically-grown and bacterially-grown cells. Differences between the pH dependence and stability of the individual proteinases were detected. Inhibitor studies suggested that the faster proteinases A—D may be cathepsin B-like, whilst the slower enzymes E, E′ and F do not fit readily into any known group of proteinases since they were sensitive to $HgCl_2$ but not to other inhibitors of cathepsin B and not to inhibitors of cathepsin D-like proteinases under standard conditions. None of the proteinases was apparently formed during or after preparation of extracts and the proteinases could be re-run on polyacrylamide gels to give only the band expected from the first run. The bands are believed to reflect multiple proteinase activities within the cell.

Introduction

Differentiation in a variety of microorganisms is accompanied by increased proteolytic activity observed as both an elevated rate of intracellular protein breakdown [1—5] and increased levels of proteinase activity [2,5—10]. Although little is known about the regulation of proteolysis during microbial

Abbreviations: PMSF, phenylmethylsulphonyl fluoride; TLCK, *n*-α-*p*-tosyl-L-lysine chloromethyl ketone HCL; TPCK, 1-chloro-4-phenyl-3-tosylamido-L-butan-2-one; DAN, diazoacetyl-DL-norleucine methyl ester.

differentiation, it is probable that proteinases are involved in a number of important processes including the release of amino acids from pre-existing proteins to allow de novo synthesis of developmental proteins during conditions of starvation, the modification of proteins to produce specific changes in property (this could initiate a cascade of secondary effects) and the selective inactivation of proteins which might interfere with the developmental process.

The cellular slime mould *Dictyostelium discoideum* has been used extensively as a model system for the study of differentiation [11]. During the formation of fruiting bodies from myxamoebae the cellular protein level decreases [12–15] and the amino acids released are believed to provide an important source of energy for this developmental phase of the life cycle. It has been suggested that the ammonia formed during amino acid catabolism has a role in morphogenesis [16]. A number of enzymes are also inactivated at specific times during development (see ref. 11).

Although proteinases must be involved in these processes, little study of them has been made in *D. discoideum*. Acid proteinase activity has been described: its specific activity remains constant throughout development [17, 18]. In a more recent study [19] it was shown that the properties of acid proteinase activity in crude extracts differ from those reported for many other proteinases with low optimum pH values, the cathepsin D-like proteinases, from a number of other organisms.

It would appear unlikely that all of the proteolytic events in the life cycle of *D. discoideum* could be due to a single acid proteinase. Indeed the protection of proteins both in vivo [20] and in vitro [20,21] by proteinase inhibitors which did not affect the acid proteinase activity in cell extracts (unpublished data) suggests that other proteinases must be present. We have therefore undertaken a more detailed study of proteinase activities in *D. discoideum* using electrophoresis on polyacrylamide gels containing denatured haemoglobin [22] to analyze cell extracts. We report the detection of multiple acid proteinases, describe their properties and the changes in their activity that occur during development.

Materials and Methods

Organism and culture conditions. Unless otherwise indicated cells of *D. discoideum* strain AX2 were used and were grown in HL5 medium containing 86 mM glucose as described by Watts and Ashworth [23]. When required, bacterially-grown cells were obtained by inoculating *D. discoideum* onto SM agar plates [24] together with *Klebsiella pneumoniae*. For developmental studies, cells were harvested, washed and deposited on Millipore filters over pads saturated with buffered salts solution exactly as described by Hames and Ashworth [15]. All incubations were carried out at 22°C.

Extract preparation. Extracts were prepared by harvesting and washing the cells (by centrifugation for vegetative cells and by washing them directly off filters for developing cells) and finally resuspending them in water. Occasionally the cells were resuspended in buffer: the details of the buffers used are given in the Results. Triton X-100 was added to the cell suspension to give a final concentration of 0.1% (v/v) and the suspension centrifuged at 7500 × *g*

for 10 min. The supernatant was retained and stored frozen when necessary.

Proteinase assay. The total proteinase activity in cell extracts was assayed by the method described previously [19] using Hide Powder Azure as substrate.

