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Protease Activity During Cell Differentiation of the Cellular Slime Mold *Dictyostelium discoideum*

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Individual aggregates, migrating pseudoplasmodia, and sorocarps of *Dictyostelium discoideum* were assayed for proteolytic activities by colorimetric and fluorometric techniques. Cathepsin D-like and cathepsin B-like acid protease activities were found to decrease throughout development, but the patterns of decrease were different for the two enzymes. A gradual decrease was found for cathepsin D, whereas a sharp decrease between aggregates and migrating pseudoplasmodia was detected for cathepsin B. By using microdissection techniques and fluorometric assays for amino acids and peptides, prestalk cells and prespore cells exhibited no difference in cathepsin D activity, whereas cathepsin B activity was higher in the prestalk cells. Similarly, stalk cells and spores in the sorocarps showed no difference in cathepsin D activity, but showed a fivefold higher cathepsin B activity in the stalk cells. This finding suggests a possible role for cathepsin B in stalk cell differentiation.

During morphogenesis of the cellular slime mold *Dictyostelium discoideum*, amoebae aggregate to form a multicellular migrating pseudoplasmodium, which later differentiates into a sorocarp composed of stalk cells and spores (5, 13). Aggregation and differentiation from one initial cell type to two final cell types are induced by starvation, especially of amino acids (15, 16). Protein degradation occurs during this process, as measured by decreasing cellular protein content from amoebae to sorocarps (6, 7, 27, 31). Thus, information on protease activities during development of *Dictyostelium* would be of interest, especially with regard to stalk cell and spore differentiation, because the former undergoes aging and cell death, whereas the latter is a storehouse for future germination. Sussman and Sussman (23) and Wiener and Ashworth (30) reported acid protease activity at pH optima of 2.3 and 2, respectively, with minor changes in activity during development (30). Recently, in a related species, *Polysphondylium pallidum*, O'Day (18) reported two peaks of acid protease activity (pH optima 3.5 and 6.0) during microcyst germination.

Results of experiments described in the present report indicate that cathepsin-like protease activities are present at different stages of development. (Reasons for attributing the proteases to be cathepsins will be discussed in a later section.) Single slime mold individuals (aggregates, migrating pseudoplasmodia, or sorocarps) were used for these assays. Moreover, with microdissection and a fluorometric reaction for amino acids and peptides, protease activity differences in differentiating cell types were studied. No difference was detected in cathepsin D activity between prestalk and prespore cells at the slug stage and between stalk cells and spores at the sorocarp stage. However, cathepsin B activity was higher in the pseudoplasmodial prestalk cells than in prespore cells. Similarly, cathepsin B activity in stalk cells was fivefold higher than in spores.

MATERIALS AND METHODS

Culture conditions. Lyophilized individual pseudoplasmodia of *D. discoideum* (strain NC4) were prepared as described previously (11, 20). Briefly, vegetative amoebae harvested from *Escherichia coli* B/r were allowed to differentiate on filter paper disks. Samples at different time intervals were collected, lyophilized, and stored at −76°C. Axenic strains of *D. discoideum* (strains Ax2 and Ax3) were grown in HL-5 medium in liquid culture (26). Amoebae were harvested at the exponential phase of growth and stored at −20°C until used.

Protease characterization studies. To avoid possible bacterial contamination, axenically grown amoebae were used in all of the enzyme inhibitor assays. *Dictyostelium* extracts were made by sonic treatment of the amoebae (Branson Sonifier, setting 5, 1 min) and centrifuging to obtain the supernatant (15,000 rpm, 10 min, in a Sorvall SS-34 rotor). For diazoacetyl norleucine methyl ester inhibition of cathepsin D, the method of Keilova (12) was followed, with inhibition by diazoacetyl norleucine methyl ester and cupric acetate at pH 5 and subsequent assay for

hemoglobin hydrolysis at pH 2.5. For pepstatin inhibition of cathepsin D, the method of Umezawa and Aoyagi (25) was adopted. Pepstatin was dissolved in methanol before dilution in water. Chloroquine inhibition of cathepsin B was performed by the method of Wibo and Poole (28), except that benzoylarginine nitroanilide was used as substrate. For iodoacetate, iodoacetamide, and tosyl lysyl chloromethyl ketone inhibitions of cathepsin B, the methods of Barrett (2) and Snellman (22) were used. Enzyme extracts were first incubated with inhibitors before benzoylarginine nitroanilide addition at pH 5.5. Leupeptin inhibition of cathepsin B was similar except that benzyl arginine naphthylamide was used together with an improved color reagent (3).

