A prestalk-cell-specific acid phosphatase in *Dictyostelium discoideum*

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**SUMMARY**

A simple and reliable method of separating and quantifying acid phosphatase activities in *D. discoideum* is described, and one of the electrophoretically distinguishable forms of the enzyme is shown to be specific for prestalk cells. Accumulation of this product commences some 2–3 h later in development than the prespore specific enzyme UDP-galactose polysaccharide transferase. This finding is discussed in terms of recent observations on the control of prestalk cell differentiation in this organism.

**INTRODUCTION**

During development in *D. discoideum* starved amoebae aggregate to form multicellular structures consisting of an anterior region of prestalk cells and a posterior region of prespore cells. Under normal conditions the anterior cells give rise to the stalk of the fruiting body eventually formed, while the bulk of the remainder becomes spores (Raper, 1940). It is well established that the ratio of numbers of prestalk and prespore cells is largely independent of the total size of the cell aggregate (Williams, Fisher, MacWilliams & Bonner, 1981), and that surgically produced fragments of individual aggregates (slugs) can regulate to reestablish the normal ratio of precursor cell types (Raper, 1940; Sampson, 1976). Both results point to the existence of a mechanism which regulates the proportion of the two cell types. The nature of this mechanism is being investigated in a number of laboratories (for reviews see Loomis, 1975; MacWilliams & Bonner, 1979; Gross et al. 1981). For such investigations it is becoming essential to have reliable and quantitative measures of the formation of the two types of differentiated precursor cells. Such criteria exist for prespore cells in the form of a prespore (and spore) specific antigen (Takeuchi, 1963) and of an enzyme, UDP-galactose polysaccharide transferase, involved in the formation of this antigen (Newell, Ellingson & Sussman, 1969). Using these markers it has been shown that prespore specific gene products begin to accumulate prior to tip formation when cells have aggregated to form mounds (Takeuchi, Okamoto, Tasaka & Takemoto, 1978; Kay, 1979) and that from the earliest time of their

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appearance prespore cells are absent from the anterior region of the slug (Takeuchi et al. 1978).

The situation with regard to criteria for prestalk cell differentiation is much less satisfactory. Several enzymes have been reported to show higher levels of activity in prestalk cells than in prespore cells (Krivanek & Krivanek, 1958; Jefferson & Rutherford, 1976; Armand, Stetler & Rutherford, 1980; Tsang & Bradbury, 1981). However, these enzymes are not useful as prestalk cell markers because the differences in activity between the two cell types are not large or the enzymes are already present in vegetative amoebae. Moreover, although accumulation of prestalk specific polypeptides has been demonstrated by 2-D gel electrophoresis (Alton & Brenner, 1979; Morrissey, Farnsworth & Loomis, 1981) this method is time consuming and hard to quantify. In a previous publication I identified a unique form of acid phosphatase present in the anterior region of the slugs of another Dictyostelium species, D. mucoroides (Oohata & Takeuchi, 1977). However in this species mature stalk cells are produced during migration (Bonner, 1967) so that the slug cells may be more nearly equivalent to culminating stalk cells than to the prestalk cells of D. discoideum. In the present work I demonstrate the existence of a prestalk specific form of acid phosphatase in D. discoideum, and describe a simple, reliable and quantitative assay for this activity. Evidence is then presented that prestalk cell differentiation, as defined by the accumulation of this enzyme, occurs about 3 h later than prespore cell differentiation (as evidenced by appearance of the prespore specific enzyme UDP-galactose polysaccharide transferase).

MATERIALS AND METHODS

Organisms and development

Dictyostelium discoideum strain V12M2 was grown in two-membered culture with Klebsiella aerogenes on SM agar (Sussman, 1966) at 22 °C for 45 h. Morphogenesis was initiated by plating washed amoebae on 1.8% agar containing 40 mM-Na2HPO4–KH2PO4 (pH 6.2) and 2.5 mM-MgCl2, as described by Tsang & Bradbury (1981).

