A PROGRESSIVE CHANGE IN THE ELECTROPHORETIC MOBILITY OF PREAGGREGATION CELLS OF THE SLIME MOULD, DICTYOSTELIUM DISCOIDEUM

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SUMMARY

The electrophoretic mobility of slime-mould preaggregation cells has been found to decrease progressively as they near the chemotactic aggregation stage. This appears to be a spontaneous event dependent on some aspect of cell metabolism. Its possible relevance to cell adhesion and morphogenesis is discussed.

INTRODUCTION

During the aggregation stage of the slime-mould life-cycle a dramatic change occurs in the nature of cellular interaction. The locomotor reaction of feeding cells towards each other tends to be one of repulsion (Samuel, 1961; Shaffer, 1957, 1962), but at aggregation they move together to form a multicellular body. In aggregation the cells are guided by chemotaxis and by their mutual contacts (Bonner, 1947; Shaffer, 1962, 1964). In this paper we deal with cells between the feeding and aggregation stages and shall refer to them as preaggregation cells. We report the results of an investigation of the surface properties of preaggregation cells by cell electrophoresis.

Our interest in the surface properties of preaggregation cells was stimulated by the finding (Born & Garrod, 1968) that cells in suspension in distilled water could be made to adhere by adding solutions of simple salts (sodium and calcium chlorides). The most obvious explanation of this seemed to be that the addition of a salt to cells in distilled water lowered their surface potentials, thus reducing the electrostatic repulsive force between the cells and allowing them to approach each other sufficiently for the forces involved in adhesion to operate. Further, as the cells neared the spontaneous, chemotactic aggregation stage, the amount of salt required to make them adhere became progressively smaller. This suggested a possible change in surface properties of the cells during the period leading up to aggregation. The results also seemed of interest in relation to the colloid-electrostatic double-layer theory of cell adhesion (Curtis, 1962, 1967; Pethica, 1961), which suggests that cells adhere in a position of minimal potential energy where attractive London–van der Waals forces and electrostatic repulsive forces are exactly equal.
MATERIALS AND METHODS

Cells were grown on 'S.M.' agar (Sussman, 1966) at 22 °C in association with Escherichia coli B/r. They were harvested just before aggregation in cold distilled water and washed free of bacteria by centrifugation. Next the cells were pipetted on to Millipore filters resting on support pads saturated with distilled water and contained in plastic Petri dishes. (Storing the cells in this way enables a suspension of single cells to be obtained for up to 6 h after harvesting (Born & Garrod, 1968) in contrast to storage on washed agar, in which case it is necessary to treat the cells with papain in order to separate them after 4 h incubation (Yanagida & Noda, 1967).) The dishes were incubated at 22 °C until the cells were required for experiment.

Prior to electrophoresis, the cells were washed from the filters with cold distilled water and stored in iced water until required for use. Born & Garrod (1968) have found that the cells remain healthy and retain their properties when stored in this way.

Electrophoresis was carried out in 0.01M phosphate buffer at pH 6.8–7.2. (Equal volumes of cell suspension in distilled water and 0.05M phosphate buffer were mixed.) Cell suspensions were placed in the chamber of the electrophoresis apparatus (Zeiss Cytopherometer) which was maintained at 23 °C, and allowed to stand for 7–10 min. This allowed cell clumps to settle out of suspension since turbulence produced by settling cell clumps causes the cells to drift in the chamber, making accurate measurement impossible. The mobilities of some cell clumps were measured to ensure that they were not different from single cells. A current of 1 mA, supplied by a modified Baird and Tatlock Electrophoresis Power Pack, was used and the cells were timed to the nearest 0.1 s over a distance of 0.4 μm.

RESULTS

First, it should be mentioned that cells incubated on Millipore filters with distilled water were found to develop synchronously, as do cells incubated with buffered salt solution (Ashworth & Sussman, 1967). Moreover, the different stages of the life-cycle occur at approximately the same time as found by these workers. Initially, the cells form a flat lawn on the surface of the filter. After 5–6 h a rippling of the surface of the lawn occurs and chemotactic aggregation begins 7.5–8.5 h after placing the cells on the filter. The reason the cells can aggregate under the conditions of storage in these experiments is probably that substances are leached out of them into the medium, thus increasing its ionic strength. Measurements of conductivity of the medium when cells were allowed to aggregate on a glass surface beneath distilled water showed a marked conductivity increase towards aggregation.

Our most important result is that the mobility of the cells decreases with time after the cells have been placed on the filter (Fig. 1), i.e. as they near the spontaneous aggregation stage. The average decrease in mobility for the period 1–6 h is about 17%. Since all electrophoretic experiments were performed at the same ionic strength, temperature and pH, this means that the surface charge density of the cells is spontaneously reduced, lowering the zeta potential. The average zeta potentials and surface-charge densities of cells after 1 and 6 h on the filter are shown in Table 1. The averages are calculated from 292 measurements on 1-h cells and 271 on 6-h cells.

