CELL ELECTROPHORESIS OF THE CELLULAR SLIME MOULD DICTYOSTELIUM DISCOIDEUM

II. RELEVANCE OF THE CHANGES IN CELL SURFACE CHARGE DENSITY TO CELL AGGREGATION AND MORPHOGENESIS

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SUMMARY

The reduction in the cell surface charge density of Dictyostelium discoideum during differentiation has been studied by the technique of cell electrophoresis. It was abolished under conditions in which cell aggregation was inhibited (e.g. low temperature, the presence of actinomycin D or cycloheximide). In the presence of nutrients incapable of supporting growth, cell aggregation occurred without a reduction in surface charge density. Cell adhesion in these aggregates was impaired, and a reduction in surface charge density appeared to be necessary for further development.

Brief treatment of exponential phase and aggregating cells with agents which disaggregate slugs failed to alter their electrophoretic mobilities. Low concentrations of magnesium chloride caused extensive agglutination, especially in aggregating cell suspensions, but little change in their electrophoretic mobilities. Magnesium chloride could agglutinate cells by association with cell surface components undetectable by cell electrophoresis. This, together with immunological evidence from other workers, supports the possibility of involvement of specific surface macromolecules in cellular slime mould aggregation. It was concluded that changes in surface charge density, though important for cell adhesion and morphogenesis, cannot account for all aspects of cell interactions in D. discoideum.

INTRODUCTION

The electrophoretic mobility (and hence the surface charge density) of Dictyostelium discoideum amoebae decreases progressively as they approach cell aggregation (Garrod & Gingell, 1970). This phenomenon is probably due to a decrease in the density of cell surface carboxyl groups, and an increase in that of amino groups (Lee, 1972). The reduction in surface charge density of the cells on incubation in a non-nutrient medium can be roughly correlated with an increase in their agglutinability by simple salts (Born & Garrod, 1968), and the increased resistance of cell clumps to mechanical dispersion. It is not known if a reduction in surface charge density is obligatory for cell aggregation, or is merely an unrelated phenomenon associated with starvation. One approach to this problem is to investigate the effect of various agents which inhibit cell differentiation or disaggregate pseudoplasmodia (slugs) on the electrophoretic mobility of the cells.

Factors inhibiting cell aggregation and morphogenesis in D. discoideum include low temperature (Garrod & Gingell, 1970), actinomycin D, cycloheximide and nutrients.
Actinomycin D is a recognized inhibitor of RNA synthesis, and was used to inhibit slime mould morphogenesis (Pannbacker & Wright, 1966; Wright & Pannbacker, 1967; Sussman, Loomis, Ashworth & Sussman, 1967; Hirschberg, Ceccarini, Osnos & Carchman, 1968), and as a specific inhibitor of RNA synthesis in studies of developmentally linked enzymes (Sussman, 1966b; Loomis, 1970; Telser & Sussman, 1971; Newell, Longlands & Sussman, 1971). Cycloheximide (actidione), a well known inhibitor of protein synthesis in eukaryotic cells, was used to inhibit protein synthesis and formation of developmentally linked enzymes in D. discoideum (Sussman, 1965, and the papers on actinomycin D).

The agents which disaggregate slugs and aggregates include EDTA and proteases. Sussman & Sussman (1960) used trypsin to disperse aggregates of a ‘fruity’ mutant of D. discoideum (Strain Fty-1) for cell counting, but Whitfield (1964) could not repeat this for wild type D. discoideum, and found that only papain was effective under a limited range of conditions. Papain was also used by Yanagida & Noda (1967). Takeuchi & Yabuno (1970) reported that D. discoideum slugs could be disaggregated by a variety of proteases in the presence of 2,3-dimercapto-propan-1-ol and simple salts.