Cell separation

Prestalk and prespore cells of the slug were separated by means of a self-generating Percoll gradient using the method of Tsang & Bradbury (1981). After centrifugation, the percentage of prespore cells in each fraction was determined in order to measure their purity; some of each cell fraction was fixed with methanol on coverslips and stained with prespore-specific antigen by the method of Forman & Garrod (1977). The percentage of cells in each fraction that was stained was determined by fluorescence microscopy.
Polyacrylamide gel electrophoresis

Cells at various stages of development were harvested in 20 mM-KK₂ phosphate buffer containing 2 mM-MgCl₂ (pH 6-2), washed with cold distilled water and stored frozen. For use, cell pellets were thawed and incubated in 0.1 % of Triton X-100 with occasional vortexing for 20 min at 4 °C, and the lysates spun in a microcentrifuge for 5 min. Sucrose solution was added to the supernatant to a final concentration of 15%.

Polyacrylamide slab gel electrophoresis was performed in essentially the same way as the Disc electrophoresis described previously (Oohata, 1976). The following buffer systems were used: 0.2 g of imidazole and 5.52 g of barbituric acid per litre for the running buffer, and imidazole-HCl buffer for the stacking gel (pH 6-2) and the resolving gel (pH 7-8). The stacking gel and resolving gel contained 3-6 % and 6 % acrylamide respectively. The electrophoresis was conducted at 4 °C, and consisted of 15 min at 100 V, followed by 70–90 min at 300 V. After electrophoresis, acid phosphatases were visualized by the simultaneous azo-coupling method as follows:- after preincubation in 0.1 M-citric acid–citrate buffer pH 4-5 for 15 min at room temperature, the gels were incubated for 20 to 30 min at 25 °C in the same buffer at pH 4-8 containing 0.5 mg/ml or 1.5 mg/ml of α-naphthyl acid phosphate sodium salt (Sigma) and 0.4 mg/ml of fast garnet GBC (Sigma). The reaction was stopped by replacing the solution with 7% acetic acid. The stained gels were scanned on a Joyce Loebl Chromoscan 200/201 system at 520 nm.

Enzyme assays

Glycogen phosphorylase and UDP-galactose polysaccharide transferase were assayed as described by Tsang & Bradbury (1981) according to the methods of Town & Gross (1978) and Kay (1979) respectively.

Protein determination

Protein concentration was determined by the method of Bradford (1976).

RESULTS

Identification of electrophoretically distinct acid phosphatases

Extracts of cells of D. discoideum V12M2 at various developmental stages were prepared and subjected to polyacrylamide gel electrophoresis. Extracts of vegetative amoebae gave a single band of acid phosphatase (acid phosphatase 1) which was present throughout development. Another more slowly migrating band (acid phosphatase 2) was absent in vegetative cells but present in slugs (Fig. 1). Another band which migrated still more slowly than acid phosphatase 2 was detected in extracts of vegetative amoebae when acetate buffer pH 4.5 was used.
Fig. 1. Densitometric tracing of *D. discoideum* acid phosphatases. Extracts of V12M2 cells (15 μg protein) were subjected to electrophoresis and the gel stained histochemically as described in Materials and Methods using 1.5 mg/ml α-napthyl acid phosphate as substrate. The arrows indicate the position of the tracking dye. (A) Vegetative amoebae (t₀) (B) migrating slugs (t₁₈).

For the enzyme reaction instead of citric acid–citrate buffer (data not shown). It was not studied further.

*The electrophoretic patterns of acid phosphatase in prestalk and prespore cells*

To determine whether the developmentally controlled form of acid phosphatase (acid phosphatase 2) is localized specifically in the prestalk cells of the slug, as is the case in *D. mucoroides* (Oohata & Takeuchi, 1977), slug cells
Prestalk enzyme in D. discoideum were disaggregated and separated into prestalk and prespore cells by Percoll gradient centrifugation. Their electrophoretic patterns were then compared. As shown in Fig. 2, acid phosphatase 1 was common to both cell types, whereas acid phosphatase 2 was found only in the prestalk fraction.