Polyacrylamide gel electrophoresis. The method used was adapted from that of Andary and Dabich [22]. Separating gels (pH 8.5) were prepared containing 12% (w/v) acrylamide, 0.075% (w/v) bisacrylamide and 0.2% (w/v) haemoglobin (denatured by incubation of a 2.5% (w/v) solution in 0.1 M Tris-HCl (pH 7.5) at 70°C for 5 min followed by sonication for 5 min) and polymerized by ammonium persulphate. 0.5 cm stacking gels (pH 6.8) were prepared containing 2.5% (w/v) acrylamide, 0.6% (w/v) bisacrylamide and were photopolymerized. Samples of cell extract containing up to 250 μg protein in 20% (w/v) sucrose were placed on top of the stacking gel. The upper chamber (cathode) buffer was 0.03 M sodium tetraborate, 0.01% (w/v) bromophenol blue and the lower chamber (anode) buffer was 5 mM Tris/38.5 mM glycine (pH 8.5). Electrophoresis was carried out at 4°C at 1 mA/gel until the tracking dye had entered the separating gel and then at 2 mA/gel. Because the tracking dye was lost during the development of proteinase bands its final position was marked by cutting off the small portion of the gel below it.

The proteinase bands were developed by transferring the gels to an appropriate buffer: for pH 2—3.5, 0.1 M glycine HCl, for pH 3.5—5.5, 0.1 M sodium acetate/acetic acid and above pH 5.5, 0.1 M sodium phosphate. At least two changes of buffer were made during the initial 30 min. The band development was stopped by transferring the gels to 0.1 M Tris-HCl (pH 7.5), 0.1% (w/v) nigrosin. After staining for at least 8 h, the gels were destained by repeated washing with 0.1 M Tris-HCl (pH 7.5). Densitometric scans of the gels at 550 nm were made using a Unicam SP1800 spectrophotometer.

A similar procedure was adopted for electrophoresis with polyacrylamide gels containing *D. discoideum* protein. Axenically-grown cells were harvested, washed and resuspended at a density of 2.5×10^8 cells/ml (approx. 25 mg protein/ml) in 0.1 M Tris-HCl (pH 7.5) and heated at 70°C for 5 min. The suspension was sonicated for 5 min and then used for the preparation of gels containing 0.2% (w/v) protein.

Protein assay. Protein was assayed by the Coomassie G250 method of Sedmak and Grossberg [25] using bovine albumin as standard.

Chemicals. Haemoglobin (bovine type II), bovine albumin (fraction V), nigrosin, Triton X-100, Brilliant Blue G (Coomassie Brilliant Blue G), ϵ-aminocaproic acid, phenylmethylsulphonyl fluoride (PMSF), *n*-α-*p*-tosyl-L-lysine chloromethyl ketone HCl (TLCK) and 1-chloro-4-phenyl-3-tosylamido-L-butan-2-one (TPCK) were obtained from Sigma London Chemical Co. Ltd., Poole, U.K. Hide Powder Azure was a product of Calbiochem. Ltd., Bishops Stortford, U.K. Leupeptin and pepstatin were purchased from the Protein Research Foundation, Osaka, Japan. Diazoacetyl-DL-norleucine methyl ester (DAN) was prepared by the method of Rajagopalan et al. [26].

Results

Detection of multiple proteinases in extracts of axenically-grown myxamoebae

Following electrophoresis proteinase activities were detected as clear bands

where the haemoglobin in the polyacrylamide gel had been digested. With cell extracts of axenically-grown myxamoebae multiple bands developed when gels were incubated at pH 5 or less. Below pH 3 incubation times had to be limited to less than 2.5 h as the haemoglobin solubilizes and is lost from the gel making bands increasingly more difficult to detect with time. Above pH 3 the gels could be incubated for a period of several days if necessary, but the standard incubation period was 18 h.