EDTA has been used for disaggregating slugs or aggregates (Dehaan, 1959; Gerisch, 1961; Shaffer, 1962; Whitfield, 1964; Takeuchi & Yabuno, 1970), but the results of different authors are conflicting and may reflect the experimental conditions used. EDTA prevents clumping of D. discoideum cells which have been incubated for 2 h, but does not appreciably alter their zeta potential (Gingell & Garrod, 1969; Gingell, Garrod & Palmer, 1969). These authors concluded that EDTA does not inhibit cell aggregation simply by increasing the net electrostatic repulsive force between cells through chelation of divalent cations. However, unlike other electrophoretic studies on divalent cation binding (Weiss, 1968; Seaman, Vassar & Kendall, 1969; Patinkin, Schlessinger & Doljanski, 1970; Patinkin, Zaritsky & Doljanski, 1970), magnesium ions were not present in the electrophoresis buffer of control cells, and it was not known whether their presence would lower the cell surface charge density. Another criticism is that the cells used for the experiments had been incubated for only 2 h, and the clumping of these cells is not the same as cell aggregation 4-5 h later, even though EDTA may inhibit both by the same mechanism.

The object of the work described in this paper was to establish the relevance of the surface charge density changes to cellular slime mould aggregation and morphogenesis by investigating changes in electrophoretic mobility under conditions unfavourable to cell differentiation or the existence of aggregates.

**Materials and Methods**

**Growth and differentiation of Dictyostelium discoideum**

The methods for growth of D. discoideum Ax-2 in axenic culture and differentiation on Millipore filters, agar or in non-nutrient liquid culture have been described (Lee, 1972).

In some experiments, differentiation was conducted statically in liquid medium. Cells were harvested during the exponential phase of growth, washed twice at 0 °C, and resuspended in medium M (10 mM KCl, 5 mM MgCl₂, 10 mM sodium phosphate, pH 6.0). The cell suspension
was pipetted into 6- or 9-cm plastic Petri dishes so that each dish contained $10^7$ cells and 3 ml medium or $3 \times 10^7$ cells and 10 ml medium respectively. The cells soon settled to the bottom, and were not resuspended by slight movement of fluid in the dish. The presence of magnesium ions was essential for firm attachment of the amoebae to the bottom of the dish. The dishes were incubated statically in the dark at 23 °C. Under these conditions, the formation of streams and aggregation centres was detectable after 9-10 h, and aggregation completed by 16-20 h. Sussman's medium (Sussman, 1966a) can be used instead of medium M, but differentiation is slower.

The following additions were made to medium M in the Petri dishes to prevent cell aggregation: (1) 1.62 % (w/v) glucose. (2) An amino acid mixture of the same composition as casein hydrolysate. Final concentrations in Petri dishes (mM) Asp, 2.43; Thr, 0.94; Ser, 1.80; Glu, 4.96; Pro, 2.82; Gly, 1.17; Ala, 1.77; Val, 0.93; Met, 0.43; Ile, 0.48; Leu, 1.45; Tyr, 0.26; Phe, 0.48; Lys, 1.48; His, 0.30; Arg, 0.39. Total: 21.7 mM. (3) A vitamin mixture. Final concentrations in Petri dishes (μg/ml); biotin, 0.02; calcium pantothenate, and folic acid, 6.0; pyridoxine, nicotinic acid, inositol, thiamine and β-amino-benzoic acid, 66.0.

Cell electrophoresis

This was performed as described elsewhere (Lee, 1972).

Materials

Crystalline trypsin (EC 3.4.4.4) and papain (EC 3.4.4.10) were purchased from Worthington Biochemical Corp., Freehold, N. J.; pronase (B grade) from Calbiochem Ltd.; snail gut digestive juice and cycloheximide from Koch Light Laboratories Ltd.; 2,3-dimercaptopropan-1-ol (British Anti-Lewisite, BAL) from British Drug Houses; and actinomycin D was a gift from Merck, Sharp and Dohme Ltd., Hoddesdon, Herts.

RESULTS

Effect of agents which disaggregate slugs on the electrophoretic mobility

EDTA. EDTA has been used by a number of workers to disperse aggregates and slugs of *D. discoideum*, and its effect on the electrophoretic mobility of preaggregation cells was studied by Gingell & Garrod (1969) and Yabuno (1970).

