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The Isolation and Characterization of Lysosomal Particles from Myxamoebae of the Cellular Slime Mould *Dictyostelium discoideum*

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1. The myxamoebae of the cellular slime mould *Dictyostelium discoideum* possess several typically lysosomal enzyme activities. 2. These enzymes are present in the cell in association and in a lysosome-like particle. 3. The lysosomes of myxamoebae grown axenically have a different enzymic composition and a different density from those grown on bacteria. 4. During cell differentiation the specific activities of the lysosomal enzymes change. 5. It is suggested that both during growth and differentiation the amounts of lysosomal enzymes present in the cell are regulated.

The life cycle of the cellular slime mould *Dictyostelium discoideum* is divided into two mutually exclusive phases. In the first, feeding, phase the organism exists as solitary amoeboid cells (myxamoebae), and in the second, differentiation, phase the myxamoebae associate together to form aggregates that, after a period of concerted movement, form fruiting bodies consisting of a mass of spores borne on a cylindrical stalk (Bonner, 1967). Cell differentiation in this organism thus occurs in the absence of growth and at the expense of endogenous food reserves (White & Sussman, 1961). The myxamoebae contain considerable amounts of a glycogen-like polysaccharide (White & Sussman, 1961; J. M. Ashworth & D. J. Watts, unpublished work), and this seems to be mobilized during differentiation. In addition, there is a considerable net loss of protein during differentiation, and this occurs in spite of the appearance of some new or enhanced enzymic activities (Sussman & Sussman, 1969). Considerable attention has been devoted to the mechanisms leading to the synthesis of proteins during the cell differentiation processes (Roth, Ashworth & Sussman, 1968), but little is known of the ways in which the endogenous food materials are mobilized for energy production and biosynthetic purposes. In other differentiating systems it has been suggested that lysosomal enzymes play a key role in the redistribution of cellular materials (de Duve & Wattiaux, 1966).

We report the isolation and characterization of intracellular particles from the myxamoebae of the cellular slime mould. These particles possess properties that are in some respects similar to those of the lysosomes of *Tetrahymena* (Muller, Baudhuin & de Duve, 1966), leucocytes (Cohn, Hirsch & Wiener, 1963) and liver (de Duve, 1959), which have been studied by others. We discuss the role that these organelles might play in the cell differentiation process characteristic of this organism.

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MATERIALS AND METHODS

Growth and harvesting of myxamoebae. The axenic strain of *Dictyostelium discoideum* that we used was obtained from *D. discoideum* NC4 by selecting for ability to grow in a semi-defined liquid medium in the absence of bacteria (J. M. Ashworth & D. J. Watts, unpublished work). It is similar to, but probably not identical with, the strain described and designated Ax-1 by Cocucci & Sussman (1970). We therefore describe our strain *D. discoideum* Ax-2.

The medium used for the axenic growth of the myxamoebae had the following composition: bacteriological peptone (Oxoid), 10g; yeast extract (Oxoid), 5g; Na_2HPO_4,H_2O, 0.89g; KH_2PO_4, 0.34g; glucose, 10.8g; water to a total volume of 700ml. This medium is similar to the HL-5 medium of Cocucci & Sussman (1970). Cells were grown at 22°C with shaking and were harvested in the late exponential phase at a cell density of approx. 5×10^6 cells/ml.

Myxamoebae of strain Ax-2 were also grown by using bacteria (*Aerobacter aerogenes*) as nutrient on agar plates according to the procedures described by Sussman (1966). They were harvested at the first sign of disappearance of the bacterial lawn.

Cells from the axenic liquid cultures were harvested by centrifugation in the cold at 400*g* for 10min. The cells were washed once in 0.25M-sucrose and resuspended in this solution to give a suspension containing 10^8 cells/ml. Cells grown on bacteria were collected from the agar plates in cold 0.25M-sucrose and washed four times in this solution by centrifugation at 400*g*. This procedure removes most of the contaminating bacteria from the myxamoebae. They were finally resuspended at 10^8 cells/ml in 0.25M-sucrose. For purely enzymological studies,

where structural integrity of the subcellular organelles was not required, the cell suspensions were frozen and stored until assayed.

Cell fractionation. Freshly harvested cells in 0.25M-sucrose were homogenized in a Teflon–glass homogenizer with a clearance between pestle and tube of 0.002–0.003in. Nine strokes of the pestle rotating at 500rev./min resulted in breakage of 80–90% of the cells. Unbroken cells were removed by centrifugation at 400*g* for 5min and the supernatant was further fractionated by centrifugation at 15000*g* for 15min in an MSE18 centrifuge ($2.25\times 10^5 g$-min). The crude lysosomal pellet so obtained was washed once in 0.25M-sucrose and was then further fractionated by isopycnic centrifugation in sucrose density gradients. Lysosomal preparations from axenically grown cells were layered on a linear 35–50% (w/v) sucrose gradient ($\rho = 1.16$–1.24), whereas particles from cells grown on bacteria were layered on a similar gradient mounted on a cushion of 61% (w/v) sucrose ($\rho = 1.30$). In both cases the gradients were centrifuged for 2.5h at 100000*g* in the 30K swing-out rotor of the MSE Superspeed 50 centrifuge at 4°C. Fractions of constant volume were collected by puncturing the bottom of the centrifuge tubes with an MSE tube piercer and collecting 15-drop fractions.

