USE OF AQUEOUS TWO-PHASE PARTITION TO DETECT CELL SURFACE CHANGES DURING GROWTH OF DICTYOSTELIUM DISCOIDEUM

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SUMMARY
Changes in the cell surface properties of amoebae of Dictyostelium discoideum during growth in different culture conditions have been studied by aqueous two-phase partitioning on a thin-layer countercurrent distribution apparatus. Changes in cell surface properties were not dependent on the source of nutrients but only on cell density. There was a progressive increase in cell surface hydrophobicity with cell density in both axenic cultures and cultures grown with a bacterial substrate. It is proposed that it is these cell-density-related surface changes that account for the ability of amoebae grown in different conditions to sort out during subsequent development in a manner related to cell fate.

INTRODUCTION
Aqueous two-phase partition is a technique that separates cells, on the basis of differences in surface properties, by partitioning the cells between two immiscible, aqueous solutions of polymers (Albertsson, 1971; Walter, 1977). The two aqueous polymer solutions most commonly used are dextran and poly(ethyleneglycol), which, when mixed at certain concentrations, form the two immiscible phases. The upper phase is rich in poly(ethyleneglycol) and is relatively more hydrophobic than the lower phase, which is rich in dextran. Cells partition between the interface and one of the phases to different degrees depending on their surface properties. The phases can be buffered and rendered isotonic and cell partition can be affected by either charge or non-charge associated surface properties (or both) depending on the salt composition of the phase system (Walter, 1977; Gerson, 1982).

This technique has previously been used to detect surface changes during early stages of development of the cellular slime mould Dictyostelium discoideum (Sharpe, Treffry & Watts, 1982). The phase system used was a 'low potential' (neutral) system giving little or no potential difference between the two phases (Walter, 1977). In this phase system D. discoideum amoebae partitioned between the interface and the upper phase mainly on the basis of non-charge related surface properties (i.e. cell surface hydrophobicity).

It has been shown by Leach, Ashworth & Garrod (1973) that growth conditions can affect the fate of D. discoideum amoebae during subsequent development. Thus when

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two populations of amoebae that had been grown in two different culture conditions were mixed and allowed to form fruiting bodies, there was a marked tendency for the amoebae from one population to differentiate into spores and for the amoebae from the other population to differentiate into stalk cells. This was possible because the amoebae in the two populations were able to sort out from one another during development (Leach et al. 1973) and such behaviour seems to imply that the amoebae grown in the different culture conditions must have had different cell surface properties. Aqueous two-phase partition has therefore been used to determine whether growth-related differences could be detected in the cell surface properties of *D. discoideum* amoebae.

**MATERIALS AND METHODS**

**Phase partition**

Full details of the method of thin-layer countercurrent distribution (TLCCD), sample preparation and unloading used for *D. discoideum* amoebae have been previously described (Sharpe et al. 1982). The most suitable phase system was found to comprise 5-5% (w/w) dextran T500 (batch 4094 Pharmacia Fine Chemicals), 5-5% (w/w) poly(ethylene glycol) 4000 (batch 6444220 BDH Chemicals), 0-05 mol/kg NaCl, 0-01 mol/kg Na2SO4 and 0-001 mol/kg KH2PO4/K2HPO4 buffer (pH 7-8) (Sharpe et al. 1982). Partition was carried out at 4°C on a Bioshef-MK1 TLCCD apparatus.

**Dictyostelium discoideum**

All experiments were carried out with strain Ax-2 (wild-type) amoebae (Watts & Ashworth, 1970). Amoebae were grown axenically at 22°C in HL5 medium containing 86 mM-glucose (Watts & Ashworth, 1970), harvested at various densities from exponential through to stationary phase of growth, and washed once with distilled water at 4°C. Amoebae were also grown axenically in HL5 medium without glucose (NS amoebae) and harvested at a density of 2-0×10⁶ cells ml⁻¹.

Growth of amoebae with bacteria in liquid culture was with *Aerobacter aemgenes* NCTC 418 suspended (10¹⁰ bacteria ml⁻¹) in 50 mM-phosphate buffer (pH 6-5). The *A. aemgenes* had been grown in minimal medium E containing 0-4% (w/v) glucose (Vogel & Bonner, 1956). Amoebae were harvested at densities of 1-0×10⁶ cells ml⁻¹ (exponential phase) and 1-5×10⁶ cells ml⁻¹ (stationary phase) and washed four times in distilled water at 4°C to remove residual bacteria.

Growth of amoebae on nutrient agar plates with *A. aemgenes* NCTC 418 was as described by Sussman (1966). Amoebae were harvested when the plates had been cleared of bacteria, but before any signs of aggregation were visible, and were washed four times in distilled water at 4°C.