Incubation of exponential phase cells, aggregating cells and aggregation-competent cells with 10 mM EDTA for 30 min had only marginal effects on their electrophoretic mobilities (Table 1). Although EDTA dispersed aggregates under the conditions employed, the cell suspensions obtained formed clumps of 10-20 cells on standing in 10 mM sodium phosphate (pH 6.8) at room temperature for 20 min, even in the presence of 1 mM EDTA. Under the same conditions, exponential phase cell suspensions remained essentially single-celled. These findings are in agreement with those of Gerisch (1961, 1968) that aggregation-competent cells formed clumps in the presence of EDTA. Aggregating cells dispersed with EDTA rapidly recovered and completed their development (Takeuchi & Yabuno, 1970). Although EDTA causes loss of surface macromolecules from bacteria (Leive, Shoulin & Mergenhagen, 1968), no evidence was obtained for removal of specific adhesive substances from the surface of slime mould cells by a 30-min treatment with EDTA. However, when cells were incubated on Millipore filters in the presence of 10 mM EDTA, cell aggregation and the fall in electrophoretic mobility were inhibited. After incubating for 10 h in the presence of EDTA, only 10% of the cells completed their development on removal of EDTA. It
Table 1. Effect of EDTA treatment on the electrophoretic mobility of *D. discoideum*

<table>
<thead>
<tr>
<th>Cells</th>
<th>Electrophoretic mobility ((-\mu m/s \cdot V^{-1} cm \pm S.E.M.))</th>
<th>Student's t-test probabilities, control v. treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exponential phase</td>
<td>(1.42 \pm 0.02) (10) (1.37 \pm 0.01) (10)</td>
<td>(0.1 &lt; P &lt; 0.5)</td>
</tr>
<tr>
<td>Aggregation-competent (10^5) h in medium M</td>
<td>(1.15 \pm 0.02) (17) (1.22 \pm 0.02) (21)</td>
<td>(0.01 &lt; P &lt; 0.05)</td>
</tr>
<tr>
<td>Aggregating cells (12) h on Millipore filters</td>
<td>(0.83 \pm 0.01) (11) (0.88 \pm 0.02) (10)</td>
<td>(0.01 &lt; P &lt; 0.05)</td>
</tr>
</tbody>
</table>

Cells harvested during the exponential phase of growth were incubated on Millipore filters or in suspension in medium M for 12 and \(10^5\) h respectively. They were harvested, washed and resuspended in \(0.145\) M NaCl, \(10\) mM Tris-EDTA, \(10\) mM Tris-HCl (pH 7.5) at \(10^7\) cells/ml. The suspension was shaken for 30 min at 23 °C, 150 cycles per min in an orbital incubator. Electrophoresis was carried out in \(10\) mM sodium phosphate (pH 6.8, \(I = 0.02\) g ion l.\(^{-1}\)) after treatment. Student's t test probabilities (control v. treated) are shown. The numbers of observations made are in parentheses.

Fig. 1. Effect of magnesium ions on the electrophoretic mobility of *D. discoideum*. Exponential phase cells and aggregating cells (after \(10\) h on agar) were washed once and resuspended in sodium phosphate (pH 6.8) containing different concentrations of magnesium chloride and the electrophoretic mobilities were determined. The ionic strength of the solution was maintained at \(0.02\) g ion l.\(^{-1}\). ●, exponential phase cells; ○, aggregating cells. The histograms show the electrophoretic mobilities of exponential phase cells (hatched) and aggregating cells (unhatched) in \(1\) mM Na, EDTA, \(7\) mM sodium phosphate (pH 6.95-7.00, \(I = 0.02\) g ion l.\(^{-1}\)).
Slime mould cell electrophoresis. II  

was probable that prolonged treatment resulted in irreversible interference with cell metabolism.