Microtechniques. Microdissection with handmade microscalpels on lyophilized tissues had been described (11, 20). Samples were weighed on a quartz fiber balance (with a sensitivity of 1.46 μg per deflection); and then transferred to Kimble disposable borosilicate glass culture tubes (6 by 50 mm). Buffer and substrate were added, and the tubes were then covered with Parafilm and incubated at 37°C. Constriction micropipettes (14) were used for the transfer of liquids.

Colorimetric protease assay. For cathepsin D, the buffer used was 0.05 M citrate, pH 2.75. For cathepsin B, the buffer was 0.05 M acetate, pH 5.5, with added activators 4 mM Na_2-ethylenediaminetetraacetic acid and 10 mM dithiothreitol. In colorimetric assays, 94.9 μl of buffer was added to the tube, together with 19.3 μl of 50-mg/ml azoalbumin (Sigma Chemical Co.), pH 7.2. Incubation at 37°C was 15 h for cathepsin D and 20 h for cathepsin B. The reaction was stopped by adding 111 μl of cold 10% trichloroacetic acid. Low-speed centrifugation (3,000 rpm, 15 min, International centrifuge, refrigerated model PR-J) gave a trichloroacetic acid-soluble fraction. A 111-μl amount of the trichloroacetic acid-soluble fraction was added with an equal volume of 0.5 N NaOH, and readings were taken at 440 nm (model 240 Gilford spectrophotometer) by the method of Tomarelli et al. (24).

Fluorometric protease assay. Fluorometric assays were similar to those of Hirsch and Parks (8), except that *o*-phthalaldehyde (Eastman Organic Chemical Division of Eastman Kodak Co.) and β-mercaptoethanol (Sigma) were used instead of fluorescamine. For cathepsin D assay, 1% (wt/vol) hemoglobin (Worthington Biochemicals Corp.) was added to 0.05 M citrate buffer at pH 2.75. Either 58.3 or 100 μl was used per tube, with a 10-h incubation at 37°C. For cathepsin B assay, 1% (wt/vol) bovine serum albumin (Sigma) was added to 0.05 M acetate buffer (pH 5.5) and activators (4 mM Na_2-ethylenediaminetetraacetic acid and 10 mM dithiothreitol). Either 51.4 or 58.3 μl was used per tube, with a 20-h incubation at 37°C. Reactions were stopped with an equal volume of cold 10% trichloroacetic acid, and centrifuged for trichloroacetic acid-soluble fractions. Portions of the trichloroacetic acid-soluble fractions, e.g., 27.2 and 46.5 μl, were assayed fluorometrically for released amino acids and peptides, by the method of Roth (19), by using unincubated controls as blanks. Each trichloroacetic acid-soluble portion was added to 1 ml of *o*-phthalaldehyde buffer (50 ml of 0.4 M borate adjusted to pH 10.4 with KOH, with 7.5 mg of *o*-phthalaldehyde in 0.75 ml of methanol, and with 50 μl of β-mercaptoethanol). This was Vortex mixed, incubated in the dark for 30 min at room temperature, and finally read in a Farrand fluorometer model A4, with appropriate filters for *o*-phthalaldehyde (excitation at 340 nm, emission at 455 nm).

RESULTS

Characterization of acid protease activities. A preliminary study of inhibitors of *Dictyostelium* acid proteases is shown in Table 1. Cathepsin D activity was inhibited by diazoacetyl norleucine methyl ester and partially inhibited by pepstatin. The latter finding suggested that other acid proteases may also be involved because crude cell extract was used in this enzyme assay. Cathepsin B activity was inhibited by iodoacetate, iodoacetamide, tosyl lysyl chloromethyl ketone, chloroquine, and leupeptin. In conclusion, besides the pepstatin result, all properties of enzyme inhibition correlated well with known cathepsin D and cathepsin B activities (4).

