Acid-activatable Cysteine Proteinases in the Cellular Slime Mold
Dictyostelium discoideum*

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Studies of the cysteine proteinases of the cellular slime mold Dictyostelium discoideum have been aided by a simple acid treatment step that was incorporated into the standard one-dimensional gelatin-sodium dodecyl sulfate-polyacrylamide gel electrophoresis assay procedure. The step involved immersing the separating gel in 10% (v/v) glacial acetic acid for 30–60 s immediately after electrophoresis. This modified approach revealed the presence of acid-activatable forms of some enzymes with noticeable increases in their ability to hydrolyze gelatin, a substrate present in the sodium dodecyl sulfate-polyacrylamide gels, and peptidyl amidomethylcoumarins. The activation has been analyzed using extracts of dormant spores from which cysteine proteinase activity had previously appeared low or virtually absent. The major acid-activatable proteinase had an apparent molecular mass of 48 kDa. Its activation was not due to autocatalysis as it was not prevented by mercuric chloride, an inhibitor of the enzyme, and was not accompanied by a significant change in electrophoretic mobility. It was most likely due to a conformational change and/or the removal of a low molecular weight inhibitor. The acid treatment has also revealed the presence of acid-activatable cysteine proteinases in vegetative cells, in which cysteine proteinase activity is present at high levels, as well as among enzymes from the developmental cells which have much lower cysteine proteinase activity. Indeed novel developmental forms were detected at some stages. These results provide additional insight concerning cysteine proteinase expression at various stages during development in the slime molds. A developmental model is presented which suggests that the crypticity of the cysteine proteinases in dormant spores may be governed by proton pumps and endogenous lysosomotropic agents.

Dictyostelium discoideum possesses multiple forms of cysteine proteinase (ddCP) (1) which have been shown to differ from one another during growth (2) and development (3). Net cysteine proteinase activity is high in vegetative cells but decreases during the developmental phase (3), partly as a result of secretion (3, 4). Consequently, both the stalk cells and the dormant spores that are formed during development possess very low levels of detectable activity; however, some cysteine proteinase activity is found in the extracellular matrix surrounding spores in fruiting bodies (5). Net intracellular cysteine proteinase activity returns to appreciable levels during spore germination (5). In addition to the cysteine proteinases, D. discoideum also produces an aspartic proteinase (ddAP58) whose level remains relatively constant throughout the life cycle.

There are three developmentally regulated genes present in D. discoideum whose sequences indicate that they encode cysteine proteinases. Predicted products of the cprA (CP1) and cprB (CP2) genes have considerable similarity to members of the papain superfamily (6, 7), whereas the product of the third gene (CP3) may be a truncated cysteine proteinase (8). Intriguingly, all three genes are transcribed at times when net cysteine proteinase activity is decreasing, and hence their transcription appears inconsistent with activity levels of the cysteine proteinases detected during various stages of development (1). To date, attempts to show a meaningful relationship between the transcription of the developmentally regulated cysteine proteinase genes and the activity levels of the cysteine proteinases recorded during development have been unsuccessful. However, the possibility that gene products may have been present as inactive forms was not examined in detail.

Studies of many lysosomal enzymes, including cysteine proteinases, have indicated that the mechanism involved in the production of a mature and active conformation requires the cleavage of a pro-sequence. This cleavage, with mammalian cathepsin L (9–12) and cathepsin B (13) for example, may be brought about in vitro at acid pH, suggesting that the lower pH of the lysosome may be responsible for triggering activation in vivo. In D. discoideum, comparisons of preprocessed and mature forms of various lysosomal enzymes have indicated molecular weight differences that could be clearly resolved using SDS-PAGE techniques (14). It has also been reported that “increasing the lysosomal/endosomal pH from 5.4 to 6.4 with ammonium chloride does not prevent proper sorting of lysosomal hydrolases, but does prevent the cleavage of the lysosomally localized intermediate forms of the enzymes” (15). Thus, it appears plausible that in vivo, the optimal activity of the lysosomal cysteine proteinases might require an acidic pH.

We will describe the conditions required for an in vitro acid activation of the cysteine proteinases in D. discoideum. The results of this work provide information that may relate to the transcriptional and translational events governing the developmentally regulated cysteine proteinase genes in the cellular slime molds and support a proposed model whereby the crypticity of the cysteine proteinases in dormant spores is controlled by proton pumps and endogenous lysosomotropic agents (16).