An alternative explanation for the dispersal of aggregates by EDTA is that it chelates divalent cations bound to the cell surface, and increases the zeta potential. The effect of a divalent cation (Mg$^{2+}$) on the electrophoretic mobilities of exponential phase and aggregating cells was studied (Fig. 1). Magnesium ions had little effect on the electrophoretic mobilities of both cell preparations at concentrations up to 2 mM, but produced extensive clumping, especially in suspensions of aggregating cells. At 5 mM MgCl$_2$, the electrophoretic mobilities of both cell preparations were reduced by about 20%. It was not possible to determine electrophoretic mobilities at greater magnesium ion concentrations due to massive cell clumping. The presence of EDTA (1 mM) in the electrophoresis buffer caused a significant ($P < 0.001$) but small increase in the electrophoretic mobility of exponential phase cells, but not in that of aggregating cells ($P > 0.5$).

**Enzymes.** Dictyostelium discoideum pseudoplasmodia are effectively disaggregated by papain and pronase in the presence of 2,3-dimercapto-propan-1-ol (BAL) (Takeuchi & Yabuno, 1970), and the effect of these and other enzymes on the electrophoretic mobility of exponential phase and aggregating cells was studied (Table 2). It is clear that treatment with enzymes, irrespective of their ability to disaggregate slugs on agar, produced only marginal effects on the electrophoretic mobility of both cell preparations, even though some of the changes were statistically significant ($P < 0.05$).

**Factors affecting cell aggregation and development**

**Temperature.** Growth and development of *D. discoideum* did not occur at 4 °C, and at this temperature there was no fall in electrophoretic mobility when incubated in a non-nutrient medium (Fig. 2). This finding is in agreement with the results of Garrod & Gingell (1970) for pre-aggregation cells.

**Inhibitors of nucleic acid and protein synthesis.** The 2 inhibitors used most extensively in studying cellular slime mould development are actinomycin D and cycloheximide, and the effects of these on the changes in electrophoretic mobility which occur during the early stages of differentiation were investigated. Cells harvested during the exponential phase of growth were incubated on Millipore filters, and after various time intervals the filters were transferred to support pads containing actinomycin D. Cells were harvested at intervals, and their electrophoretic mobilities determined (Fig. 3). Addition of actinomycin D at any time up to 5 h inhibited cell aggregation and abolished any further decrease in electrophoretic mobility. There was a rise in electrophoretic mobility towards the initial value for cells which had been incubated for 4 and 5 h before addition of the inhibitor. Addition of actinomycin D at 6.5 h did not prevent the fall in electrophoretic mobility or inhibit cell aggregation. This failure to inhibit aggregation has been attributed by Sussman *et al.* (1967) to the presence of mRNA synthesized before addition of the inhibitor.

In contrast to actinomycin D, cycloheximide inhibits differentiation immediately, and its addition to cells at 0 and 6.5 h immediately prevented any further fall in electrophoretic mobility, and inhibited cell aggregation (Fig. 4).
Table 2. Effect of enzymic treatment on the electrophoretic mobility of *D. discoideum*

<table>
<thead>
<tr>
<th>Enzyme and duration of treatment</th>
<th>Effectiveness in dispersing slugs on agar</th>
<th>Electrophoresis buffer</th>
<th>Exponential phase cells</th>
<th>Aggregating cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Treated</td>
<td>Control</td>
</tr>
<tr>
<td>Trypsin 1 h</td>
<td></td>
<td>1.35 ± 0.02 (16)</td>
<td>1.34 ± 0.01 (25)</td>
<td>1.25 ± 0.03 (15)</td>
</tr>
<tr>
<td>Snail gut juice 1 h</td>
<td></td>
<td>10 mM sodium phosphate</td>
<td>(pH 6.8)</td>
<td>—</td>
</tr>
<tr>
<td>Papain/BAL 30 min</td>
<td>+</td>
<td>1.33 ± 0.03 (11)</td>
<td>1.41 ± 0.03 (21)</td>
<td>1.15 ± 0.02 (24)</td>
</tr>
<tr>
<td>Pronase/mercaptoethanol</td>
<td>±</td>
<td>10 mM sodium phosphate</td>
<td>(pH 6.8)</td>
<td>0.1 &lt; (P &lt; 0.5)</td>
</tr>
<tr>
<td>Pronase/BAL 30 min</td>
<td>+</td>
<td>1.46 ± 0.02 (12)</td>
<td>1.40 ± 0.02 (11)</td>
<td>1.02 ± 0.01 (12)</td>
</tr>
<tr>
<td>Pronase/BAL 30 min</td>
<td>+</td>
<td>0.145 M NaCl 3 \times 10^{-4} M NaHCO(_3)</td>
<td>(pH 7.2)</td>
<td>0.01 &lt; (P &lt; 0.05)</td>
</tr>
</tbody>
</table>