Cathepsin activity of whole individuals. Single slime mold pseudoplasmodia were used to assay for acid protease activities by using azoalbumin as a substrate. The colorimetric protease assay results are shown in Table 2. Protease activity per unit (dry weight) decreased throughout development. At any one stage of

TABLE 1. *Effects of inhibitors on acid protease activities in crude D. discoideum extracts*[a]

Determination	Inhibitor	Final concn	% Inhibition
Cathepsin D assay	DNME	1 mM	26.6
		10 mM	68.5
		50 mM	81.5
	Pepstatin	0.16 μg/ml	31.2
		0.8 μg/ml	35.5
		4 μg/ml	37.8
Cathepsin B assay	Iodoacetate	0.01 mM	94.8
		1 mM	100
	Iodoacetamide	1 mM	100
	TLCK	1 μM	73.3
		10 μM	93.5
		100 μM	96.3
	Chloroquine	5 mM	32.3
		25 mM	74.1
		50 mM	84.4
	Leupeptin	0.32 μg/ml	53.0
		1.6 μg/ml	80.7
		8 μg/ml	89.7

[a] Amoebae extracts were tested for acid protease activities in the presence and absence of inhibitors. DNME, Diazoacetyl norleucine methyl ester; TLCK, tosyl lysyl chloromethyl ketone.

development, cathepsin D activity was always higher than cathepsin B activity. This last result is similar to that reported by O'Day (18), whose pH 3.5 protease activity was higher than a pH 6 protease activity in a related slime mold species.

To achieve greater sensitivity, a fluorometric assay for amino acids and peptides was used. Individual *D. discoideum* sorocarps of different sizes were chosen to check cathepsin D activity at pH 2.75 with respect to protease concentration. The assay was found to be enzyme (tissue) concentration dependent (Fig. 1). The same assay was done to check cathepsin B activity at pH 5.5, except that culminating pseudoplasmodia of different sizes were selected for enzyme assay. Again, the results showed concentration dependence for the enzyme reaction (Fig. 2).

TABLE 2. *Cathepsin activities during Dictyostelium development*[a]

Determination		Aggregate	Migrating pseudoplasmodium	Sorocarp
Cathepsin assay[b]	D	12.57 ± 0.97	6.82 ± 0.37	3.67 ± 1.24
Cathepsin assay[c]	B	8.47 ± 0.29	2.55 ± 0.46	1.13 ± 0.35

[a] One unit of enzyme activity is defined as (optical density at 440 nm) × 10^{-4} per hour per microgram (dry weight). Cathepsin D was assayed at pH 2.75 for 15 h. Cathepsin B was assayed at pH 5.5 for 20 h. Means and standard deviations are for three determinations (n = 3).

[b] Tissue dry weight varied from 2.39 to 16.8 μg; readings were at least twice the blank value.

[c] Tissue dry weight varied from 3.47 to 10.1 μg. All readings were less than twice the blank value, except the aggregates.

We also examined the protease activity with the fluorometric method during three different stages of development by using individual aggregates, migrating pseudoplasmodia, and sorocarps. To show time dependence of the protease assay, reactions were stopped at various time intervals by trichloroacetic acid, and products were tested fluorometrically for released amino acids and peptides. Cathepsin D activity during development is shown in Fig. 3, and cathepsin B activity is shown in Fig. 4. In agreement with results obtained from colorimetric assays, both cathepsin activities in the cellular slime mold decreased from vegetative amoebae to mature sorocarps. However, the decrease pattern for cathepsin D was different from that of cathepsin B. Decrease in cathepsin D activity seemed to be gradual, whereas there was a sharp decrease in cathepsin B activity from aggregation stage to migrating-pseudoplasmodium stage.

Cathepsin activity in differentiating cell types. The fluorometric method was used to investigate cathepsin activity in the two cell types. By using microscalpels, the tip or prestalk region (the front fourth of the migrating slug) was cut away from the midsection of the slug,