Two points of interest emerge from the changes in mobility of groups of cells in 9 different experiments shown in Fig. 1. First, the results are always quantitatively slightly different. Taking the results as a whole, it is apparent that in some experiments cells at 6 h had a higher mobility than cells at 1 h in other experiments. Secondly, although the results are quantitatively different, in 6 out of 9 cases the lines represen-
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The rate of change of mobility for different groups of cells are almost exactly parallel to each other, indicating that over the 5-h period the rate of change of mobility is nearly always the same in different experiments, irrespective of the initial value.

Fig. 1. Graph showing reduction in electrophoretic mobility of preaggregation cells with length of incubation at 22 °C on Millipore filters saturated with distilled water. Each line represents a different experiment. Under the conditions of incubation chemotactic aggregation begins between 6.5 and 8.5 h after placing the cells on the filter. Each point is the average of between 20 and 70 measurements. For clarity, standard errors are not shown here, but are indicated in Figs. 2 and 3 for individual experiments.

<table>
<thead>
<tr>
<th>Period of incubation (h)</th>
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<tbody>
<tr>
<td><strong>Zeta potential (mV)</strong></td>
<td>21.99</td>
<td>18.35</td>
</tr>
<tr>
<td><strong>Surface charge density (e.s.u. cm⁻¹)</strong></td>
<td>$2.105 \times 10^5$</td>
<td>$1.649 \times 10^5$</td>
</tr>
</tbody>
</table>

Zeta potentials calculated using the Helmholtz-Smoluchowski equation and surface charge densities as by Gingell & Garrod (1969), assuming an ion-impenetrable surface.

There seemed to be two reasons why the mobilities of cells stored in this way should change progressively. Either during the preaggregation stage the cells undergo a change in surface properties which is under metabolic control, or the change is a response of the cells to being stored in distilled water. For example, storage in distilled water might
result in the desorption of anions from the cell surface thus decreasing the electrophoretic mobility. We performed two experiments to try to distinguish between these possibilities.

Born & Garrod (1968) showed that the change in surface properties of slime-mould cells demonstrated by adhesion tests was dependent on temperature and did not occur when the cells were stored on filters in the cold, and the cells do not aggregate under these conditions. We therefore stored cells at 3 °C and measured their mobilities at

![Graph of electrophoretic mobility of preaggregation cells plotted against length of incubation on Millipore filters, saturated with distilled water at 22 °C (solid line) and 3 °C (broken line). Mobility measurements were made at 23 °C in both cases. Vertical lines through points represent standard errors.](image1)

![Graph showing reduction in electrophoretic mobility of preaggregation cells with length of incubation at 22 °C on Millipore filters saturated with 0.01M phosphate buffer. Vertical lines through points represent standard errors.](image2)
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23 °C at zero time, 1 and 6 h. The result (Fig. 2) shows that there is virtually no change in the mobility of cells stored for 6 h in distilled water at 3 °C.

Next we carried out an experiment in which the cells were stored at 22 °C on filters saturated with 0.01 M phosphate buffer. Mobilities were measured at 0, 1 and 4 h at 23 °C. The results (Fig. 3) indicate that a decrease in mobility occurs when the cells are stored in phosphate buffer. Spontaneous chemotactic aggregation also occurs about 8 h after the beginning of incubation with phosphate buffer. However, it was not possible to make electrophoretic measurements later than 4 h after the beginning of incubation because extensive clumping of the cells occurred after this time.

These results suggest that the reduction in electrophoretic mobility is under metabolic control and is not a reaction of the cells to storage in distilled water.

DISCUSSION

Our main observation is that the surface-charge density of slime-mould preaggregation cells is spontaneously and progressively reduced as the cells near the chemotactic aggregation stage. It seems that this result may be of importance in relation to cell adhesion generally as well as to slime-mould morphogenesis. We shall discuss each of these topics separately.

Born & Garrod (1968) found that less salt was required to produce the same rate of adhesion between older preaggregation cells than between young ones. One possible reason for this appeared to be that less salt was required with old cells in order to reduce the electrostatic repulsive force between cells to a critical value below which stable contact could occur. In view of the present result this seems likely, since a reduction in cell surface charge density would indeed mean that less salt would be required to reduce electrostatic repulsive force to a given value.

Superficially our results would seem to support the colloid-electrostatic double-layer theory of adhesion (Curtis, 1962, 1967), since there does seem to be a relationship between surface charge density and adhesion in this system. Significant here, however, are the further findings (Gingell & Garrod, 1969; D. Garrod, D. Gingell & G. V. R. Born, unpublished) that two factors, EDTA and low temperature, can largely inhibit the adhesion of slime mould cells without markedly affecting the electrostatic repulsive force between them. Further, we must emphasize that our results reveal nothing about the nature of the forces responsible for cell adhesion; we cannot say whether London-van der Waals forces are responsible, as the colloid theory suggests, or whether, for example, some intercellular ligand is involved as appears to be the case with sponge cells (Moscona, 1968). Probably all we can conclude is that under some conditions (see Yanagida & Noda, 1967; Born & Garrod, 1968) the electrostatic repulsive force between cells may be a limiting factor in determining adhesive stability. This conclusion is not particularly helpful in relation to the specific problem of cell adhesion, since the same is true of inert particles and the phenomenon is well known in colloid chemistry (see Verwey & Overbeek, 1948). It is worth emphasizing that, in any case, gross surface properties measured by cell electrophoresis may be of limited significance in relation to cell adhesion (Weiss, 1968). It also seems possible that
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colloidal forces might be involved in initial cell-to-cell contact in suspension, but may have little subsequent relevance to the mechanism of cell adhesion.