Electrophoretic mobility \((-\mu m \ s^{-1} \ V^{-1} \ cm \ ± \ S.E.M.)\)

Exponential phase cells and aggregating cells (after 10 h incubation on 2% agar) were harvested and incubated at a cell density of 107 cells/ml at 23°C, 150 cycles per min in an orbital incubator in the following enzyme solutions: (i) Trypsin: 0.1 mg/ml in 10 mM CaCl\(_2\), 50 mM Tris-HCl (pH 8.0); (ii) Snail gut digestive juice: 2% (v/v) in 10 mM sodium phosphate (pH 7.0); (iii) Papain: 0.3 mg/ml in 20 mM NaCl, 20 mM KCl, 2.5 mM BAL (2,3 dimercapto-propan-1-ol) in 10 mM sodium phosphate (pH 6.0); (iv) Pronase: 1 mg/ml in 20 mM NaCl, 20 mM KCl, 2.5 mM BAL or mercaptoethanol and 30 mM Tris-HCl (pH 7.4). After the period of time shown in the table, the cells were harvested, washed and resuspended for electrophoretic measurements. The numbers of observations made are in parentheses. Student's t-test probabilities (control vs. treated) are shown. Controls were incubated under identical conditions but without the enzymes. The experiments using different enzymes were performed on separate occasions. This and the different incubation conditions could explain the differences in the controls.

* "Aggregation-competent" cells incubated in suspension in 10 mM sodium phosphate (pH 6.8) for 12 h were used.
Fig. 2. Effect of incubation at low temperature on the electrophoretic mobility of *D. discoideum*. Cells harvested during the exponential phase of growth were incubated in liquid culture at a cell density of $10^7$/ml in 10 mM sodium phosphate (pH 6.8) in an orbital incubator at 23 °C or 4 °C. Samples were taken at various times, and their electrophoretic mobilities determined in 10 mM sodium phosphate (pH 6.8, $I = 0.02$ g ion l$^{-1}$). ○, cells incubated at 23 °C; □, 4 °C.

Fig. 3. Effect of actinomycin D on the electrophoretic mobility of differentiating cells of *D. discoideum*. Cells harvested during the exponential phase of growth were incubated on Millipore filters. At 0, 4, 5, and 6.5 h, filters were transferred to support pads containing 2.5 ml 10 mM sodium phosphate (pH 6.8) with 125 μg/ml actinomycin D, and incubation was continued. Cells from 2 or more filters were harvested at intervals, and their electrophoretic mobilities determined in 10 mM sodium phosphate (pH 6.8, $I = 0.02$ g ion l$^{-1}$). The arrow indicates the onset of aggregation in the control. ○, control; □, treated with actinomycin D.
Effect of nutrients. *Dictyostelium discoideum* did not develop aggregation competence in the growth medium, and on continued incubation into the stationary phase of growth, lost the ability to differentiate. Watts & Ashworth (1970) reported that *D. discoideum* cells would grow and form colonies on agar containing the growth medium, but rarely formed fruiting bodies. It was decided to look for simple mixtures of nutrients which would inhibit aggregation.