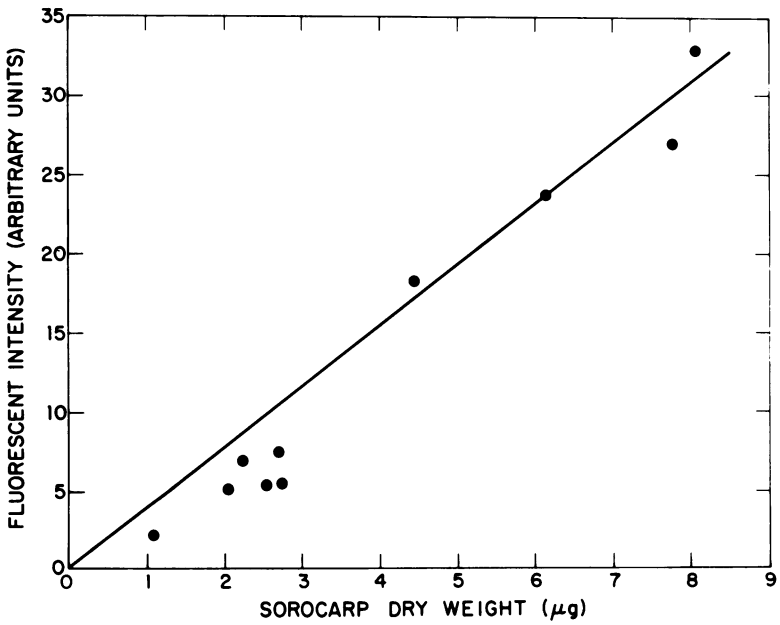

FIG. 1. *Cathepsin D activity in D. discoideum sorocarps. Individual sorocarps of different sizes were weighed and assayed for enzyme activity with 58.3 μl of hemoglobin-citrate buffer at pH 2.75. A 27.2-μl amount of the acid-soluble fraction was added to 1 ml of o-phthalaldehyde buffer to measure fluorescence. The blank value was 4 U.*

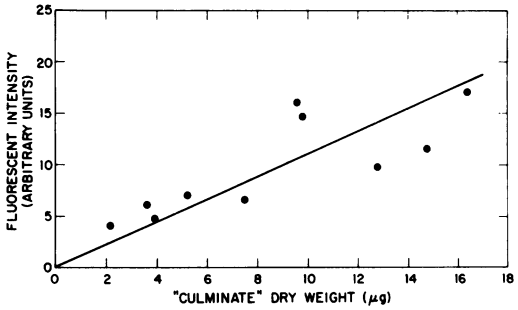

FIG. 2. *Cathepsin B activity in D. discoideum during early culmination. Individual culminating masses of different sizes were weighed and assayed for enzyme activity with 51.4 μl of albumin-acetate buffer at pH 5.5. A 51.4-μl amount of acid-soluble fraction was added to 1 ml of o-phthalaldehyde buffer to measure fluorescence. The blank value was 6.27 U.*

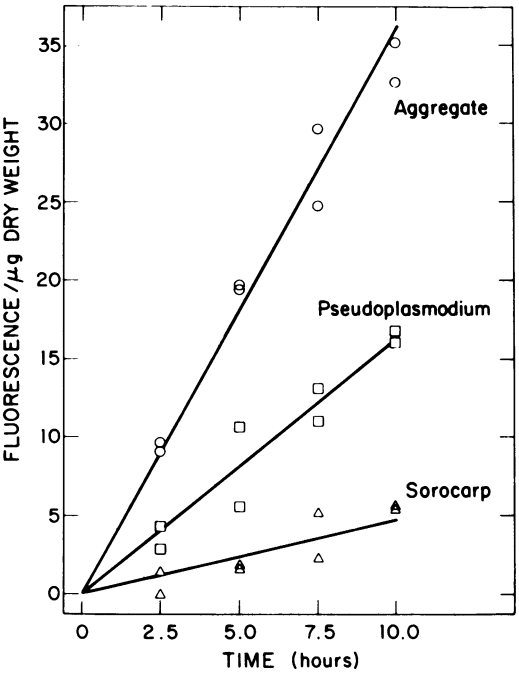

FIG. 3. *Cathepsin D activity during D. discoideum development. Individuals were weighed and assayed for enzyme activity in 100 μl of hemoglobin-citrate (pH 2.75) at 37°C. A 88.4-μl amount of the acid-soluble fraction was added to 1 ml of o-phthalaldehyde buffer to measure fluorescence. Unincubated individuals served as blank values. Fluorescent intensities are in arbitrary units. Tissue dry weight varied from 1.40 to 5.99 μg; the average value was 2.91 μg. Aggregates (○), migrating pseudoplasmodia (□), and sorocarps (△).*