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§ The abbreviations used are: PAGE, polyacrylamide gel electrophoresis; Boc, N-(tert-butoxycarbonyl); Bz, N-benzoyl; CHN2, diazomethane; NMec, 7-(4-methyl)coumarylamide; Suc, N-succinyl; Z, N-benzoxycarbonyl.
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EXPERIMENTAL PROCEDURES

Materials

Gelatin (porcine type I), phenylmethylsulfonyl fluoride, Boc-Val-Leu-Lys-NMec, Z-Phe-Arg-NMec, Bz-Phe-Arg-NMec, Suc-Lys-Tyr-NMec, and Z-Arg-Arg-NMec were obtained from Sigma. E-64 and pepstatin were from Scientific Marketing Associates, Barnet, U. K. H-Leu-Val-Tyr-NMec, Z-Phe-Phe-CHN₂, and Z-Phe-Ala-CHN₂ were from Bachem, Bubendorf, Switzerland. All other chemicals used were of the highest purity available from their commercial suppliers.

Methods

Organisms Used and Sample Preparation—Experiments were performed using D. discoideum strains SG1 (ATCC 48440) and, for axenically grown myxamoebae, AX2 (ATCC 24397). Strain SG1 was grown in the presence of Escherichia coli for spore production (5) and in association with Klebsiella aerogenes when myxamoebae and developmental cells were required. Axenically grown AX2 were grown on HL5 medium (2). Extracts from dormant spores as well as from the late stages of development were prepared by vortexing with glass beads (5). Spore casings were obtained from dormant spores by centrifuging at 4,500 × g for 2 min to pellet the glass beads and any spores that remained intact, then centrifuging the supernatant for 2 min at 15,000 × g in an Eppendorf multicentrifuge and resuspending the pelleted spore casings in ice-cold H₂O. Cells from all other stages were harvested and lysed with 0.1% (w/v) Triton X-100 (4) and then either stored frozen at −20 °C or used immediately in experiments. Freezing had no apparent effect on the levels of proteinase activity observed.

Electrophoresis and Proteinase Detection—Proteinases were detected following their electrophoretic separation using the gelatin-SDS-PAGE assay system, which consisted primarily of 30% (w/v) acrylamide mini-gels (0.75 mm) containing 0.2% (w/v) gelatin, Tris-HCl buffer, pH 8.8, and 0.1% (w/v) SDS (3, 17). For acid activation, the standard treatment involved immersing the separating gel in 10% (v/v) acetic acid for 30–60 s immediately after the electrophoretic separation was complete and prior to its subsequent washing with 2.5% (v/v) Triton X-100 for 30 min. Proteinase bands were then developed by incubating the gel at 25 °C for 16 h in acetic acid/sodium acetate buffer, pH 4.0, containing 1 mM dithiothreitol. Alternatively, proteinase bands were detected with fluorogenic peptidyl amidomethylcoumarins using a modification of the method described previously (17). Unless otherwise indicated, gelatin was not included in these gels. Following electrophoresis, gels were transferred to 0.1 x sodium phosphate buffer, pH 6.0, containing 20 mM cysteine, either immediately or after a 30-s incubation in 10% (v/v) acetic acid. Gels were incubated for 10 min with one change of buffer and then transferred to the same buffer containing 0.05 mM substrate. Bands of proteinase activity were recorded as described (17) during a 60-min incubation at room temperature.

When a second electrophoretic separation was required, samples were first run under standard conditions using narrow lanes (15-well comb in the Bio-Rad Mini Protein system). One method involved loading two identical samples in adjacent lanes in the center of the gel and, after electrophoresis, the gels were cut between these two lanes. One section of the gel was treated with 10% (v/v) acetic acid as above. The second section was then washed for 5 min five times in Tris-HCl buffer (0.375 M Tris HCl, pH 8.8) containing 0.1% (w/v) SDS and then placed between glass plates for the Mini Protean system so that the original sample lanes ran along the top edge of the gel sections from left to right (bottom). Gaps were then filled with fresh separating gel mix and electrophoresis continued until the marker dye, added to fresh electrophoresis running buffer, had reached the bottom of the gel. Activity was then detected using the fluorogenic substrates Boc-Val-Leu-Lys-NMec as described above. After activity had been detected the gel was stained with Coomassie Blue R-250. An alternative method, used with either gelatin or fluorogenic substrates, involved the following. Samples were run in narrow lanes, as above. After electrophoresis, strips the width of a single lane were cut from the gel, and these were treated, if required, with 10% (v/v) acetic acid for 30 s. Gelatin gels were then incubated with 2.5% (v/v) Triton X-100 for 30 min, and all gels were finally immersed in electrophoresis running buffer (0.25 M Tris, 0.192 M glycine, 0.1% (w/v) SDS). Two gel strips were laid horizontally on the top of a new gel so that they covered the gel, and electrophoresis was carried out until marker dye had reached the bottom of the gel. Activity was detected as described above.