Cell aggregation under water did not occur in the presence of glucose, a mixture of amino acids with the same composition as casein hydrolysate and vitamins at the same concentrations as Ashworth's partially defined growth medium for *D. discoideum Ax-3* (Ashworth, unpublished). This nutrient mixture (designated full mixture) did not support the growth of the amoebae, but the decrease in cell dry weight and protein content which normally occurs during differentiation (White, & Sussman, 1961) was prevented. Glucose or amino acids plus vitamins alone did not prevent aggregation, but the aggregates formed were more diffuse and disorganized than those formed in the absence of nutrients. If a fall in electrophoretic mobility of cells is obligatory for cell aggregation, it will be expected to happen in the presence of either glucose or amino acids plus vitamins but not the full mixture. However, very little reduction in electrophoretic mobility occurred in the presence of either glucose or amino acids plus vitamins (Table 3). Subsequently, it was shown that the electrophoretic mobility of cells incubated in suspension in medium M with additions of glucose, amino acids plus vitamins or the full mixture remained essentially constant up to 12 h. Similar results were obtained for cells incubated on Millipore filters in the presence of glucose (Fig. 5).

Although cell aggregation occurred on Millipore filters in the presence of glucose
Table 3. Effect of incubation with nutrients on the electrophoretic mobility of Dictyostelium discoideum

<table>
<thead>
<tr>
<th>Cells</th>
<th>Nutrients present during incubation</th>
<th>Stage of life cycle when harvested</th>
<th>Electrophoretic mobility ((-\mu m \text{s}^{-1} \text{V}^{-1} \text{cm} \pm \text{S.E.M.}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>10 mM sodium phosphate (pH 6.8)</td>
</tr>
<tr>
<td>Exponential phase</td>
<td>Not incubated</td>
<td>Exponential phase</td>
<td>1.45 ± 0.01 (20)</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>Late aggregation</td>
<td>1.11 ± 0.02 (16)</td>
</tr>
<tr>
<td></td>
<td>Glucose (1-22%)</td>
<td>Late aggregation</td>
<td>1.39 ± 0.02 (22)</td>
</tr>
<tr>
<td></td>
<td>Amino acids + vitamins</td>
<td>Late aggregation</td>
<td>1.31 ± 0.01 (14)</td>
</tr>
<tr>
<td>After 13 h incubation</td>
<td>Glucose, amino acids and vitamins (full mixture)</td>
<td>No visible morphogenesis</td>
<td>1.41 ± 0.02 (18)</td>
</tr>
</tbody>
</table>

Cells harvested during the exponential phase of growth were suspended in medium M and incubated under water with addition of nutrients. They were harvested after 13 h, washed in the appropriate electrophoresis buffer (10 mM sodium phosphate, pH 6.8, \(I = 0.02 \text{ g ion l}^{-1}\) or 0.145 M NaCl, 3 x 10^{-4} M NaHCO₃, pH 7.2, \(I = 0.145 \text{ g ion l}^{-1}\)), and their electrophoretic mobilities were determined. The numbers of observations made are in parentheses.
without a fall in the electrophoretic mobility of the cells, the aggregates produced (Fig. 11) appeared less firm and compact than those produced in the control (Fig. 7), and were readily dispersed to form a suspension of single cells and some small clumps. The control aggregates required several washes in ice-cold buffer and vortex-mixing for dispersal (Fig. 15). It is clear that in the presence of glucose (and amino acids plus vitamins), cell aggregation occurred in the absence of a fall in electrophoretic mobility, but cell adhesiveness was reduced.

Fig. 5. Effect of incubation with glucose on the electrophoretic mobility of differentiating cells of *D. discoideum*. Cells harvested during the exponential phase of growth were incubated on Millipore filters with or without 1.62% (w/v) glucose. Cells from two or more filters were harvested at various times, and their electrophoretic mobilities determined in 10 mM sodium phosphate (pH 6.8, I = 0.02 g ion L\(^{-1}\)). The arrows indicate the times at which cell aggregation occurred. ○, incubated with glucose; ●, without glucose.

