SHORT COMMUNICATION

The Cell Surface in Amoeboid Locomotion: Behaviour of *Naegleria gruberi* on an Adhesive Lectin Substrate

By T. M. PRESTON* AND D. S. O'DELL

Department of Zoology, University College London, Gower Street, London WC1E 6BT

(Received 1 October 1979)

The behaviour of *Naegleria gruberi* amoebae on coverslips coated with concanavalin A was followed by reflection interference microscopy. Locomotion continued briefly on the protein and much material was shed before the cells halted. The effect was overcome by perfusion with hapten sugars. These carbohydrates dissociated those trails formed in water from the substrate save for areas of focal contact, whereas trails generated in 10 mM-KCl were unaffected by hapten. These results argue for direct cell–substrate contact over a limited portion of the cell surface during this type of locomotion.

INTRODUCTION

During amoeboid locomotion one major function of the cell surface is to form adhesions to the substrate through which traction may be applied. Without traction, crawling movement would be impossible. If the establishment of adhesions is a necessary prelude to traction, it is clear that their controlled termination is also implicit in such a means of locomotion. Intuitively, one would expect that if de-adhesion were prevented, locomotion would be grossly impeded. It is these phenomena of adhesion and de-adhesion which we have investigated with the soil protozoon *Naegleria gruberi*. This amoeboflagellate has two advantages of special relevance to this study: firstly, it has a surface lacking a substantial glycocalyx but rich in receptors for the lectin concanavalin A (Con A) (Preston *et al.*, 1975) and, secondly, it tolerates a wide range of ionic conditions. Thus we have been able to investigate the behaviour of amoebae on Con A-coated coverslips and follow both adhesion to the lectin and de-adhesion when appropriate competing sugars were added. Ionic conditions were varied since this modulates reversibly the gap between the bulk of the ventral surface of the cell and the substrate (Preston & King, 1978*b*). The dynamic morphology of interactions between cell and adhesive substrate was monitored by reflection interference microscopy. We show here that on Con A surfaces the movement of *Naegleria gruberi* is severely impaired and is only possible at the expense of shedding considerable amounts of cellular material in the form of trails emanating from the uroid (posterior) region. This impediment can be overcome by perfusion with sugars which release Con A receptors from their interaction with the substrate, while the extent to which the cell comes into contact with this substrate in the first place depends on the ionic environment. These observations have allowed consideration of the extent of direct (molecular) contact between cell and substrate during this type of locomotion, and may argue for the special nature of limited areas of 'focal contact'.

METHODS

Cell culture. *Naegleria gruberi* (stock 1518/1c, Culture Collection of Algae and Protozoa, Cambridge) was maintained at 25 °C in monoxenic culture with *Escherichia coli* on 2% (w/v) peptone agar plates and harvested as described previously (Preston & King, 1978b).

Preparation of substrates. Circular glass no. 2 coverslips were cleaned as follows: they were boiled in a solution of Pyroneg (Diversey Ltd, Cockfosters), then washed in several changes of deionized water followed by methanol from which they were air-dried. Lectin-coated coverslips were prepared by pipetting on to one face 50 µl of a 1 mg ml⁻¹ solution of concanavalin A (grade IV, Sigma) in 1 M-acetic acid. The solvent was allowed to evaporate at 37 °C producing a fairly homogeneous and stable film that even after prolonged washing in deionized water continued to bind human red blood cells. Denatured Con A was prepared by dialysing 10 ml of a 1 mg ml⁻¹ solution of native lectin against four changes of 1.5 l 1 M-acetic acid, and was tested by gel diffusion in Ouchterlony plates against a 50 mg ml⁻¹ solution of glycogen; no precipitin lines developed in 1 week at room temperature, whereas native Con A at one-fiftieth of the concentration gave a strong positive reaction after 2 d. Further coverslips were prepared with a coat of the denatured Con A.

Chemicals. Deionized water (specific conductance between 0.5 and 2 µS, details in Preston & King, 1978b) was used routinely. Stock solutions (1 M) were made of methyl- α -D-glucopyranoside, methyl- β -D-glucopyranoside, methyl- α -D-mannopyranoside (Koch-Light), maltose and sucrose (AR grade, BDH). KCl and NaCl (AR grade) were from BDH.