the prespore region. The last fourth of the slug was not used because this includes potential basal disk cells, similar to prestalk cells. Mature stalks were similarly dissected out from the spore masses in fruiting bodies. However, due to sensitivity problems in the protease assay, it was necessary to pool together 20 isolated stalks (after cutting away their basal disks) to get enough dry weight for one datum point. An equivalent weight of spore material was assayed for comparison in a separate tube. Moreover, the o-phthalaldehyde reagent used had significant background fluorescence, so efforts were made to obtain readings at least twice the blank values. Because of possible day-to-day variation in fluorescence readings, the higher value of one cell type was taken to be 100, and the other value was calculated as a percentage of the higher value, in that way showing the relative fluores-

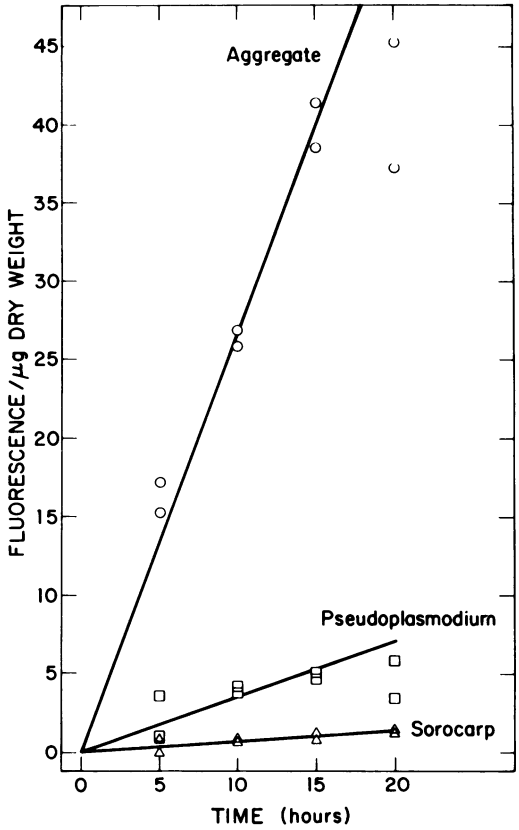

FIG. 4. *Cathepsin B activity during D. discoideum development. Individuals were weighed and assayed for enzyme activity in 58.3 μl of albumin-acetate (pH 5.5) at 37°C. A 46.5-μl amount of the acid-soluble fraction was added to 1 ml of o-phthalaldehyde buffer to measure fluorescence. Unincubated individuals served as blank values. Fluorescent intensities are in arbitrary units. Tissue dry weight varied from 0.85 to 5.96 μg; the average value was 2.31 μg. Aggregates (○), migrating pseudoplasmodia (□), and sorocarps (△).*

cence between the two cell types. Within both the slug and sorocarp stages of development, cathepsin D activity was similar between developing stalk cells and spores (Tables 3 and 4). Thus, this enzyme activity did not change during cell type differentiation at any stage of development.

However, cathepsin B activity gave a totally different picture. In the migrating pseudoplasmodium, cathepsin B activity was 1.7-fold higher in prestalk cells than prespore cells (Table 3). Also, in the sorocarp, cathepsin B activity averaged 5.6-fold higher in the stalk cells in the spores (Table 4).

Some values in Table 4 for spore-specific activity were less than twice the blank values, so it was necessary to determine whether this represented actual cathepsin B activity in the spores. We assayed for cathepsin B activity in isolated spore tissue of increasing dry weights. As spore dry weight increased, cathepsin B activity also increased (data not shown), indicating that spores did contain cathepsin B activity, although at much lower levels than stalk cells.

TABLE 3. *Cathepsin activities in D. discoideum migrating pseudoplasmodia*

Determination	Prestalk cells	Prespore cells
Cathepsin D assay [a]		
(1)[b]	100	78.9
(2)	97.1	100
(3)	100	94.2
Cathepsin B assay[c]		
(1)[d]	100	66.3
(2)	100	60.6
(3)	100	60.0
(4)	100	66.1
(5)	100	56.3
(6)	100	51.0

[a] A 100-μl amount of hemoglobin-citrate buffer at pH 2.75 was used (10 h of incubation at 37°C), and the reaction was terminated by trichloroacetic acid. Acid-soluble product was assayed fluorometrically with *o*-phthalaldehyde buffer. The fluorescent reading minus blank was then divided by dry weight. Relative units were achieved by assuming the higher reading to be 100, so the other reading became a percent value. All fluorescent readings were at least twice the blank values.