Enzyme assays. In all assays for total hydrolase activities Triton X-100 (BDH Chemicals Ltd., Poole, Dorset, U.K.) was added to the reaction mixtures to a final concentration of 0.1% (Wattiaux & de Duve, 1956). All assays were conducted at 25°C except for β-*N*-acetylglucosaminidase, which was assayed at 35°C. The incubation conditions adopted for the routine assay of hydrolase activities are summarized in Table 1. These conditions are slight modifications of methods previously used by others (Muller *et al.* 1966; de Duve, Pressman, Gianetto, Wattiaux & Appelmans, 1955; Gianetto & de Duve, 1955). Amylase was assayed by a modification of the method of Bernfeld (1955) and β-*N*-acetylglucosaminidase as described by Loomis (1969).

Enzyme assay mixtures were usually incubated for 1h, except for β-*N*-acetylglucosaminidase, when the incubation time was 30min. During these times it was found that the enzymic activities were stable and, in all cases, enzyme activity was proportional to time of incubation and amount of enzyme protein added. Control incubation mixtures without added enzyme were always included in each assay and, in the case of assays for ribonuclease, deoxyribonuclease, proteinase and amylase, further control mixtures without added substrates were also included. Values of enzymic activity obtained were corrected, where necessary, for the apparent activity obtained in these controls.

Citrate synthase was assayed at 30°C by the method of Srere, Brazil & Gonen (1963) and catalase at 20°C by the method of Machley (1954). Protein was assayed by the method of Lowry, Rosebrough, Farr & Randall (1951).

Enzyme activities are expressed, where possible, in milliunits, 1 unit being the amount of enzyme necessary to release 1μmol of product/min under the stated assay conditions. For the nucleases the product was assessed in terms of mononucleotide equivalents, for amylase in terms of glucose equivalents and for the proteinase in terms of chromogenic equivalents of 1μg of bovine serum albumin.

Chemicals. Commercially available enzyme substrates were used: disodium *p*-nitrophenyl phosphate (Sigma 104 phosphatase substrate), yeast RNA type XI, calf thymus DNA type V, *p*-nitrophenyl *N*-acetylglucosaminide, phenolphthalein glucuronide and *p*-nitrocatechol sulphate were obtained from Sigma Chemical Co., St Louis, Mo., U.S.A.; bovine haemoglobin was from Calbiochem, Los Angeles, Calif., U.S.A.; soluble starch (analytical grade) was from Fisons Scientific Apparatus Ltd., Loughborough, Leics., U.K.

Examination of fractions in the electron microscope. Pelleted material was suspended in 2% glutaraldehyde in 0.2M-sucrose–10mM-tris–HCl buffer, pH7.6, for 2h at 0°C. The fixed material was collected by centrifugation, post-fixed in buffered 2% osmic acid (30min), dehydrated through an ethanol series and infiltrated with propylene oxide and Araldite, which was polymerized for 2 days at 60°C. Silver and grey sections were cut on a LKB Ultratone III microtome and were stained with uranyl acetate [freshly prepared 2% (w/v) solution in 50% (v/v) ethanol]. The sections were examined in a Siemens Elmiskop IA electron microscope at an accelerating voltage of 80kV with a 200μm condenser aperture and an electron-optical magnification of ×8000.

RESULTS

Acid hydrolase activities. The specific activities of some typically lysosomal acid hydrolases in homogenates of myxamoebae grown either in liquid axenic culture or on bacteria are shown in Table 2. The pH-dependence of these enzymes in extracts from axenically grown cells is shown in

Table 1. *Incubation conditions for enzyme assays*

Enzyme	Buffer	Substrate	Final vol. (ml)
Phosphatase	0.1M-Glycine–HCl, pH3.0	7mM-*p*-Nitrophenyl phosphate	2.5
Ribonuclease	0.07M-Glycine–HCl, pH3.5	3mg of dialysed RNA	2.0
Deoxyribonuclease	0.07M-Glycine–HCl, pH2.5	2.5mg of dialysed DNA	2.0
Proteinase	0.07M-Glycine–HCl, pH2.0	30mg of dialysed haemoglobin	2.0
Amylase	0.1M-Sodium acetate–acetic acid, pH4.5	25mg of soluble starch	2.0
β-*N*-Acetylglucosaminidase	0.02M-Sodium acetate–acetic acid, pH5.0	0.01M-*p*-Nitrophenyl *N*-acetyl-β-glucosaminide	0.5

Table 2. *Specific activities of acid hydrolases*

Results are given as means±S.E.M., with the numbers of determinations in parentheses. Assay conditions are given in the Materials and Methods section.