RESULTS

Acid Activation of Spore Proteinases—The standard one-dimensional gelatin-SDS-PAGE technique used for analyzing the proteinases present in D. discoideum (3) involves electrophoretic separation of the enzymes in a sample containing 1% (w/v) SDS and 2.5% (v/v) mercaptoethanol, followed by a wash with 2.5% (v/v) Triton X-100, and then incubation of the gel in an appropriate buffer system, usually acetate buffer, pH 4.0, containing a reducing agent such as 1 mM dithiothreitol or 20 mM cysteine. During an analysis of proteinases present in extracts from dormant spores, we found that if this procedure was interrupted by treating the gels briefly in 10% (v/v) acetic acid before washing with Triton X-100, greater activity levels of some proteinase bands were observed. Without this acid treatment step, the major detectable band of activity corresponded to the aspartic proteinase ddAP58 (5); minor bands apparently corresponding to cysteine proteinases ddCP48 and ddCP43 were occasionally observed using a 16-h incubation time, depending on the amount of protein loaded. After acid treatment the ddAP58 band was unchanged, but the activity of the cysteine proteinases, especially that corresponding to ddCP48, was greatly enhanced (Fig. 1).

Without acid activation, high levels of ddCP48 activity only appeared when heat-activated spores were germinated (5) and rarely if germination occurred by autoactivation. All of the activatable proteinases were present at higher levels in extracts from dormant spores than in samples of spore casings (Fig. 1). Some extracellular matrix proteinases (5), with apparent molecular masses in the 20–30-kDa range, were also activated by the acid treatment. However, the major enzyme detected in the absence of acid treatment (ddCP18 with an apparent molecular mass of 18 kDa) had reduced activity after acid treatment (Fig. 1).

All of the acid-activated enzymes were cysteine proteinases whose activities were blocked when the specific inhibitors E-64 (28 μM), Z-Phe-Phe-CHN₂ (10 μM), or Z-Phe-Ala-CHN₂ (10 μM) were included in the acetic acid/sodium acetate buffer, pH 4.0 (not shown). Inhibitors of other classes of proteinases (serine, 1 mM phenylmethylsulfonyl fluoride; aspartic acid, 14 μM pepstatin; metallo-1, 1 mM EDTA) were without effect.

The activation was due to an in vitro effect on the enzyme and not on the gelatin substrate. When gelatin-containing gels were treated with acetic acid, the subsequent band pattern was identical to that obtained with nontreated gelatin gels. In addition, pretreatment of gelatin stock solutions with 10% (v/v) acetic acid, whether in the presence or absence of SDS, did not affect the observed results (data not shown).

Confirmation that the effect of the acid was on the enzyme rather than substrate was obtained by using peptidyl amidomethylcoumarins. These were not added until after the acid and
subsequent washing steps were completed. Detection of proteinases using these fluorogenic substrates did not necessarily require treatment of the gels with Triton X-100, and gels could simply be washed in phosphate buffer, pH 6.0, before addition of substrate. After acid treatment the same 48-kDa proteinase detected with gelatin as substrate could be seen using Boc-Val-Leu-Lys-NMec as described under "Experimental Procedures." Photographs were taken after 45 min, and then lane 4 was transferred to acetic buffer, pH 4.0, containing 0.02% (w/v) formic acid, which are both stronger acids than acetic acid, was used. Neither 1.7 M HCl nor 10% (v/v) acetic acid for 30-60 s being optimal. Neither 1.7 M HCl nor 10% (v/v) acetic acid, which are both stronger acids than acetic acid, was effective, but 10% (v/v) propionic acid, also a weak acid, did activate the enzymes. Sodium acetate (1.7 M) had no effect. Activation could also be achieved using 0.1 M glycyglycine buffer, pH 2.0, although a longer incubation step was required (2 min). The standard length of the acetic acid treatment was 30-60 s, but even a 15-s incubation period proved sufficient for activation. However, if the treatment was prolonged for more than 60 s less activity was observed, presumably because of subsequent denaturation of the activated enzyme.