Scanning electron microscopy. Amoebae suspended in 2 mM-Tris/HCl, pH 7.4 (specific conductance 160 µS) were applied to Con A-coated coverslips and after 15 min any unattached cells were removed by gentle washing. The preparations were then fixed in 1% (w/v) OsO₄ at room temperature and dehydrated in a graded series of acetone/water mixtures before critical point drying. The specimens were coated with carbon then gold before being examined in a Cambridge S4-10 microscope at 20 kV. Ilford FP4 35 mm film was used for photographic records.

Light microscopy. Bright field and reflection interference images were obtained from the same fields with a Vickers M41 Photoplan stand bearing a Zeiss $\times 100$ and a Cooke $\times 45$ oil immersion lenses. Green light was selected from the output of a 200 W mercury lamp by a Balzers K55 interference filter. A television camera (Link 106; Andover, Hants.), connected to a videotape recorder, was positioned on the microscope to monitor the locomotory behaviour of amoebae during experiments. When white-light interference patterns were required for estimation of the cell-substrate gap, incident illumination was provided by a 100 W quartz halogen source. A brief account of the optical principles involved in reflection interference microscopy is given in Preston & King (1978a).

Observation of cells. The test coverslip was mounted as one window of a tissue culture perfusion chamber (W. Prior, Bishops Stortford, Herts.) and deionized water was run through until the specific conductance of the effluent fell to less than 4 µS and its pH was between 5.5 and 6.0. Perfusion was then halted and amoebae (about 10⁵) were inoculated into the chamber and allowed several minutes to settle under gravity on to the test coverslip. The chamber was then inverted and perfusion with deionized water was recommenced to remove any unattached cells. The chamber was then locked in this inverted position on to the stage of the reflection interference microscope. Individual amoebae could now be observed whilst different solutions were perfused through the chamber. Separate tests with dye solutions had previously been used to calibrate the apparatus; we thus have confidence that the given concentrations of reagent represented that to which the cells were exposed when observations were made. Further details of the perfusion system are given in Preston & King (1978b).

RESULTS

Behaviour of cells on glass and on lectin films

In deionized water, amoebae readily moved across a glass surface and when viewed by reflection interference microscopy gave the pattern previously described (Preston & King, 1978a), i.e. a large whitish area of 'associated contact' about 100 nm from the substrate and linked to it by filopodia which generated small black 'focal contacts'. Such focal contacts were left behind as the cell progressed. In contrast, on films of native Con A, amoebae moved only for a variable but short distance before coming to a halt. Inspection of these arrested cells showed that they still displayed vigorous cytoplasmic activity, although new pseudopodia were no longer extended. The general appearance under phase contrast optics was of rounded amoebae billowing over a firm ventral attachment. This ventral face under reflection interference optics generated a uniform destructive interference

pattern that was black, i.e. zero order, in white-light illumination. Smaller focal contacts could not be resolved under these optical conditions. This interference pattern continued for some distance posteriorly beyond the limits of the cell profile as seen in bright field microscopy (Fig. 1*a, b*). This 'trail' marked the path taken by the cell across the lectin film and its development was observed before the cell became arrested. The trail consisted of an axial part, variable in both length and width, contiguous with the ventral surface of the amoeba and decorated laterally with very fine processes of a fairly constant width (Fig. 1*c*). Scanning electron microscopy clearly showed this dendritic appearance (Fig. 1*d*) with an axial section of thin folds drawn out from the cell body. These sheets were closely applied to the lectin film and from them emanated cylindrical microextensions of fairly constant diameter despite branching (Fig. 1*e*). Both the cell body and the dendritic trails could be shown by fluorescence microscopy to bind antibody to *Naegleria gruberi*, the preparation of which we have previously described (Preston *et al.*, 1975), and thus the trail was indeed of cellular origin.

When Con A had been applied to only half of the face of a coverslip, amoebae moved normally on the uncoated region, as judged by phase contrast and reflection interference microscopy. On the coated portion, they became immobilized, as described above. On films of dialysed Con A, cell behaviour was variable with normal unhindered locomotion in places and intermittent trail production elsewhere.