Morphogenesis of *D. discoideum* after cell aggregation did not occur under water, and incubation on Millipore filters was used to study further development of aggregates formed in the presence of glucose. The majority of these aggregates formed slugs and fruiting bodies (Fig. 11–14). The variation in electrophoretic mobility of *D. discoideum* incubated on Millipore filters with or without glucose is shown in Fig. 5. There was virtually no change in the electrophoretic mobility of cells incubated in the presence of glucose during the first 12 h, even though cell aggregation was discernible after 8 h. The aggregates dispersed fairly easily on washing off the filters, and the distribution of electrophoretic mobilities was unimodal at all times up to 12 h. After 14.5 h incubation, cells with lower electrophoretic mobilities were evident, and after 16.5 h, the distribution of electrophoretic mobilities was distinctly bimodal (Fig. 6). Only the lower mean value of electrophoretic mobilities is plotted in Fig. 5 for the 16.5-h time point. No evidence of a bimodal distribution was found for cells incubated in the absence of glucose (Lee, 1972).
At 16.5 h (Fig. 6), high electrophoretic mobilities were associated almost exclusively with single cells which showed little or no pseudopodial activity in the electrophoresis chamber. This behaviour was characteristic of non-aggregating cells. Low electrophoretic mobilities were associated with single cells and many clumps which showed the rapid pseudopodial activity characteristic of aggregating cells.

The appearance of cells with low electrophoretic mobilities after 12 h incubation

Fig. 6. Distribution of electrophoretic mobilities of *D. discoideum* cells incubated with glucose. Cells incubated on Millipore filters in the presence of 1.62% (w/v) glucose were harvested after various times for electrophoretic measurements. The electrophoretic mobilities are represented by the time intervals taken by the cells to traverse 10 graticule divisions (142.8 \( \mu \)m) when the applied voltage was 100 V. The times in hours, numbers of observations made and mean electrophoretic mobilities (\( -\mu m \ s^{-1} \ V^{-1} \ cm \)) were: A, 12, 25, 1.42 \( \pm \) 0.01; B, 14.5, 123, 1.27 \( \pm \) 0.02; and C, 16.5, 106 and high, 1.50 \( \pm \) 0.02, low 1.02 \( \pm \) 0.02.
with glucose correlated approximately with the transition from the loose-looking aggregates (Fig. 11) into more compact aggregates (Fig. 12) on Millipore filters. A variable proportion, occasionally as high as 50%, of aggregates remained as shapeless clumps (Fig. 16) and failed to transform into slugs and fruiting bodies (Fig. 17). During migration on Millipore filters in the presence of glucose, the slugs sometimes left behind large numbers of cells, some of which formed separate slugs, and some failed to develop further (Fig. 13). This could reflect an impairment of cell-cell adhesion.

The distributions of electrophoretic mobilities in Fig. 6 for 14.5 and 16.5 h are unreliable, as a proportion (up to 20%) of the cells harvested at these times remained in large tight aggregates which were not dispersed by vortex-mixing. The proportion of cells with low electrophoretic mobilities is therefore an underestimate. Development after aggregation is not possible if the cells are fully submerged, and the volume of liquid in the support pads and the cell concentration on the Millipore filters were carefully adjusted to give consistent results. Although cells incubated in the presence of glucose exhibited small variations in different experiments in the times at which cell aggregation began and cells of low electrophoretic mobilities appeared, no appreciable fall in electrophoretic mobility was observed before cell aggregation occurred.

**DISCUSSION**

During the early stages of differentiation in *D. discoideum* there was a linear reduction in electrophoretic mobility, and an increase in cell adhesiveness associated with increased resistance of cell clumps to mechanical dispersion. These changes were not dissociated from cell aggregation by incubation at low temperature or in the presence of actinomycin D or cycloheximide. When the cells were incubated in the presence of glucose (or amino acids plus vitamins), there was no reduction in electrophoretic mobility before cell aggregation occurred, and cell adhesion in the resulting aggregates was impaired. The transformation of these aggregates on Millipore filters into more compact cell masses and eventually fruiting bodies was associated with the appearance of cell clumps with increased resistance to mechanical dispersion and lower electrophoretic mobilities. These changes may be due to a localized depletion of glucose in the aggregates. It is likely that a decrease in the surface charge density, although unnecessary for cell aggregation, is essential for cell adhesion in aggregates and their subsequent development.