It is possible that under these conditions other proteolytic enzymes inactivated the cysteine proteinases. The activity of the spore enzymes toward the fluorogenic substrate, an effect that has also been noted with cysteine proteinases from other stages of D. discoideum. For this reason gelatin was routinely omitted from the gels whenever fluorogenic substrates were used. For the 48-kDa proteinase, Boc-Val-Leu-Lys-NMec was the best fluorogenic substrate tested, and the enzyme also hydrolyzed Z-Phe-Arg-NMec well. The enzyme had lower activity toward H-Leu-Val-Tyr-NMec, Bz-Phe-Val-Arg-NMec, and Suc-Leu-Leu-Arg-NMec. No activity was detectable with Z-Arg-Arg-NMec. In its preference for substrates with bulky amino acids at the Pro2 and Pro3 positions and inability to hydrolyze Z-Arg-Arg-NMec the enzyme resembled mammalian cathepsins L and S rather than cathepsin B (18, 19).

Boc-Val-Leu-Lys-NMec was adopted as the preferred low molecular weight substrate for the detection of acid-activatable spore proteinases. The activity of the spore enzymes toward this substrate was not totally dependent on a reducing agent (not shown), but since a reducing agent was required for the detection of cysteine proteinases at other stages of the D. discoideum life cycle and cysteine was found to be most appropriate, the latter was included routinely at a concentration of 20 mM.

**Conditions Required for Activation of the Spore Cysteine Proteinase**—The activation of the 48-kDa proteinase required a weak acid with an incubation in 10% (v/v) (1.7 M) glacial acetic acid for 30-60 s being optimal. Neither 1.7 M HCl nor 10% (v/v) formic acid, which are both stronger acids than acetic acid, was effective, but 10% (v/v) propionic acid, also a weak acid, did

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2 M. J. North and K. Nicol, unpublished data.
Electrophoretic mobility of the activated proteinase was examined. Since activation has only been achieved with proteinases in gels, two-dimensional electrophoresis was used. Various procedures were tested to minimize a loss of activity of the acid-treated enzyme during the second run, which posed a major problem. However, whenever activation was detectable after the second run, regardless of the procedure used (see "Methods"), the mobility of the major acid-activatable proteinase was not apparently affected by the acid treatment. For example, Fig. 4 shows the result of using large blocks of the first gel for the second run and Boc-Val-Leu-Lys-NMec as the substrate. After the first electrophoresis run one gel block was acid treated, the other not, but the mobility of the proteinase during the second run was the same in both. The lack of effect of acid treatment was confirmed by the location of the activity band on the diagonal line of Coomassie Blue-stained proteins. Any protein whose mobility had changed between the two runs would lie above or below this line.

Acid Activation of Proteinases Present at Other Stages—Vegetative myxamoebae have much higher cysteine proteinase activity than spores (1, 3, 5). Acid treatment did have some effect on the myxamoebal proteinase pattern, although only certain proteinases were affected. With Boc-Val-Leu-Lys-NMec as substrate two proteinases, ddCP48 in bacterially grown cells and ddCP51 in axenic cells were detectable only after acid treatment. The other proteinases did not require acid treatment (Fig. 5A). With gelatin-SDS-PAGE, however, it was not always possible to distinguish any effect of acid treatment (Fig. 5B). The difference between the effects observed with fluorescent substrates and gelatin is likely to be due to the time required for detecting activity (less than 1 h and 16 h, respectively) and may reflect the fact that the vegetative proteinases, unlike the spore proteinase, are activated slowly during incubation at pH 4, the pH of the buffer used to detect bands in gelatin gels.

As development proceeds, cysteine proteinase activity declines. This has been shown using a number of different detection methods, including the standard gelatin-SDS-PAGE technique (1, 3). During the aggregation stage acid treatment enhanced the activity of some of the proteinases, but no new proteinases were detected. Following aggregation, acid treatment revealed the presence of a number of activatable enzymes. These were not always readily detectable using gelatin-SDS-PAGE but could be clearly seen with Boc-Val-Leu-Lys-NMec as substrate (Fig. 6). A proteinase with an apparent molecular mass of 60 kDa was detected only after acid treatment, whereas the activity of another, with an apparent molecular mass of 24 kDa, was enhanced significantly by acid treatment. The level of these two enzymes, together with ddCP18, increased after aggregation (from 12 h onward). All were confirmed as cysteine proteinases by their sensitivity to the cystine proteases (left, bacterially grown cells; right, axenic cells) are those determined previously (see Ref. 3) and were confirmed by reference to the positions of standard markers.