Fig. 1 shows typical patterns of the bands that develop in the pH range 2—6. At pH 2 four relatively fast-moving bands were detected, named A, B, D and E in order of decreasing electrophoretic mobility (towards the abode). Band E represents the major proteolytic activity in most extracts. In addition two slow moving bands G and H were sometimes, although not always, observed. At higher pH values longer incubation time was required for the appearance of bands. This was consistent with the low optimum pH (approx. pH 2) determined for total proteinase activity in cell extracts [17—19]. At pH 4 bands A and E developed, together with band C which ran at a position between those observed for bands B and D at pH 2. A slower band E′ sometimes appears above band E (see Fig. 3). At pH 5 band C was more prominent and it would appear that the optimum pH of the enzyme responsible is higher than that of the other proteinases. Gels incubated at or above pH 6 failed to develop any

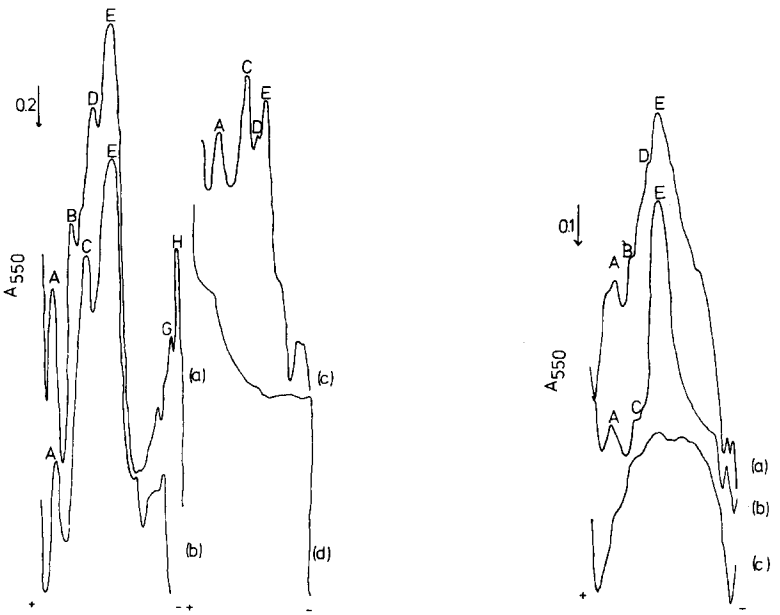

Fig. 1. The effect of pH on the development of proteinase bands. Samples of an extract from axenically-grown myxamoebae containing 200 μg protein were electrophoresed and the gels incubated for 2 h at pH 2 (a); for 18 h at pH 4 (b); pH 5 (c) and pH 6 (d). In this and all other figures the scans from entire gels are represented and all visible bands are labelled.

Fig. 2. Band patterns observed after electrophoresis on gels containing denatured *Dictyostelium* protein. Samples of an extract from axenically-grown myxamoebae containing 250 μg protein were electrophoresed and the gels incubated for 2 h at pH 2 (a); for 18 h at pH 4 (b) and pH 6 (c).

bands. Thus transfer to pH 7.5 buffer prevented all further development of bands and was used for staining the undigested haemoglobin remaining with nigrosin.

Table I indicates the amounts of protein that must be loaded onto gels for the development of bands A—E′. The development of bands G and H was variable and did not always correlate with the amount of protein loaded. Although the method does provide information about the relative levels of proteinases present in an extract, it can not be used for the precise determination of quantitative levels of activity.

The band patterns observed were not significantly altered if cell extracts were prepared by sonication instead of Triton treatment, nor if extracts were dialyzed overnight or the total protein precipitated with ammonium sulphate and redissolved in fresh buffer before electrophoresis.

The electrophoretic mobility of the proteins responsible for the bands was not affected by the presence of the haemoglobin in the gel. Although the bands were more difficult to observe in gels containing less haemoglobin, those that were visible ran in the same position regardless of the concentration of haemoglobin used. When gels were prepared from denatured *Dictyostelium* protein a similar pattern was observed (Fig. 2), but the bands were less well resolved.