Enzyme	Specific activity (m-units/mg of protein)	
	Cells grown axenically	Cells grown on bacteria
Acid phosphatase	124± 10 (8)	91±11 (5)
Ribonuclease	184± 30 (7)	88±11 (4)
Deoxyribonuclease	8±1.0 (5)	5± 0.3 (3)
Proteinase	126± 26 (5)	69± 9 (4)
Amylase	48± 8 (2)	50± 5 (5)
β-*N*-Acetylglucosaminidase	442±108 (2)	122±27 (4)
β-Glucuronidase	Not measurable	Not determined
Arylsulphatase	Not measurable	Not determined

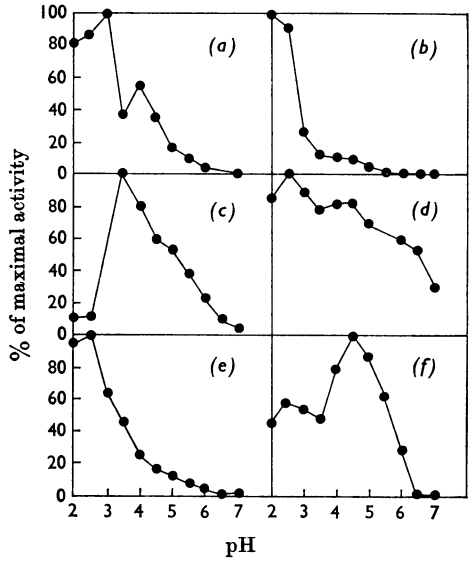


Fig. 1. Influence of pH on the activity of various acid hydrolases. Glycine–HCl buffers were used at pH values up to 3.5 and arsenate–borate–citrate buffers (de Duve, 1949) at pH values above 3.5 in all cases except the amylase assays, when citrate–phosphate buffers were used above this pH. (*a*) Acid phosphatase; (*b*) proteinase; (*c*) ribonuclease; (*d*) amylase; (*e*) deoxyribonuclease; (*f*) *N*-acetylglucosaminidase.

Fig. 1. All the six enzymes studied have a peak activity at very low pH values and three (acid phosphatase, amylase and β-*N*-acetylglucosaminidase) have a second optimum at pH values between 4 and 5. However, only one enzyme, β-*N*-acetylglucosaminidase, shows maximal activity at this higher pH value. The pH-dependence of the enzymic activities of cells grown on bacteria were found to be similar to those found in glucose-grown cells.

Intracellular distribution of acid hydrolases. Acid hydrolases are usually found to be associated together in a lysosomal particle (de Duve, 1959), and to test whether this is also true of the cellular slime mould the sedimentability of the hydrolases was determined in homogenates prepared from cell suspensions in 0.25M-sucrose. The results are summarized in Table 3. About 70–80% of the acid hydrolase activity of either bacterial or axenically grown cells could be recovered in the $2.25\times10^5 g$-min pellet, whereas only some 23–35% of the cellular protein could be recovered in this fraction. This association between acid hydrolase activity and a particle sedimentable at $2.25\times10^5 g$-min was sensitive to freezing and thawing, addition of detergents such as Triton X-100 and temperature. Thus after incubation of washed particles at 22°C for 2.5h in 0.25M-sucrose only 21% of the acid phosphatase activity was still found in the $2.25\times10^5 g$-min pellet, whereas after incubation at 4°C for the same time 55% of the acid phosphatase activity was found in this fraction. The acid phosphatase activity in the particle fraction was also latent. The $2.25\times10^5 g$-min pellet was resuspended in 0.25M-sucrose and samples of this suspension were frozen and thawed twice, diluted 1:10 with water and treated with Triton X-100 (final concn. 0.1%). The apparent acid phosphatase activity of the original suspension before and after these various procedures was then determined as described above but with the inclusion of sucrose (final concn. 0.25M) in the incubation mixture and with an incubation time of only 10min. All three of the above procedures increased the apparent acid phosphatase activity of the original particle suspension by between two- and three-fold. The acid phosphatase activity thus appears to be present in a particle

Table 3. *Intracellular distribution of acid hydrolases*

Results are given as means±S.E.M., with the numbers of determinations in parentheses.