**DISCUSSION**

During the final stages of sporulation in the life cycle of eukaryotic microorganisms, it seems reasonable to assume that the activity of lysosomal enzymes such as proteinases should become minimal, consistent with the dormant state of the organisms. In addition to the normal reductions of transcriptional and translational events during sporulation (22), there
are several other strategies that an organism might utilize to minimize lysosomal enzyme activity, including the secretion of enzymes, the synthesis of inhibitors, or alterations in the conformational state of the enzymes. Conversely, during the germination of dormant structures, increases in the activity of the lysosomal enzymes could result from de novo synthesis, the loss of inhibitors from preexisting enzymes, or conformational changes in preexisting enzymes.

The results described here show that to detect some of the Dictyostelium cysteine proteinases using substrate-SDS-PAGE it was necessary to include a brief acid treatment step. The changes in cysteine proteinases during the different stages of the life cycle detected previously by gelatin-SDS-PAGE, i.e., without acid treatment, were consistent with the changes in net activity detected in cell extracts using cysteine proteinase-specific substrates such as peptide nitroanilides (5, 23). Thus it seems likely that the activatable enzymes were initially in an inactive form in the samples analyzed. We conclude that the lack of activity of these enzymes was not an artifact resulting simply from the procedures involved in the electrophoretic analysis and is therefore of physiological significance. The cysteine proteinases in D. discoideum may thus be synthesized and packaged in lysosomes as relatively inactive forms. It is not sufficient to free the enzymes from the spore lysosomes and incubate them at their optimal pH with protein or peptide derivatives to demonstrate enzymatic activity.

Mammalian lysosomal cysteine proteinases are synthesized as pro-enzymes containing an amino-terminal pro-region consisting of 62 amino acids, in the case of cathepsin B, or approximately 100 amino acids in the case of other cysteine proteinases such as cathepsins H, L, and S. The enzymes are activated by removal of the pro-region shortly after leaving the Golgi apparatus and entering the lysosome. Although there is some evidence suggesting the involvement of other proteolytic enzymes, for example metalloproteinases and aspartic proteinases (24, 25), it is more likely that an autocatalytic process is involved (see Ref. 13). Typically, a 35-40-kDa inactive pro-enzyme is converted to a 22-33-kDa active enzyme that may then be further proteolytically processed. The cysteine proteinases of D. discoideum for which sequence information is available (6–8, 26) are, like the aforementioned cathepsins, members of the papain superfamily (peptidase family C1 (27)), and it is likely that all of them are synthesized as precursors containing a typical pro-region. A possible explanation for activation, therefore, might have been the acid-induced removal of the pro-peptide, as has been observed with cysteine proteinases from mammalian cells (9–12), tick eggs (28), and Leishmania mexicana mexicana (20). However, this would have resulted in a detectable shift in electrophoretic mobility, and this was not apparent for the 48-kDa spore enzyme or any of the other bands detectable on gels (Fig. 4). A second possibility was that the pro-enzyme was activated without the removal of the pro-region; the apparent molecular mass of the activated 48-kDa enzyme was sufficiently large to have included the pro-region. However, sequence data for two of the active enzymes produced by vegetative cells of D. discoideum suggest that this is unlikely. ddCP38 from bacterially grown cells and ddCP42 from axenic cells (respective molecular masses of approximately 38 and 40 kDa as determined by SDS-PAGE), are both larger than cysteine proteinases found in other organisms but have amino-terminal sequences that align closely with those of other mature cysteine proteinases.3 Thus D. discoideum cysteine proteinases can be large, even without a pro-region at the amino terminus, and this is most likely due to inserts within the mature sequence and glycosylation (26). It is therefore probable that the activatable enzymes had lost the pro-peptide prior to being treated with acid.