[b] Prestalk and prespore cells were dissected from the pseudoplasmodia. Dry weights in micrograms (prestalk/prespore): (1) 6.86:7.18; (2) 6.15:6.19; and (3) 6.06:6.06.

[c] A 58.3-μl amount of albumin-acetate buffer at pH 5.5 was used for (1) to (4), and 51.4 μl was used for (5) and (6), with a 20-h incubation at 37°C, and other treatments same as footnote *a*.

[d] Prestalk/prespore dry weights in micrograms: (1) 6.95:7.37; (2) 7.02:7.88; (3) 8.19:7.64; (4) 8.57:7.96; (5) 7.64:7.65; and (6) 7.50:7.40.

TABLE 4. *Cathepsin activities in D. discoideum sorocarps*

Determination	Stalk cells	Spores
Cathepsin D assay[a]		
(1)[b]	99.4	100
(2)	100	89.1
(3)	72.5	100
(4)	100	75.1
(5)	77.2	100
Cathepsin B assay[c]		
(1)[d]	100	20.3
(2)	100	17.0
(3)	100	24.3
(4)	100	13.5
(5)	100	10.3
(6)	100	27.9
(7)	100	11.1

[a] Same as Table 3. A 58.3-μl amount of hemoglobin-citrate was used in (1); in all others, 100 μl was used. All readings were at least twice the blank values. Relative units achieved by assuming the higher value to be 100.

[b] Dry weights in micrograms: (1) Stalks with basal disks, 3.17 from 6 sorocarps; spores, 3.08; (2) 8 stalks with bases, 7.3; spores, 7.46; (3) 8 stalks with bases, 6.1; spores, 5.99; (4) 20 dissected stalks, 6.73; spores, 6.83; (5) 21 dissected stalks, 5.99; spores, 6.37.

[c] Same as Table 3. A 51.4-μl amount of albumin-acetate was used for (1), (2), (3), and (7); 58.3 μl was used for (4), (5), and (6). In (1) to (3), readings were at least twice the blank values; in (4) to (7) the spore values were less than twice the blank values.

[d] Dry weights in micrograms: (1) stalks with basal disks, 8.54 from 10 sorocarps; spores 8.26; (2) 10 stalks with bases, 6.41; spores, 6.60; (3) 16 dissected stalks, 5.93; spores, 5.97; (4) 13 stalks, 7.39; spores, 7.34; (5) 15 stalks, 6.64; spores, 6.80; (6) 20 stalks, 7.96; spores, 8.02; (7) 25 stalks, 7.53; spores, 7.74.

DISCUSSION

Differences in cathepsin activity during *Dictyostelium* development have been demonstrated by using microtechniques and fluorometric amino acid and peptide assays. Although cathepsin D activity was similar in prestalk and prespore cells, and in stalk cells and spores, cathepsin B activity was higher in prestalk than prespore cells and again much higher in stalk cells than spores.

We have used the terms cathepsin D and cathepsin B because our enzymes showed many of the characteristics of these two cathepsins (Table 1). Studies involving activators, inhibitors, substrates, and pH optima were performed to distinguish the two cathepsins. The terms are chosen because cathepsins D and B are the major known lysosomal acid proteases, since

distribution of related enzymes, cathepsins H and L, and cathepsin E has only been confirmed in rat liver and rabbit bone marrow, respectively (4).

There may be other proteases in the cellular slime mold. Partial inhibition by pepstatin indicated the possibility of additional acid proteases. Native hemoglobin-acrylamide gels showed three protease bands with amoebae extract at pH 2.75, and at least three peptidase bands had been detected by using dipeptides as substrates (D. Fong, unpublished data).

Our finding of fivefold higher cathepsin B activity in stalk cells than spores is consistent with earlier results of Gregg et al. (6), who reported that spores retained higher protein content than stalk cells. Because cathepsin D activity was similar in the two cell types, it is possible that cathepsin B was responsible for higher stalk cell proteolysis.