Enzyme	% of total activity recovered in pellet: Cells grown axenically	Cells grown on bacteria
Acid phosphatase	73±5 (3)	69±3 (5)
Ribonuclease	70±7 (3)	70±5 (4)
Deoxyribonuclease	81±2 (2)	74±5 (2)
Proteinase	68±9 (3)	68±2 (4)
Amylase	77±2 (2)	67±4 (5)
β-*N*-Acetylglucosaminidase	79±5 (2)	68±6 (4)
Protein	23±2 (6)	35±1 (4)

having the properties of a lysosome as defined by de Duve (1959) and this particle also appears to contain the other hydrolases listed in Table 3.

In view of the possible role of lysosomes during cell differentiation (see de Duve & Wattiaux, 1966) we also studied the stability of the association between hydrolase activity and the 2.25×10^5g-min particle. Washed suspensions of particles were resuspended in sucrose solutions buffered at various pH values between 2 and 7 and incubated at 4°C for 2.5h. The suspensions were then centrifuged and the protein and hydrolase activity of the resulting 2.25×10^5g-min pellet and supernatant fractions were measured. Incubation in these acid buffers causes a decrease in the amount of sedimentable protein; after incubation at pH3 only 40% of the total protein is sedimentable whereas after incubation at pH6 at least 80% of the total protein is still sedimentable. This presumably reflects the stability, in acid buffers, of the mitochondria, which are also present in the original 2.25×10^5g-min fraction. Not all the hydrolase activities were equally stable under these conditions of incubation. β-*N*-Acetylglucosaminidase, ribonuclease and proteinase activities decreased by approx. 25% during incubation at pH3 but were quite stable at higher pH values; acid phosphatase, deoxyribonuclease and amylase activities were stable at all the pH values tested. The percentage of the acid hydrolase activities remaining after the incubation period that were present in the 2.25×10^5g-min pellet is shown in Fig. 2. All the profiles are similar in that the sedimentability of the hydrolases decreased sharply above pH4 with little change in the pH5–6 region. Below pH4 the profiles differ considerably.

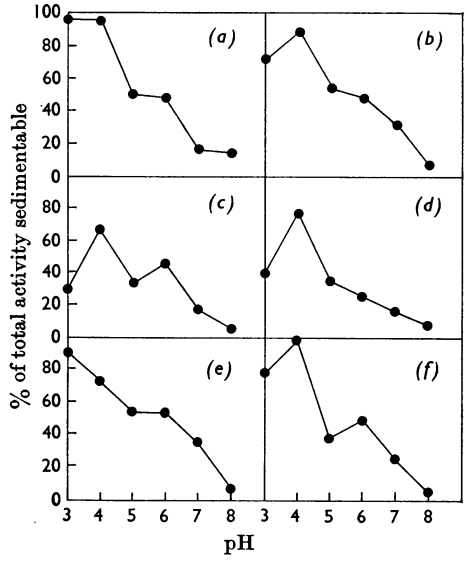


Fig. 2. Influence of pH on the stability of the lysosomal particles from axenically grown myxamoebae. The 2.25×10^5g-min pellet from axenically grown myxamoebae was resuspended in 0.25M-sucrose solutions containing 0.025M buffers at various pH values. After incubation at 4°C for 2.5h these suspensions were centrifuged and the acid hydrolase activities of the 2.25×10^5g-min pellet and supernatant were determined. The buffers used were: pH3, glycine–HCl; pH4 and 5, sodium acetate–acetic acid; pH6, 7 and 8, KH_2PO_4–Na_2HPO_4. (*a*) Acid phosphatase; (*b*) proteinase; (*c*) ribonuclease; (*d*) amylase; (*e*) deoxyribonuclease; (*f*) *N*-acetylglucosaminidase.

Isolation of lysosomes. The crude 2.25×10^5g-min pellet would be expected to contain particles other than lysosome-like structures, and in particular mitochondria and peroxisomes (de Duve, 1969). The crude 2.25×10^5g-min pellet was washed in 0.25M-sucrose and then layered over a sucrose density gradient and subjected to isopycnic centrifugation as described above. Citrate synthase was taken as a marker enzyme for mitochondria, catalase for peroxisomes, and acid phosphatase and β-*N*-acetylglucosaminidase were selected as representative acid hydrolases. At the end of the centrifugation (2.5h at 4°C) a considerable amount of the