Changes in band pattern during development

The specific activity of total acid proteinase activity does not change during the developmental phase of the life cycle [17,18]. However a change in band pattern was observed. Proteinase activity was released from fruiting bodies by Triton treatment or by sonication for 3 min and with both treatments a new proteinase band F was observed. This proteinase was found both in extracts of spores, removed from fruiting bodies by touching the spore masses with a glass slide, and in extracts of the cells remaining on the filter after spore removal (these were predominantly stalk cells although some spores were probably present) (Fig. 3). Only a small proportion (10% or less) of the total spore protein was released into the spore extract, but with the "stalk cell" extract most of the protein was solubilized. When extracts were prepared from cells at earlier

TABLE I

BANDS DETECTED ON GELS LOADED WITH VARYING AMOUNTS OF CELL PROTEIN (MYXAMOEBAL EXTRACT)

—, no band observed; +, no distinct visible band but band appears on densitometric scan; ++, distinct visible band.

Protein/ gel (μg)	Gels incubated at pH 2 for 2 h				Gels incubated at pH 4 for 18 h				
	A	B	D	E	A	C	D	E	E′
5	—	—	—	—	—	—	—	+	—
10	—	—	—	++	—	—	—	++	—
20	—	+	—	++	—	++	—	++	—
50	+	++	—	++	++	++	+	++	+
100	++	++	+	++	++	++	+	++	+
200	++	++	++	++	++	++	+	++	—

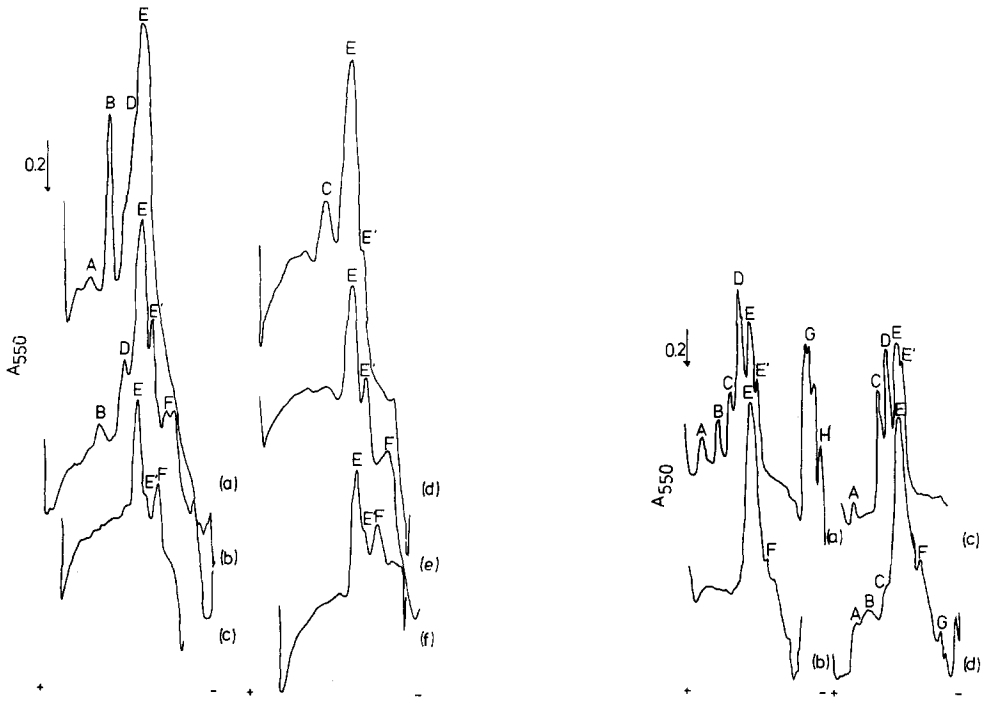

Fig. 3. Comparison of band patterns with samples from myxamoebae and cells from fruiting bodies. Samples of extracts were electrophoresed and the gels incubated for 2 h at pH 2 (a—c) or for 18 h at pH 4 (d—f). Myxamoebal extract containing 100 μg protein (a,d); "stalk cell" extract containing 100 μg protein (b,e) and spore extract containing 120 μg protein (c,f). The latter two extracts were prepared from fruiting bodies after 26 h of development.