Since cell aggregation occurs in the absence of a reduction in surface negative charge density, other surface components must be involved, for example, acrasin receptors, species-specific recognition sites and intercellular adhesive substances. Cell aggregates and slugs are dispersed by treatment with proteolytic enzymes, but this treatment does not affect the electrophoretic mobility of the cells. It is possible that the dispersal results from non-specific damage to the cell surface. This dispersal of aggregates without changes in surface charge density is consistent with the hypothesis that the intercellular electrostatic repulsive force is not the only determining factor in cellular slime mould aggregation.

The electrophoretic mobilities of exponential phase and aggregating cells were not changed appreciably by treatment with EDTA or by the presence of EDTA in the
electrophoresis buffer. The presence of magnesium ions in the electrophoresis buffer at concentrations which caused extensive clumping, especially of aggregating cells, did not alter the electrophoretic mobilities of these cells. There is no evidence that EDTA inhibits cell clumping by chelating divalent cations. Magnesium ions could cause clumping by association with surface components undetectable by cell electrophoresis.

Yabuno (1970) reported that pronase or EDTA treatment resulted in an increase in the electrophoretic mobility of *D. discoideum* (strain NC-4) which had been incubated on agar for 3-5 h. It is not possible to assess the reliability of this result in the absence of statistical data, but the cells used had been grown on bacteria, and their electrophoretic mobilities would be expected to show considerable variation (Garrod & Gingell, 1970). Attempts to repeat this finding in *D. discoideum* (strain Ax-2) were not successful (Lee, unpublished).

There is evidence for the presence of intercellular binding substances in sponges (Humphreys, 1963, 1965, 1970), mammalian and avian cells (Moscona, 1962, 1968; Lilien, 1970). Complementary glycoproteins isolated from opposite mating types of the yeast, *Hansenula wingei* are believed to be involved in specific recognition during mating (Crandall & Brock, 1968a, b). There is a large body of immunological evidence in the cellular slime moulds for antigenic differences between non-aggregating and aggregating cells (Sonneborn, Sussman & Levine, 1964; Gerisch, 1968; Beug, Gerisch, Kempff, Riedel & Kremer, 1970), between species (Gerisch, Malchow, Wilhelms & Lüderitz, 1969) and between wild type cells and their aggregateless mutants (Gregg & Trygstad, 1958; Gerisch & Beug, 1971). Although most of these differences were demonstrated in crude preparations, and not always unequivocally established to be on the cell surface, they may be involved in cell adhesion.

Cell aggregation in *D. discoideum* involves at least 2 processes: formation of aggregates by chemotaxis, and cell adhesion. The linear reduction in cell surface charge density during the early stages of differentiation is not specifically associated with the onset of cell aggregation and chemotaxis, but it can be correlated with a progressive increase in agglutinability and adhesiveness of the cells (Born & Garrod, 1968). This change in electrophoretic mobility is probably essential for strong cell adhesion in aggregates, and for their subsequent development. It is reasonable to suppose that the existence of pseudoplasmodia of 10⁶ cells or more and the complex morphogenetic movements involved in migration and fruiting require strong cell-cell adhesion. However, changes in cell surface charge density cannot account for all aspects of cell recognition, interaction and morphogenetic movements during aggregation, and surface components undetectable by cell electrophoresis are probably involved.

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REFERENCES


Slime mould cell electrophoresis. II


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Figs. 7-10. Differentiation of *Dictyostelium discoideum* on Millipore filters; after 10, 16.5, 24, 36 h incubation respectively. × 3.

Figs. 11-14. Differentiation of *Dictyostelium discoideum* on Millipore filters in the presence of 1.62% (w/v) glucose; after 10, 16.5, 24, 36 h incubation respectively. The arrows indicate cells that failed to fruit (Fig. 14). × 3.

Fig. 15. Cells from one Millipore filter (5 × 10⁶ cells) were washed off with 15 ml 10 mM sodium phosphate (pH 6.8), and vortex-mixed for 5 s after 10 h incubation in the presence (A) or absence (B) of glucose. Actual size.

Figs. 16, 17. *Dictyostelium discoideum* after 16.5- and 36-h incubations in the presence of glucose, showing cells that did not develop after aggregation (arrows). × 3.