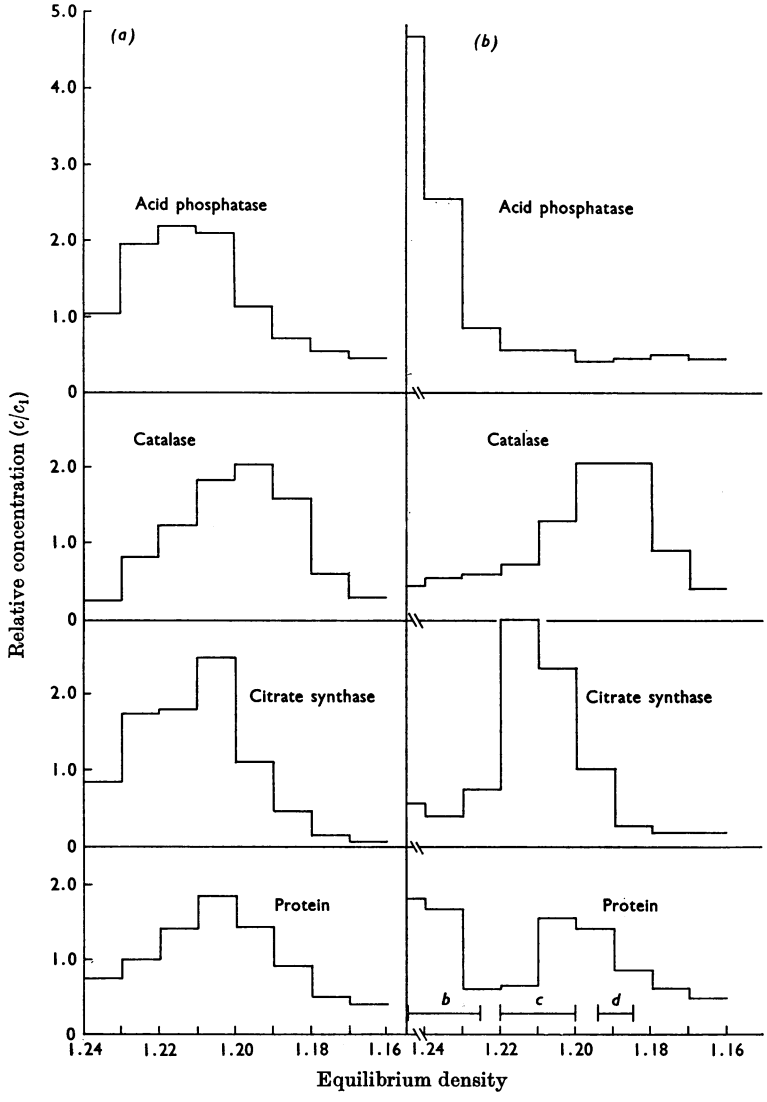


Fig. 3. Distribution of protein and various enzymic activities after isopycnic sucrose-density-gradient centrifugation of the $2.25\times10^5 g$-min pellet of myxamoebae. Details of the centrifugation techniques are given in the text. (*a*) Particles from axenically grown cells; (*b*) particles from cells grown on bacteria. The bars show the source of the samples taken for examination in the electron microscope (Plate 1*b*, 1*c* and 1*d*). The interruption on the abscissa marks the change from the cushion ($\rho = 1.3$) to the gradient.

acid phosphatase and β-*N*-acetylglucosaminidase activities could be recovered from the sample layer. Subsequent centrifugation of this layer did not yield any pellet, and it was therefore concluded that this activity represented hydrolases released from the particles during centrifugation. The amount of enzyme activity present in the sample layer was disregarded in Figs. 3(*a*) and 3(*b*), which show the relative distributions of protein and the various marker enzymes in the sucrose density gradient. The distribution of β-*N*-acetylglucosaminidase activity was identical with that of acid phosphatase. Fig. 3(*a*) shows the distribution of particles from cells grown in axenic medium. The major protein band coincides with the citrate synthase activity at densities of 1.20–1.21. Particles containing

hydrolase activities are only slightly denser (equilibrium density range 1.20–1.23) and hardly separated from the mitochondria. The catalase-rich particles are located at lower densities (1.19–1.20). Particles obtained from cells grown on bacteria were much more clearly separated, particularly if a cushion of sucrose of density 1.30 was used in addition to the gradient. Fig. 3(*b*) shows the distribution of particles from such cells and shows a clear separation of lysosomal particles ($\rho > 1.23$), mitochondria ($\rho = 1.20$–1.22) and peroxisomes ($\rho = 1.18$–1.20). The fractions from the areas of the gradient labelled *b*, *c* and *d* were collected, diluted with 0.25M-sucrose and centrifuged. The pelleted material was fixed, stained, sectioned and examined in the electron microscope as described above. Examination of these sections showed that these three areas of the gradient contained different types of particles, and representative fields from such electron micrographs are shown in Plate 1. Plate 1 also shows a section of a cell grown on bacteria.