Fig. 4. Proteinase band patterns with extracts prepared from cells grown on *K. pneunomiae*. Wild type strain NC4 was used. Samples of extract were electrophoresed and the gels incubated for 2 h at pH 2 (a,b) or for 18 h at pH 4 (c,d). Extract from myxamoebae containing 100 μg protein (a,c) and extract from fruiting bodies (formed 24 h after the plates had cleared of bacteria) containing 130 μg protein (b,d).

developmental stages and analyzed, band F was detected only after 21 h of development, the culmination stage when differentiation of stalk cells and spores occurs. The proteinase was not observed if cells were incubated on filters in the presence of 60 mM ϵ-aminocaproic acid, an agent which blocks post-aggregation development [27], and so its appearance is dependent on a normal developmental programme. It was still detected when extracts of myxamoebae were mixed with fruiting body extracts. Therefore the change in activity was not due to the loss of an inhibitor present in myxamoebal extracts. Proteinase F was active from pH 2 to 5.

Other changes in band pattern also occurred. In general the faster bands A—D were smaller with extracts of developing cells. Proteinase E remained the major activity throughout.

Proteinase in bacterially-grown cells

The band patterns obtained after electrophoresis of extracts of bacterially-grown myxamoebae (washed free of bacteria by centrifugation) were similar to

those observed with axenically-grown cells. Fig. 4 shows results from the wild-type strain NC4 and identical scans were obtained using AX2. The resolution of individual bands was considerably better than with extracts of axenically-grown myxamoebae. Indeed band D was much more prominent. No bands formed with extracts of *K. pneumoniae*, the bacterium on which the myxamoebae were grown. If myxamoebae were allowed to form fruiting bodies by maintaining them on SM agar plates after all the bacteria had been consumed similar changes in band pattern to those accompanying the development of axenically-grown cells were observed, including the appearance of band F.

Effects of inhibitors on proteinase bands

Proteinases can be characterized on the basis of their sensitivity to inhibitors. The acid proteinase present in crude myxamoebal extracts is completely inhibited by $HgCl_2$ [19], and the more recent finding that leupeptin also inhibits the activity in crude extracts (at pH 3, 52.5% inhibition at a concentration of 6 μg/ml) suggests that a large proportion of the activity is cathepsin B-like [28] although the optimum pH is very low. The effects of a number of inhibitors on the development of bands after electrophoresis were examined. $HgCl_2$ at concentrations above 0.1 mM prevented the development of all bands (Fig. 5). Leupeptin considerably reduced the activity of the faster proteinases A-D and there was a slight reduction in the size of the major band E (Fig. 5). $CuCl_2$ and iodoacetic acid had similar effects to leupeptin but appeared to have little effect on band E: this was consistent with their effects on the total activity in cell extracts at pH 3 (10 mM $CuCl_2$ reduced the activity by 20% and 1 mM iodoacetic acid by 27%). Other proteinase inhibitors including pepstatin, DAN,

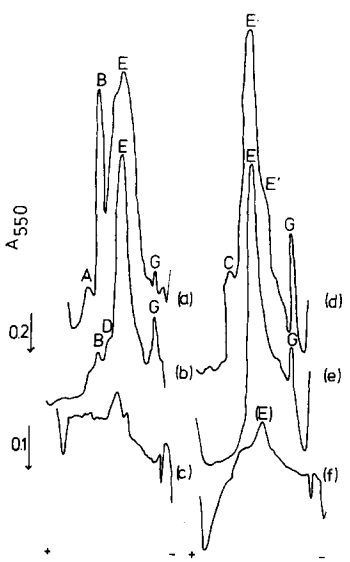

Fig. 5. The effect of leupeptin and $HgCl_2$ on the development of proteinase bands. Samples of extract from axenically-grown myxamoebae containing 200—220 μg protein were electrophoresed and the gels incubated for 2 h at pH 2 (a—c) or for 18 h at pH 4 (d—f). 7 μg leupeptin/ml (b,e) or 0.1 mM $HgCl_2$ (c, f) were added to the development buffer.