Quantitative determination of acid phosphatase activities

The bands obtained by densitometric tracing of stained gels (see Fig. 1) were found to be symmetrical when up to 30 µg protein extract was loaded. Hence the absorbance due to acid phosphatase 1 could be estimated from the outside half-area of its band, and that due to acid phosphatase 2 could be measured in the same way. Occasionally when band 2 was not well resolved from band 1 its area was estimated by subtracting twice the half-area of acid phosphatase 1 from the total area due to the two enzymes. The dependence of reaction rate on protein

Fig. 2. The electrophoretic pattern of acid phosphatases in prestalk and prespore cells. The cells of migrating slugs (117) were dissociated and separated in a Percoll gradient as described by Tsang & Bradbury (1981). After electrophoresis the gel was stained for 25 min with 0.5 mg/ml substrate. (A) Whole slugs; in this case all the cells in one Percoll gradient tube were pooled after centrifugation, and subjected to electrophoresis. 20 µg protein. (B) Top fraction from the gradient; staining with antispore serum gave 7% prespore cells. 12 µg protein. (C) Bottom fraction; 99% prespore cells. 14 µg protein.
concentration was determined using this quantitative method. Figure 3 shows a standard curve of the absorbance due to acid phosphatase 2 as a function of protein concentration. The reaction was linear to at least 16.5 μg protein with 1.5 mg/ml of substrate under the conditions described in Materials and Methods. A similar result was obtained with acid phosphatase 1.

Kinetics of appearance of the prestalk specific acid phosphatase during development

Figure 4A presents measurements of the activities of the acid phosphatases as a function of time of development. It can be seen that acid phosphatase 1 activity dropped to about half its initial value starting at about t9, while acid phosphatase 2 remained undetectable until about the same time and then rose to a plateau at t18.

In order to relate the time of appearance of the prestalk specific acid phosphatase 2 to other developmental events the accumulation of two well-known postaggregative enzymes (Town & Gross, 1978) was examined in the same experiment. Glycogen phosphorylase, present in both prestalk and prespore cells (Tsang & Bradbury, 1981), and UDP-galactose polysaccharide transferase, a prespore-specific product (Newell et al. 1969), both began to appear before t9. Thus the appearance of acid phosphatase 2 is delayed by 2–3 h compared to these two enzymes. Similar results to those shown in Figs 4A and 4B were obtained with strain NC4 of D. discoideum, though the activity of acid phosphatase 2 was lower than in strain V12M2 (data not shown).
Multiple forms of acid phosphatase have been identified previously by Solomon, Johnson & Gregg (1964) and Parish (1976). In the present study, I have focused attention on the two forms of enzyme detected when the assay is performed in citrate buffer, and have shown that one of these forms is specific
for prestalk cells. I have not attempted to identify the molecular basis of the
difference between the two enzyme forms. The function of the prestalk-specific
enzyme is unknown, though it may play a role in cell wall formation at the time
of stalk construction (see Gezelius, 1972).

In studies in this laboratory on cell differentiation in monolayers it has been
shown that amoebae incubated at low density in the presence of cyclic AMP
develop into stalk cells only if provided with a low-molecular-weight lipid-like
factor (DIF) that is liberated into the extracellular medium during development
(Town, Gross & Kay, 1976; Town & Stanford, 1979). It has been suggested by
Gross et al. (1981) that DIF is an activator of prestalk cell formation in a lateral
inhibition mechanism of the kind proposed by Gierer & Meinhardt (1972).
Recently, Brookman, Town, Jermyn & Kay (1982) have shown that DIF
accumulates in cells co-ordinately with glycogen phosphorylase and UDP-
galactose polysaccharide transferase (a prespore-specific product). My observa-
tion that the appearance of acid phosphatase is delayed by two to three hours
compared to these products is thus quite compatible with the idea that prestalk
cell differentiation is dependent upon prior accumulation of DIF. Further
evidence that synthesis of acid phosphatase 2 is DIF-dependent comes from our
recent finding that mutants that fail to make their own DIF, but can respond to
it, also fail to accumulate acid phosphatase 2. Moreover, acid phosphatase 2 is
produced when both DIF and cAMP are added in normal development. Such
mutants do accumulate high levels of prespore specific UDP-galactose
polysaccharide transferase (Kopachik, Oohata, Dhokia, Brookman & Kay (in
preparation)).

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