The evidence suggests that cathepsins and other proteases may play roles during development of an organism (9). Cathepsin D was shown to be needed for amphibian metamorphosis, because its inhibitor pepstatin selectively blocked hormone-induced tadpole tail fin regression in vitro (21). Protease activities change during development. Marks et al. (17) reported an initial decrease and a later increase for cathepsin D and neutral protease activity during maturation of the rat brain. A somewhat analogous case concerning cellular protein degradation was presented by Amenta et al. (1) in cycloheximide-treated fibroblasts, which showed decreased specific activities of the lysosomal proteases, with the pattern of decrease for cathepsin D different from that for cathepsin B. Our data also showed cathepsin D and cathepsin B activity decreases during differentiation from amoebae to sorocarps in *D. discoideum*. The pattern of decrease was different for the two enzyme activities.

Cathepsin B may play a more important role in cellular regulatory phenomena than cathepsin D. For instance, in a study of muscles of dystrophic chickens, an increase in cathepsin B activity correlated well with muscle autolysis and preceded any increase in cathepsin D activity (10). Cathepsin B and related thiol proteases indicated preferred digestion of young cytosol, whereas cathepsin D showed no preference in a study comparing protease digestion of young versus old rat liver cytosol proteins (29). Our data for *D. discoideum* also suggested a regulatory role for cathepsin B during development. There was a marked difference of cathepsin B activity between stalk cells and spores, whereas there was no difference for cathepsin D activity.

Hence, cathepsin B may play some developmental function in the cellular slime molds. Perhaps the localization of cathepsin B activity in the stalk cells is a reflection of cell-specific protein degradation. The protease activity difference between stalk cells and spores may be due to selective synthesis or degradation of the enzyme, activators or inhibitors of the enzyme, or secretion of enzyme into the medium. O'Day (18) reported that inhibitors were probably absent and proteases were being secreted, at least during microcyst germination in *Polysphondylium*. Obviously, the role of proteases in *Dictyostelium* needs further study.

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LITERATURE CITED

1. **Amenta, J. S., M. J. Sargus, and F. M. Baccino.** 1977. Control of cell protein degradation, changes in activities of lysosomal proteases. Biochim. Biophys. Acta **476**:253–261.
2. **Barrett, A. J.** 1973. Human cathepsin B1, purification and some properties of the enzyme. Biochem. J. **131**:809–822.
3. **Barrett, A. J.** 1976. An improved color reagent for use in Barrett's assay of cathepsin B. Anal. Biochem. **76**:374–376.
4. **Barrett, A. J., and M. F. Heath.** 1977. Lysosomal enzymes, p. 19–145. *In* J. T. Dingle (ed.), Lysosomes, a laboratory handbook, 2nd ed. North-Holland, Amsterdam.
5. **Bonner, J. T.** 1967. The cellular slime molds, 2nd ed., p. 1–205. Princeton University, Princeton.
6. **Gregg, J. H., A. L. Hackney, and J. O. Krivanek.** 1954. Nitrogen metabolism of the slime mold *Dictyostelium discoideum* during growth and morphogenesis. Biol. Bull. (Woods Hole, Mass.) **107**:226–235.
7. **Hames, B. D., and J. M. Ashworth.** 1974. The metabolism of macromolecules during the differentiation of myxamoebae of the cellular slime mould *Dictyostelium discoideum* containing different amounts of glycogen. Biochem. J. **142**:301–315.
8. **Hirsch, H. E., and M. E. Parks.** 1973. The quantitative histochemistry of acid proteinase in the nervous system: localization in neurons. J. Neurochem. **21**:453–458.
9. **Holzer, H., H. Betz, and E. Ebner.** 1975. Intracellular proteinases in microorganisms, p. 103–156. *In* B. L. Horecker and E. R. Stadtman (eds.), Current topics in cellular regulation, vol. 9. Academic Press Inc., New York.
10. **Iodice, A. A., J. Chin, S. Perker, and I. M. Weinstock.** 1972. Cathepsins A, B, C, D and autolysis during development of breast muscle of normal and dystrophic chickens. Arch. Biochem. Biophys. **152**:166–174.
11. **Jefferson, B. L., and C. L. Rutherford.** 1976. A stalk-