TLCK, TPCK, PMSF and ϵ-aminocaproic acid had no effect on band development. $SnCl_2$ was also without effect, although it does severely inhibit the activity against Hide Powder Azure [19]. Preincubation of extracts with DAN, TLCK, TPCK or PMSF before electrophoresis did not result in any significant changes in band pattern (see Table II).

The effects of the inhibitors suggested that the proteinases were all thiol enzymes, and dithiothreitol, cysteine and mercaptoethanol, at a concentration of 5 mM, all enhanced the development of bands. None caused additional bands to appear. The reducing agents did not have an activating effect on the total proteinase activity in myxamoebal extracts at pH 3, however.

Like the proteinases present in myxamoebal extracts, the proteinase responsible for the developmental band F was severely inhibited by $HgCl_2$. It was not inactivated by either leupeptin or pepstatin.

Effect of pre-electrophoresis conditions on the proteinase band patterns

The possibility that some of the proteinases responsible for the bands might have arisen as a result of modifications that occurred during or after extract preparation was investigated. The results in Table II do not support such a possibility. Formation of a proteinase species in vitro might be reflected by an increase in its activity during a pre-electrophoresis incubation period (probably accompanied by a loss of another species) and might be prevented by inhibiting proteinase activity during extraction.

The activity of a number of the proteinases, in particular A—D, decreased during pre-incubation, but none increased. Proteinase inhibitors including TLCK and PMSF, which protect other proteins at neutral pH values [20,21], and $HgCl_2$, which would have inhibited the proteolytic activity of the acid proteinases themselves, did not prevent the appearance of any of the proteinases in fresh extracts. It would appear that the band patterns observed with fresh extracts did represent the proteinases present at the time the extracts were prepared. Proteinase inhibitors did not block inactivation during pre-incubation and some of the proteinases must be inherently unstable.

All the proteinases survived short periods of incubation at temperatures up to 50°C. Pre-incubation at 55°C resulted initially in a decrease in the electrophoretic mobility of the proteinases and a loss of activity only after 10 min: A and C were the first activities to decrease and then B, D and E (Fig. 6).

Secondary electrophoresis of proteinases

To determine whether the proteinases responsible for the bands were unchanged by electrophoresis the following procedure was carried out. Electrophoresis of myxamoebal extract was carried out as normal and individual gels incubated for 1 h at pH 2 to determine the position of band E. The gels were then transferred to 38.5 mM Tris-HCl (pH 8.5), 0.575% (v/v) *N,N,N',N'*-tetramethylethylenediamine and allowed to equilibrate. 1 cm section of the gels were then cut, one from the bottom of each gel in the region of band A, one above the first section in the region of bands B and C, one containing band E and one at the top of the gel. These sections were then placed on top of fresh stacking gels with a small vol. of 20% (w/v) sucrose between to allow an even flow of current and material. Electrophoresis was carried out as before and the

TABLE II

EFFECT OF VARIOUS TREATMENTS PRIOR TO ELECTROPHORESIS ON THE SUBSEQUENT DEVELOPMENT OF PROTEINASE BANDS

Band patterns were observed after development at pH 2 and 4. Samples subjected to electrophoresis immediately after each treatment had commenced showed no differences in the band pattern from untreated controls.