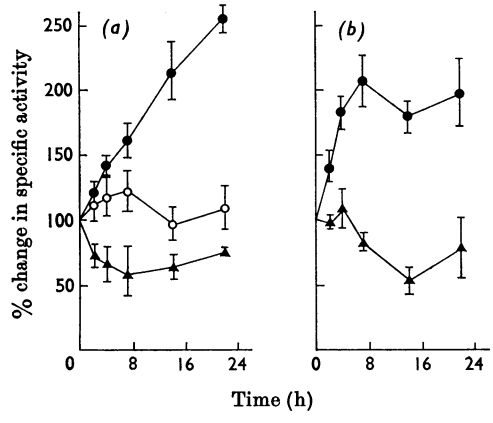


Fig. 4. Changes in specific activity of various acid hydrolases during differentiation of myxamoebae grown on bacteria. The relationship between time and morphogenetic stage is as described in Roth *et al.* (1968). The specific activities in myxamoebae at zero time are given in Table 2. (*a*) ●, Acid phosphatase; ○, proteinase; ▲, ribonuclease. (*b*) ●, *N*-Acetylglucosaminidase; ▲, amylase.

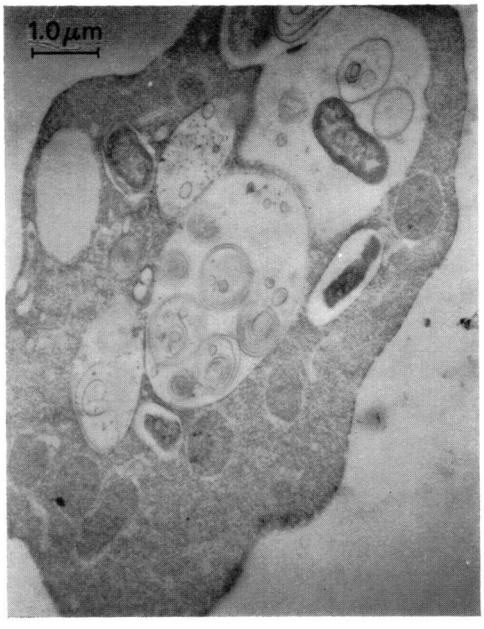
Changes in acid hydrolase activities during cell differentiation. Myxamoebae were grown with bacteria as nutrient, harvested in 0.25M-sucrose and washed four times in this solution. They were then resuspended in water to give 2×10^8 cells/ml, and 0.5ml portions of this suspension were plated out on washed Millipore filters as described by Sussman (1966). This procedure gives highly synchronous development of fruiting bodies after 24±1h at 22°C. Cells were harvested after incubation at 22°C for various periods by washing the Millipore filters with water. These suspensions were stored frozen and subsequently assayed for hydrolase activities as described above. Fig. 4 shows the results of such assays, which have been expressed in percentage changes of the enzyme activites of the bacteria-grown myxamoebae (Table 2). Since considerable changes in enzyme activities were observed it was decided to test whether the acid hydrolases were present in lysosome-like particles in cells undergoing differentiation. Consequently myxamoebae on Millipore filters were harvested in cold 0.25M-sucrose (1ml/filter), homogenized immediately and the percentage of the total hydrolase activity precipitated in the $2.25 \times 10^5 g$-min pellet was determined as described above. It was noticed that the normal method of harvesting myxamoebae gave zero-time samples in which the sedimentability of the acid hydrolases was greatly diminished. This effect seemed to be due to the change in osmotic pressure caused by harvesting myxamoebae in sucrose and then resuspending them in water before their deposition on Millipore filters.

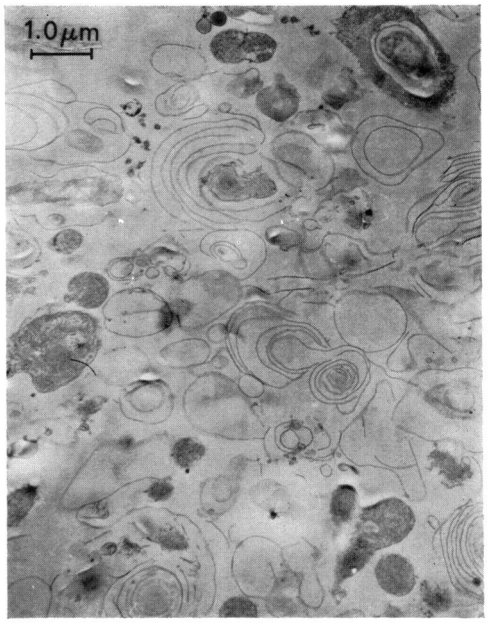
Table 4. *Intracellular distribution of acid hydrolases during cell differentiation*

Millipore filters were set up with myxamoebae that had been grown on bacteria, harvested and washed in 0.25M-sucrose and resuspended in water before deposition on the filter. After 12h aggregation has been completed and the cells are in the slug stage of development. A 'tight' homogenizer was used for preparation of the cell extracts. Results are given as means±S.E.M. of three determinations.