specific localization of trehalase activity in *Dictyostelium discoideum*. Exp. Cell Res. **103**:127–134.
12. **Keilova, H.** 1970. Inhibition of cathepsin D by diazoacetylnorleucine methyl ester. FEBS Lett. **6**:312–314.
13. **Loomis, W. F.** 1975. *Dictyostelium discoideum*, a developmental system, p. 1–214. Academic Press Inc., New York.
14. **Lowry, O. H., and J. V. Passonneau.** 1972. A flexible system of enzymatic analysis, p. 1–291. Academic Press Inc., New York.
15. **Marin, F. T.** 1976. Regulation of development in *Dictyostelium discoideum*. I. Initiation of the growth to development transition by amino acid starvation. Dev. Biol. **48**:110–117.
16. **Marin, F. T.** 1977. Regulation of development in *Dictyostelium discoideum*. II. Regulation of early cell differentiation by amino acid starvation and intercellular interaction. Dev. Biol. **60**:389–395.
17. **Marks, N., F. Stern, and A. Lajtha.** 1975. Changes in proteolytic enzymes and proteins during maturation of the brain. Brain Res. **86**:307–322.
18. **O'Day, D. H.** 1976. Acid protease activity during germination of microcysts of the cellular slime mold *Polysphondylium pallidum*. J. Bacteriol. **125**:8–13.
19. **Roth, M.** 1971. Fluorescence reaction for amino acids. Anal. Chem. **43**:880–882.
20. **Rutherford, C. L., and J. F. Harris.** 1976. Localization of glycogen phosphorylase in specific cell types during differentiation of *Dictyostelium discoideum*. Arch. Biochem. Biophys. **175**:453–462.
21. **Seshimo, H., M. Ryuzaki, and K. Yoshizato.** 1977. Specific inhibition of triiodothyronine-induced tadpole tail-fin regression by cathepsin D-inhibitor pepstatin. Dev. Biol. **59**:96–100.
22. **Snellman, O.** 1970. A study of the reactivity of the thiol group of cathepsin B, p. 29–43. *In* A. J. Barrett and J. T. Dingle (ed.), Tissue proteinases, North-Holland, Amsterdam.
23. **Sussman, M., and R. Sussman.** 1969. Patterns of RNA synthesis and of enzyme accumulation and disappearance during cellular slime mould cytodifferentiation. Symp. Soc. Gen. Microbiol. **19**:403–435.
24. **Tomarelli, R. M., J. Charney, and M. L. Harding.** 1949. Use of azoalbumin as substrate in the colorimetric determination of peptic and tryptic activity. J. Lab. Clin. Med. **34**:428–433.
25. **Umezawa, H., and T. Aoyagi.** 1977. Activities of proteinase inhibitors of microbial origin, p. 637–662. *In* A. J. Barrett (ed.), Proteinases in mammalian cells and tissues. North-Holland, Amsterdam.
26. **Watts, D. J., and J. M. Ashworth.** 1970. Growth of myxamoebae of the cellular slime mould *Dictyostelium discoideum* in axenic culture. Biochem. J. **119**:171–174.
27. **White, G. J., and M. Sussman.** 1961. Metabolism of major cell components during slime mold morphogenesis. Biochim. Biophys. Acta **53**:285–293.
28. **Wibo, M., and B. Poole.** 1974. Protein degradation in cultured cells. II. The uptake of chloroquine by rat fibroblasts and the inhibition of cellular protein degradation and cathepsin B1. J. Cell Biol. **63**:430–440.
29. **Wiederanders, B., S. Ansorge, P. Bohley, H. Kirschke, J. Langner, and H. Hanson.** 1977. The age dependence of intracellular proteolysis in the rat liver, p. 144–147. *In* V. Turk and N. Marks (ed.), Intracellular protein catabolism II. Plenum Publishing Corp., New York.
30. **Wiener, E., and J. M. Ashworth.** 1970. The isolation and characterization of lysosomal particles from myxamoebae of the cellular slime mould *Dictyostelium discoideum*. Biochem. J. **118**:505–512.
31. **Wright, B. E., and M. L. Anderson.** 1960. Protein and amino acid turnover during differentiation in the slime mold. I. Utilization of endogenous amino acids and proteins. Biochim. Biophys. Acta **43**:62–66.