Treatment of myxamoebal extract before electrophoresis				Effect on band pattern
Buffer	Addition	Time (h)	Temperature (°C)	
0.1 M Glycine HCl, pH 2 *	—	24	25	Decrease in size of A, B, C, D
0.1 M Glycine HCl, pH 2 *	0.2 mM $HgCl_2$ *	24	25	Decrease in size of A, B, C, D
0.1 M Sodium acetate/acetic acid, pH 4 *	—	24	25	Complete loss of A, B, C, D
0.1 M Sodium acetate/acetic acid, pH 4 *	0.2 mM $HgCl_2$ *	24	25	Complete loss of A, B, C, D
0.02 M Sodium phosphate, pH 6 *	—	24	25	Complete loss of A
0.02 M Sodium phosphate, pH 6 *	200 μg PMSF/ml *	24	25	Complete loss of A
0.02 M Sodium phosphate, pH 6 *	2 mM TLCK *	24	25	Complete loss of A
0.025 M Sodium acetate/acetic acid, pH 4.8	1 mM cupric acetate + 130 μg DAN/ml	2	15	No effect
0.025 M Sodium acetate/acetic acid, pH 4.8	25 mM cupric acetate + 3.25 mg DAN/ml	2	15	No effect
Water	—	2	40	No effect
Water	—	2	50	Decrease in size of all bands
Water	—	2	60	Complete loss of all bands

* Present during and after extraction; treatment times and temperatures refer to those after extract preparation was completed.

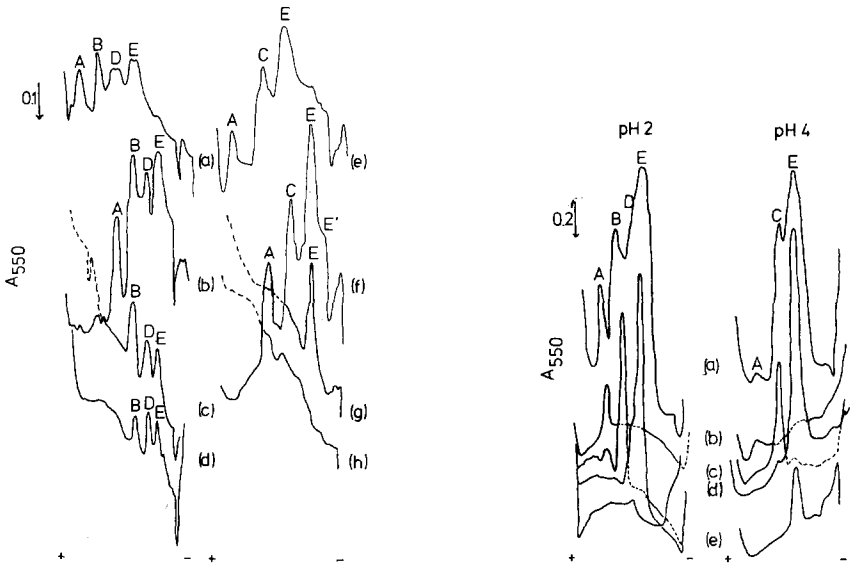

Fig. 6. The effect of preincubation at 55°C on the proteinase band pattern. An extract prepared from axenically-grown cells was incubated at 55°C and samples withdrawn at different times. Samples, each containing 230 μg protein, were electrophoresed and the gels incubated for 2 h at pH 2 (a—d) or for 18 h at pH 4 (e—h). Pre-incubation times: 0 min (a,e); 2 min (b,f); 10 min (c,g) and 20 min (d,h).

Fig. 7. Secondary electrophoresis of proteinases. Samples of an extract from axenically-grown myxamoebae containing 200 μg protein were electrophoresed. Two gels were treated as described in the text with four sections cut and the samples electrophoresed again. The secondary gels were incubated for 2 h at pH 2 or for 18 h at pH 4. Duplicate primary gels incubated as above (a); secondary gels using sections cut from bottom (b) middle (c,d) and top (e) of the primary gel.

gels developed at either pH 2 or 4. Fig. 7 shows that the band patterns on these secondary gels were characteristic of that portion of the first gel which had been used. Thus each band represented a species that was unaltered by the electrophoresis process.