**Cell sorting-out**

Cell sorting-out experiments were conducted with cells from the extreme edges of the TLCCD profiles obtained with cells harvested in the exponential phase of axenic growth. The experimental procedure involved mixing equal numbers of wild-type and mutant (acriflavin-resistant) amoebae as previously described (Leach et al. 1973; Sharpe et al. 1982). Wild-type and mutant amoebae produced identical TLCCD profiles.

**RESULTS**

**Axenic growth**

Growth of Ax-2 amoebae in axenic culture containing glucose has been described previously (Watts & Ashworth, 1970). Stationary phase is reached at a cell density of approximately 2-0×10⁷ cells ml⁻¹. Fig. 1 shows the CCD profiles obtained with Ax-2 amoebae harvested at 1-0×10⁶ cells ml⁻¹ (exponential phase), 5-0×10⁶ cells ml⁻¹.
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(late exponential phase), 1·0×10⁷ cells ml⁻¹ (early stationary phase) and 2·0×10⁷ cells ml⁻¹ (stationary phase). As cell density increased during growth the cell profiles moved to the right (i.e. to higher fraction numbers). Thus peaks for exponential, late exponential, early stationary and stationary phase cells were in fractions 20, 26, 31 and 35, respectively (Fig. 1). This indicated (Albertsson, 1971; Gerson, 1982) that there was a progressive increase in cell surface hydrophobicity with cell density. The profile obtained with stationary-phase amoebae was broader than the profiles of amoebae harvested in earlier phases of growth (Fig. 1), and this would suggest (Albertsson, 1971; Walter, 1977) that there was an increase in cell surface heterogeneity when amoebae were in stationary phase.

Amoebae grown axenically in HL5 medium lacking glucose (NS amoebae) enter the stationary phase of growth at a cell density of 4·0×10⁶ to 6·0×10⁶ cells ml⁻¹ (Watts & Ashworth, 1970). Hence amoebae harvested at 2·0×10⁶ cells ml⁻¹ from these cultures are in the early stationary phase of growth, whereas amoebae harvested at the

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**Fig. 1.** Countercurrent distribution profile of Ax-2 amoebae grown in HL5 glucose medium and harvested at various stages of growth: A, exponential; B, late exponential; C, early stationary; D, stationary.
same cell density from cultures supplemented with glucose are in exponential growth. The difference was reflected in the partitioning profile of the NS amoebae. When this was compared with profiles given by amoebae grown in the glucose-containing medium, it resembled the profile given by stationary-phase amoebae and not the profile given by exponential-phase amoebae (Fig. 2).

**Growth with bacteria in liquid culture**

Fig. 3 shows the CCD profiles obtained with amoebae grown with suspensions of bacteria and harvested in the exponential or stationary phase of growth. There appeared to be an increase in surface hydrophobicity with cell density that was similar to that shown by amoebae grown in axenic culture (Fig. 1). A small peak detected in the early CCD fractions (Fig. 3) comprised bacteria that had not been removed by repeated washing.

![Fig. 2. Countercurrent distribution profile of Ax-2 amoebae grown axenically in medium lacking glucose and harvested in early stationary phase.](image1)

![Fig. 3. Countercurrent distribution profile of Ax-2 amoebae grown with A. aerogenes in liquid culture and harvested in early exponential (A) and stationary (B) phases of growth.](image2)
Growth with bacteria on agar plates

It was not possible to study partitioning of amoebae that had been harvested during exponential growth on agar plates with a bacterial substrate because the amoebae were heavily contaminated with bacteria that could not be removed by washing. Amoebae were therefore harvested only when most of the bacteria had been consumed and hence were close to being in stationary phase or early development. This is usual practice when amoebae have been grown on agar plates with *A. aerogenes* (Sussman, 1966). The profile was broad (Fig. 4) in comparison with the profiles given by amoebae grown in shaken culture and suggested that the amoebae were very heterogeneous in cell surface properties.

Cell sorting-out during development of amoebae harvested during exponential, axenic growth

Since there appeared to be some cell surface heterogeneity even within a population of cells harvested during exponential, axenic growth, attempts were made to determine whether this had any effects on cell fate during subsequent development. When equal numbers of cells from the trailing and leading edges of the TLCCD profile were mixed and allowed to form fruiting-bodies, cells from the trailing edge preferentially formed spores (Table 1).

DISCUSSION

Growth-related differences in the cell surface properties of amoebae of *D. discoideum* were detected by partitioning. These seemed not to be dependent on the source of nutrients but only on the phase of growth in which the amoebae were harvested. Thus there was an increase in cell surface hydrophobicity as amoebae passed from the exponential phase of growth into stationary phase for both amoebae grown axenically and amoebae grown with a bacterial substrate.