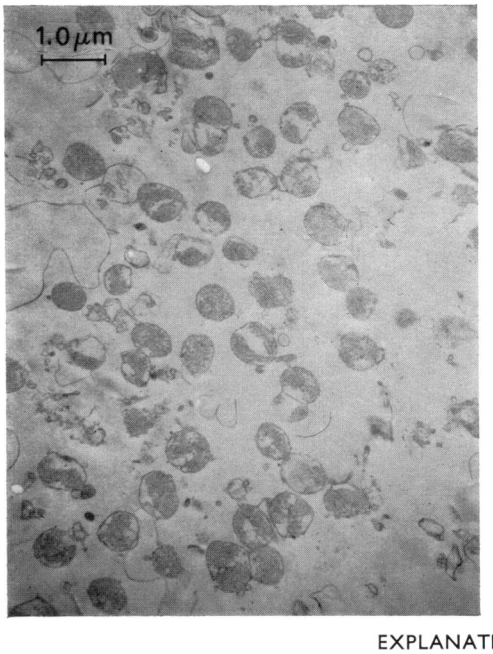
Sample (h at 22°C)	% of total activity recovered in pellet			
	Phosphatase	*N*-Acetyl-glucosaminidase	Proteinase	Amylase
Myxamoebae	69±3	68±6	67±4	68±2
0	42±3	36±6	45±6	43±5
2	39±5	33±6	42±7	40±9
4	38±3	32±5	42±4	42±6
7	50±7	50±6	41±9	51±4
12	60±5	52±6	57±7	53±2

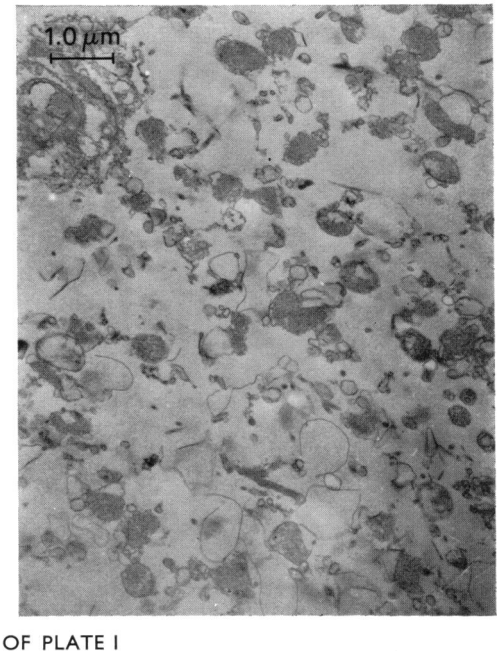
(*a*) (*b*)





(*c*) (*d*)





EXPLANATION OF PLATE I

Electron micrographs of sections of a myxamoeba grown on bacteria (*a*) and of subcellular particles isolated from such myxamoebae by isopycnic sucrose-density-gradient centrifugation (*b*, *c* and *d*). Samples (*b*), (*c*) and (*d*) are derived from the experiment described in Fig. 3(*b*).

Thus this procedure suggested that only 30–40% of the acid phosphatase activity was lysosomal compared with the value of 70% for the myxamoebae (Table 4). As cell differentiation proceeded the percentage sedimentability of the acid hydrolases increased until, at 12h, they were comparable with the values previously obtained for myxamoebae. If the osmotic shock was avoided and cells were deposited on the Millipore filters in suspension in sucrose, then fruiting-body construction was delayed and asynchronous. The percentage sedimentability of the acid hydrolases in the zero-time sample was still decreased but not to the extent observed if the cells had been suspended in water. If the myxamoebae were suspended in sucrose and deposited on Millipore filters saturated in a solution containing 0.2M-sucrose in addition to the usual salts (thus receiving no osmotic shock), cell differentiation was considerably delayed, and about one-third of the cells never differentiated at all. When plates on which the myxamoebae are grown are incubated at 22°C after all the bacteria have been consumed cell differentiation occurs and fruiting bodies are constructed. Since it is very difficult to ensure a uniform rate of disappearance of the bacterial food (and thus initiation of cell differentiation) construction of fruiting bodies under these circumstances is asynchronous. However, if the decrease in percentage sedimentability of the acid hydrolases observed during the first 12h is of significance for the initiation of the cell differentiation process, it might be expected that harvesting growth plates in 0.25M-sucrose at 2h intervals over an 8h period after the bacterial lawn has started to disappear should result in values for the percentage sedimentability of the acid hydrolases in the range 50–60%. When such experiments were carried out it was discovered that the results obtained depended critically on the homogenizer used. With a 'loose' homogenizer, where the clearance between pestle and glass sleeve was approx. 0.003in, values for the percentage sedimentability of the acid phosphatase of 69–73% were obtained at all times over an 8h period, whereas a 'tight' homogenizer with a clearance of approx. 0.002in gave values of 49–61%. Both homogenizers gave a value of 70% for the percentage sedimentability of the hydrolases of the myxamoebae. Thus the variations in apparent lysosomal content reported in Table 4 are best interpreted, not as a change in the amount of lysosome-bound hydrolase per cell, but as indicating an increased fragility of the lysosomes to homogenization during the early stages of cell differentiation.