Discussion

The results presented in this paper demonstrate multiple forms of acid proteinase in extracts of *D. discoideum*. They were present throughout the life cycle, although the relative levels of the individual enzymes did change. It is almost certain that the bands do represent multiple species within the cell, although we are not yet able to rule out the possibility that there are a smaller number of proteinases, and that differences in electrophoretic mobility may be due to the binding of other molecules to the enzyme(s). If this were the case, however, the molecules must be tightly bound (they were not removed by dialysis or precipitation of protein with ammonium sulphate) yet do not affect the activity of the enzyme. An attempt to remove any bound molecules by denaturation and dialysis in the presence of 6 M guanidium hydrochloride was unsuccessful as no activity was recovered after the removal of the denaturing agent.

The proteinases described can be divided into two groups. The faster proteinases A—D were more sensitive to pre-incubation and were sensitive to a number of inhibitors of cathepsin B [28] and thus resemble other proteinases identified in *Tetrahymena pyriformis* [29], rat liver lysosomes [30,31] and calf lymph nodes [32]. However the *Dictyostelium* proteinases generally have lower optimum pH values, unlike human cathepsin B [28] they survive electrophoresis at pH 8.5 and, although enhanced by them, are not dependent on reducing agents for activity. The slower proteinases E, E′ and F were sensitive to $HgCl_2$ but not to the other cathepsin B inhibitors. Unlike many other acid proteinases they were not inhibited by pepstatin or DAN, although a purified preparation of *Dictyostelium* proteinase (shown by electrophoresis to contain proteinase E, unpublished) was rapidly and completely inactivated by DAN [19]. It is possible that the presence of *Dictyostelium* proteins or haemoglobin in the gel prevented inactivation. Nevertheless these proteins fit neither into a cathepsin B-like group nor a cathepsin D-like group.

Whilst this work was in progress characterization of *Dictyostelium* proteinase was also being undertaken by Fong and Rutherford [33]. They have reported a cathepsin D-like activity, assayed in cell extracts at pH 2.5—2.75, and a cathepsin B-like activity assayed at pH 5.5. At pH 2.5 the predominant activity on gels was proteinase E, and so their cathepsin D-like proteinase may correspond to E, although Fong and Rutherford did observe slight inhibition with pepstatin and inactivation by DAN, albeit at high concentrations. The cathepsin B-like activity had properties similar to the faster proteinases, and since proteinase C is predominant at pH 5.0 their assay may have measured proteinase C. Interestingly, Fong and Rutherford found that this activity was inhibited by TLCK. As our results indicate, assays of crude cell extracts probably measure the sum of the activities of more than one enzyme which must be separated from one another before they can be characterized clearly. The electrophoresis method will be useful in monitoring the success of different separation procedures.

The acid proteinases described here may not represent all the cellular proteolytic activities. The properties of the slow enzymes G and H have still to be determined, and additional bands have been observed on some gels associated with bands A, D, E and F. No proteinase activity was detected above pH 6 even when electrophoresis was carried out at pH 4.5 as described by Andary and Dabich [22]. If neutral and alkaline proteinases are present in *D. discoideum* alternative substrates may be needed.

At present we can make few conclusions about the role of the individual proteinases during the life cycle. Wiener and Ashworth [17] found that 68% of the total activity at pH 2 was associated with the lysosomal pellet, but the subcellular localization of the individual proteinases is not yet known. Lysosomal proteinases, including cathepsin B, have been implicated in protein turnover in higher organisms [34,35]. The lack of any dramatic change in the proteinase pattern on starvation of the myxamoebae suggests that the switch from processing of food proteins to autolysis is not controlled through changes in the levels of acid proteinases. Proteinase F is not associated with early development and its appearance coincides with the formation of fruiting bodies. Its association with spores could be related to a need for proteolytic activity during ger-

mination. In the closely related *Polysphondylium pallidum* O'Day [36] has proposed a role for proteinases in microcyst germination. The ease with which proteinases were released from fruiting bodies suggests that some activity may be extracellular. Rossomando et al. [37] have recently reported that proteinase activity is excreted at the onset of starvation.

A more detailed study of the individual proteinases should lead to a better understanding of their role in *D. discoideum*. Studies in which proteinase activities are manipulated either by the use of inhibitors (in our hands this has been unrewarding to date) or through the isolation of proteinase mutants should be of particular importance in future.

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