The nature of the changes in surface properties occurring as amoebae enter the stationary phase of growth is not known but the changes probably do not involve cell
surface charge, since such changes would not have been detected by the phase system that was used to study the cell surface properties. Furthermore, electrophoresis studies have shown that there is little change in cell surface charge during growth of *D. discoideum* amoebae (Lee, 1972). The phase system would have detected changes in cell surface carbohydrate composition (Sharpe & Warren, 1984; Walter & Coyle, 1968) and it is probable that the increases in cell surface hydrophobicity resulted from such changes. Jaffé, Swan & Garrod (1979) have found that a carbohydrate is progressively lost from the cell surface of amoebae during axenic growth and is released into the growth medium. The carbohydrate seems to be involved in cell cohesion.

Leach et al. (1973) have shown that cell differentiation in *D. discoideum* is affected by previous growth conditions. For example, they found that, when amoebae harvested during exponential axenic growth were mixed with amoebae harvested in the stationary phase of axenic growth and allowed to form fruiting bodies, the exponential-phase amoebae differentiated into spores whereas stalk cells were formed from the stationary-phase amoebae. Similar experiments were performed with amoebae grown in other culture conditions and it became apparent that it was possible to place amoebae in an order that indicated which amoebae had the greatest tendency to form spores. The order was: exponential-phase amoebae from HL5 glucose medium > exponential-phase amoebae from HL5 medium (NS amoebae) > amoebae grown on agar plates with a bacterial substrate > stationary-phase amoebae grown axenically. Thus when amoebae from two different growth conditions were mixed and

<table>
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<th>Experiment</th>
<th>Nature of cells from Trailing Leading</th>
<th>Percentage of spores derived from Trailing Leading</th>
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<tr>
<td>1</td>
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<td>64</td>
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<tr>
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<td>61</td>
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<td>2</td>
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<td>Mutant Wild-type</td>
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**Table 1. Developmental fate of amoebae having different surface properties during the exponential phase of axenic growth**

Wild-type and mutant (acriflavin-resistant) amoebae were harvested during exponential growth and subjected to TLCCD. Cells isolated from the leading edge of the wild-type profile were mixed with an equal number of cells from the trailing edge of the mutant profile, and *vice versa*, and allowed to form fruiting bodies. Spores from the fruiting bodies were then analysed for wild-type and acriflavin-resistant character. (Trailing edge comprised fractions 11–16; leading edge comprised fractions 24–29; see Fig. 1A.)
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allowed to form fruiting bodies, spores were formed from the amoebae coming earliest in the list whilst the amoebae later in the list formed stalk cells. It was also shown (Leach et al. 1973) that this behaviour was dependent on the ability of the amoebae to sort out during development and it now seems probable that this sorting-out was possible because the amoebae had different cell surface properties. The difference in surface properties is most apparent for amoebae grown in HL5 glucose medium and harvested during the exponential and stationary phases of growth. The surface properties of amoebae harvested after growth in dual culture with bacteria on agar plates are also unique since these amoebae partition to give a broad profile, which indicates a high degree of cell surface heterogeneity. It is probable that this is because amoebae harvested off agar plates are in all phases of growth from exponential to stationary phase/early development. It is difficult to account for the ability of NS amoebae to sort out in the manner described by Leach et al. (1973) unless the NS amoebae had been harvested in the early stationary phase of growth. Unfortunately, Leach et al. (1973) omitted details of the cell density at which NS amoebae were harvested. However, it is clear that in later studies (Tasaka & Takeuchi, 1981) in which similar sorting out of NS amoebae from amoebae grown in HL5 glucose medium was described, the NS amoebae were in early stationary phase.

There was some cell surface heterogeneity even amongst amoebae harvested during exponential, axenic growth. When amoebae with the most pronounced differences in surface properties (i.e. those in the leading and trailing edges of the TLCCD profile) were mixed in equal numbers and allowed to form fruiting bodies, it was found that amoebae from the trailing edge predominantly formed spores. Thus, it may be deduced that, in agreement with experiments discussed above, there was a tendency for the cells with the more hydrophobic cell surface properties (i.e. those in the leading edge of the TLCCD profile) to differentiate into stalk.

Tasaka & Takeuchi (1981), Takeuchi et al. (1981) and Meinhardt (1983) have suggested that the cell sorting out within mixtures of NS amoebae and amoebae grown in HL5 glucose medium is a model for the sorting out that they believe must also take place during pattern formation that occurs before fruiting bodies are formed from a population of amoebae all harvested in the same phase of growth. This may well be correct because it has since been shown by Sharpe et al. (1982) that exponential-phase amoebae harvested from HL5 glucose medium and allowed to enter development can, 10 h later, be separated by partitioning into two populations of amoebae, which therefore differ in surface properties. One population seems to comprise presumptive spore cells whilst the other comprises presumptive stalk cells (Sharpe et al. 1982, 1984). Furthermore, in the studies of both Tasaka & Takeuchi (1981) and Sharpe et al. (1982, 1984), it was the amoebae with the least hydrophobic cell surface properties that differentiated into spores.

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REFERENCES


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