The reduction in cell surface-charge density during the preaggregation stage may be an important event in relation to aggregation, the next stage in the slime mould life-cycle. It does not seem to be a response of the cells to storage in distilled water since it also occurs in medium of high ionic strength. Also, it seems probable that the change may depend on some aspect of cellular metabolism, since it is inhibited in the cold, as is further morphogenesis. We shall now examine possible ways in which the reduction in surface charge could be of importance in morphogenesis.

One possibility is that the reduction in charge density might be related to increasing cellular adhesiveness prior to aggregation. The reduction in charge density might be important in itself in this respect or, as mentioned before, it might represent the synthesis of an intercellular binding material. We have, however, only circumstantial evidence for supposing that cellular adhesiveness does increase towards aggregation. Shaffer (1957) has made qualitative, light-microscope observations on the behaviour of slime-mould cells prior to and during aggregation. He observed that feeding cells and early preaggregation cells could make contact with each other but tended to separate after so doing. Late preaggregation cells tend to collect in clusters, whereas aggregating cells appeared to adhere strongly to each other in aggregation streams and centres. On the basis of these observations, Shaffer suggested that the adhesiveness of cells increased towards aggregation. It must be pointed out that, although Shaffer's interpretation may be correct, any assessment of the degree of contact between cells made on light-microscope observations cannot be reliable and the apparent differences in cellular adhesiveness could be due to differences in cell behaviour completely unrelated to adhesiveness. Slightly stronger evidence for an increase in cellular adhesiveness towards aggregation comes from the work of Yanagida & Noda (1967). They found that when preaggregation cells were incubated on non-nutrient agar at 23 °C for up to 4 h, a suspension of single cells could be obtained by washing the cells from the plate with cold distilled water. After 4 h, however, it became necessary to treat the cells with papain in order to separate them. This probably indicates that the cells had increased in adhesiveness after 4 h incubation, though other interpretations are possible. The only satisfactory way to demonstrate an increase in adhesiveness of preaggregation cells with time would be to assess quantitatively the disaggregation of aggregates formed by cells of different ages. On balance it can probably be said that there is a progressive increase in adhesiveness of slime-mould cells prior to aggregation, though we would emphasize our reservations in coming to this conclusion.

If there is an increase in adhesiveness and if the reduction in surface charge density which we have found has anything to do with it, it seems that it may not be the reduction of surface charge density itself which is important. This is because, although the reduction was always found, results were always quantitatively dissimilar. Some cells had a higher surface charge density at 6 h than others at 1 h. (Incidentally, the same lack of quantitative similarity between different adhesion experiments was found by Born & Garrod (1968).) Since aggregation under the conditions used occurs between 7.5 and 8.5 h after the beginning of incubation, it seems unlikely that the re-
duction of surface charge density to a critical level is an important factor in aggregation. We incline to the view that, if an increase in adhesiveness occurs, it may be due to the development of some adhesive property of the cell surface, such as the synthesis of an intercellular binding substance, which is manifested in our experiments by the reduction in surface charge density. (Interestingly, Gerisch (1968) has shown that an antigenic component is present at the surface of aggregation-competent *D. discoideum* cells, which is absent in feeding cells.) Further possibilities are that the reduction in surface charge might indicate the re-orientation of a polymeric adhesive material at the surface, or might induce a membrane permeability change affecting cytoplasmic systems involved in adhesion.

The change in surface-charge density could be associated with some parameter of cellular interaction, other than adhesion. For example, it might be a manifestation of some surface change involved in the liberation of, or response to, the chemotactic substance acrasin. Some recent work has suggested that acrasin may be cyclic adenosine monophosphate (Konijn, Barkley, Chang & Bonner, 1968). Cyclic AMP is a negatively charged molecule, so a reduction in cell surface negative charge density might promote the adsorption of acrasin to the surface of a responding cell.

Lastly, Gingell (1967) has suggested that the rise in surface potential experienced when charged cell surfaces approach each other in solution could play an important role in intercellular communication. The magnitude of the rise in surface potential would be greater for approaching cells having a higher surface potential at infinite separation. It would seem possible, therefore, that the difference in surface potential between old and young preaggregation cells which we have found could be involved in determining the obvious change in the nature of cellular interaction which occurs during the period.

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**REFERENCES**


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