The resolution achieved with the two-dimensional gel systems may not have allowed the detection of any change in mobility resulting from a slight reduction in molecular weight, and so the possibility that a small peptide was removed cannot be entirely discounted. However, in view of the lack of effect on activation of the cysteine proteinase inhibitor HgCl₂, it seems more likely that the activation was due to a conformational change or the removal of a small, tightly bound inhibitor. The only cysteine proteinase inhibitor reported in D. discoideum is too large (14 kDa) (29) to have had any involvement in acid activation as its removal from any proteinase would have affected electrophoretic mobility.

There are precedents for proteinase activation involving conformational changes; indeed it has been suggested that a change in conformation occurs before bond cleavage which removes the pro-region of the cathepsin L precursor (9). Another group of proteinases, the matrix metalloproteinases, are synthesized in a latent form that is inactive because of the formation of an intramolecular complex between the single cysteine residue in its pro-peptide domain and the essential zinc atom in the catalytic domain. A variety of activators including conformational perturbants, reversible sulphhydryl group modifiers,

3 A. Champion, G. Harrison, M. Wilkins, M. J. North, A. Gooley, and K. L. Williams, unpublished data.
and irreversible sulfhydryl group modifiers trigger the so-called “cysteine switch” (30). Activation is achieved through dissociation of the enzyme from the complex. It is possible that the activation described here might be achieved through a similar mechanism but in this case involving the dissociation of an ionic bond.

This in vitro activation technique worked not only for spore cysteine proteinases but was able to enhance the activity from cells at other developmental stages, although to different degrees. Thus, it is possible that many of the cysteine proteinases in *D. discoideum* can exist in at least two activity states, active and inactive, which can be altered by an acid shock. The extent to which acid treatment is needed to detect activity in vitro may reflect, in part at least, the degree of exposure to low pH already experienced by individual proteinases within the cells.

It has become clear that the mature lysosomal enzymes of *D. discoideum* become acidified with the aid of specific and discrete proton pump vacuoles (31–33). Our hypothesis for the production of dormant spore cysteine proteinases involves the synthesis of cysteine proteinases and their packaging into lysosomal vesicles that do not make immediate contact with the proton pump vacuoles. Although this would prevent the activation of new cysteine proteinases, it might not be sufficient to deactivate the existing enzymes completely. It is possible that the synthesis of a lysosomotropic agent such as ammonia deactivates the enzymes, which can subsequently be reactivated with the proton pump vacuoles. Although this would prevent the activation described here might be achieved through a similar mechanism but in this case involving the dissociation of the cysteine from the complex. It is possible that the synthesis of cysteine proteinases but was able to enhance the activity from cells at other developmental stages, although to different degrees. Thus, it is possible that many of the cysteine proteinases in *D. discoideum* can exist in at least two activity states, active and inactive, which can be altered by an acid shock. The extent to which acid treatment is needed to detect activity in vitro may reflect, in part at least, the degree of exposure to low pH already experienced by individual proteinases within the cells.

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We also suggest that the dormant spore lysosome should have a relatively high pH, unlike the low pH of the functioning lysosomes in vegetative cells. During the spore germination process the fusion of the organelle with a functioning proton pump vacuole would provide a sufficient drop in pH to convert the inactive forms of the cysteine proteinases into their active conformations.

Further research is required to determine the validity of our hypothesis and to ascertain whether acid-activatable proteinases are inactive within the cell and indeed if their activation has physiological significance. Interestingly, it was the development of a second acid treatment step in the analysis. Additional and more complex scenarios for the process of reducing lysosomal enzyme activities in vivo may involve: 1) the appearance of a development-specific inhibitor; 2) a difference in proteinase location, which affects conformation or inhibitor attachment; or 3) the synthesis of a new set of developmental cysteine proteinases that bind more tightly to an inhibitor present throughout the life cycle or which require special conditions to gain an active conformation. Additional research is also required to determine whether the acid activation phenomenon occurs with other enzymes and developmental systems. Such work is essential to understanding the specific mechanisms(s) involved and their importance in regulating development. Thus far, preliminary results from a work involving acid-activatable forms of the cysteine proteinases in *D. discoideum* suggest that at least some of the proteinases revealed by this treatment during development correspond to the developmentally regulated cpA and cpB gene products. It is not yet known whether the 48-kDa acid-activated spore proteinase is one such or if this is identical to the vegetative enzyme dcdCP48. Purification of the activatable proteinases is now in progress to establish their relationship to the predicted products of these genes, to investigate the activation mechanism in more detail, and to determine the extent to which it relates to the control of proteolysis during the development and germination of the dormant structures in the cellular slime molds.

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