After 12h of development there have been considerable changes in the specific activity of a number of acid hydrolases (Fig. 4). Yet despite this the intracellular distribution of the enzyme remains unchanged (Table 4). It is possible, however, that the lysosomes present in a 12h (early slug) sample might differ in buoyant density from those in the myxamoebae. Experiment showed, however, that fractionation of the $2.25 \times 10^5 g$-min pellet from a 12h sample on a linear 35–50% (w/v) sucrose density gradient over a cushion of 61% (w/v) sucrose gave a distribution profile indistinguishable from that of Fig. 3(*b*).

DISCUSSION

Acid hydrolases from *D. discoideum* have been studied previously by others. Gezelius (1966) reported the presence of an acid phosphatase that increased between two- and four-fold in specific activity during cell differentiation, and Solomon, Johnson & Gregg (1964) reported that the acid phosphatase activity of the myxamoebae could be separated electrophoretically into several distinct protein species. Rosness (1968) reported the presence of a β-glucosidase in myxamoebae with a pH optimum of 5.0, and Loomis & Coston (1969) have shown that the myxamoebal enzyme (β-glucosidase 1) is electrophoretically distinct from that made during cell differentiation (β-glucosidase 2). It has been shown (J. M. Ashworth, unpublished work) that in both bacteria-grown and axenically grown myxamoebae the β-glucosidase 1 enzyme occurs in association with the other hydrolases in the $2.25 \times 10^5 g$-min pellet. Trehalase (Ceccarini, 1967), which also has an acid pH optimum (at pH 5), does not, however, appear to be associated with the other hydrolases and is freely soluble in both axenically grown and bacteria-grown myxamoebae (G. Weeks & J. M. Ashworth, unpublished work).

The acid hydrolases of *D. discoideum* differ from most lysosomal acid hydrolases previously described by others in having pH optima of 3. In other organisms pH optima of 4–5 are more typical. The specific activities of the hydrolases in the myxamoebae are also high compared with those of rat liver and comparable with those reported for *Tetrahymena* (Muller *et al.* 1966). All the enzymic activities are present at the same or at higher specific activity in axenically grown cells than they are in cells grown on bacteria (Table 2). We found this surprising, since cytological evidence suggests that the lysosomal particles of the myxamoebae (like those of *Tetrahymena*) are involved in digestion of the bacterial food. In Plate 1(*a*) several bacteria can be seen inside a large vacuole at various stages of digestion. Also present in these vacuoles are characteristic whorls of (presumably) phospholipid, which provide a useful marker for the vacuoles that contain them. As can be seen in Plate 1 these whorls are only found in the lysosomal fraction (Plate 1*b* and Fig. 3*b*), thus suggesting that the

lysosome particles we are studying can be identified, at least in part, with the food vacuoles. The fact that the lysosomes of axenically grown cells (which do not possess the whorls shown in Plate 1*a* and 1*b*) have a different density from those of cells grown on bacteria provides further support for this identification. However, the significance of this difference in enzyme activities became less clear when we realized that axenically grown cells and cells grown on bacteria differed in virtually every enzymic activity that we have assayed (J. M. Ashworth & D. Watts, unpublished work) and, of course, the axenic medium still contains much of its nutrients in a polymeric form. It was also observed (Table 2) that the differences in activity between the various hydrolases in axenically grown and bacteria-grown cells are not constant. Thus whereas cells grown on either substrate have identical amylase activities and very similar acid phosphatase activities the increases observed with proteinase, ribonuclease and deoxyribonuclease (all about twofold) are very different from the increases observed with *N*-acetylglucosaminidase (3.6-fold). In both cases, however, the percentage of the total activity present in the $2.25 \times 10^5 g$-min pellet is the same. The amounts of the various lysosomal enzymes present in a cell therefore appear to be independently regulated. In principle such regulation could occur either by altering the proportions in which lysosomes with different enzymic compositions occurred in the cell or by altering the enzymic composition of a homogeneous lysosome population. It is not possible to decide between these two alternatives at present.

The apparent stability of the lysosomes at pH values below 4 varies considerably with the enzyme that is used as a 'marker' for the particle (Fig. 2). These experiments do not distinguish between hydrolase activity which is sedimentable because its lysosome remains unbroken, and activity which is sedimentable because it is absorbed on lysosomal, or other, material. The total amount of sedimentable protein decreases as the pH of incubation is decreased, whereas many of the hydrolase activities are not sedimentable after incubation at pH values above 5. This suggests that if the results shown in Fig. 2 are due to adsorption then this adsorption is not on to contaminating mitochondrial debris. If the results of Fig. 2 are, in fact, due to differential stability of lysosomes then this experiment suggests that the lysosome population is heterogeneous, but further work will be required before this conclusion can be accepted.

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