Effect of carbohydrates on locomotion over lectin surfaces

When 0.1 M-methyl- α -D-mannopyranoside was perfused into the experimental chamber to replace water there was no change in the gross behaviour or reflection interference patterns of amoebae on uncoated surfaces. Cells immobilized on Con A films, however, soon recovered their powers of locomotion. The ventral surface of the cell lifted clear of the lectin substrate to establish an area of associated contact identical to that displayed on glass with individual small focal contacts. Cells broke free from their trails, which themselves responded to the hapten. In white light, the axial sheets, like the associated contact of liberated amoebae, soon gave a white reflection interference image within which were numerous punctate black spots (Fig. 1*g*) similar in appearance to focal contacts both in the main sheet of the trail and at the end of the dendritic processes. These close contacts remained stable in the presence of further (0.5 M) sugar.

This releasing effect of methyl- α -D-mannopyranoside was also achieved by methyl- α -D-glucopyranoside, sucrose and maltose at the higher concentration of 0.5 M; methyl- β -D-glucopyranoside was ineffective at this concentration.

Influence of electrolytes on adhesion and de-adhesion

In 10 mM-KCl or NaCl cells moved normally on glass but were closer to the substrate with the associated contact generating a black interference pattern as previously described (Preston & King, 1978*a*). Focal contacts were still formed, but these contacts were not left behind, as they would be in deionized water (King *et al.*, 1979). In the presence of 10 mM-KCl, amoebae on Con A films generated trails and became arrested as though in the presence of deionized water. On perfusion with an aqueous solution of hapten, the arrested amoebae recovered and moved off, generating an area of white associated contact when the conductivity of the effluent had dropped to 10 μ S. The membrane sheet of the detached trails, however, behaved strikingly differently from those formed in deionized water. In this case it continued to produce a uniform black reflection interference image and failed to show any sign of lifting off even after 20 ml 1.0 M-methyl- α -D-mannopyranoside had been perfused through the 1 ml chamber.

DISCUSSION

Our results clearly show that *Naegleria gruberi* amoebae can initially move on a Con A substrate, but trail a relatively massive amount of cellular material and eventually halt. That this should happen is not entirely surprising, since it was already known (Preston *et al.*, 1975; O'Dell *et al.*, 1976) that Con A is a powerful ligand for the surface of *Naegleria gruberi*. This lectin binds strongly to exposed carbohydrate groups of the nature of methyl- α -D-mannopyranoside (Lis & Sharon, 1973) and thus could be expected to trap those elements of the surface which reached short-range (molecular) contact with the substrate during locomotion. This occurred both in deionized water and in 10 mM electrolyte, despite the altered features of contact in these two media (King *et al.*, 1979). The specificity of such reaction between cell and lectin substrate is shown by its abolition in the presence of the appropriate competing sugars. Methyl- α -D-mannopyranoside is the most effective of those tested and methyl- β -D-glucopyranoside the least, as would be predicted from studies on Con A-carbohydrate interactions (Goldstein *et al.*, 1965). Specificity is further confirmed by observation of normal locomotion on at least part of films of Con A which had been denatured, but not perhaps entirely irreversibly, by dialysis to remove divalent cations. Thus our observations argue for direct cell-substrate contact over a limited portion of the cell surface during this type of crawling locomotion. That this is a surface-to-surface effect, and not due to Con A escaping into solution, is shown by the observations on half-coated coverslips. Our further control experiments show that the hapten sugars themselves have no obvious short-term effect on the cells.

During locomotion on glass in deionized water only small focal contacts get entrapped and ruptured off as the cell progresses. On a Con A substrate a far greater area of the surface suffers this fate. The bond between Con A and the cell periphery may be such as to pull more of it within the ambit of short-range forces, or perhaps, once within such a range, less can escape.

Analysis of the effects of carbohydrates on trails argues, perhaps, for special features of focal contacts. Hapten sugars released the bulk of trails but not small areas apparently equivalent to focal contacts. If cells had previously been in 10 mM-KCl, however, the entire trail was held down, suggesting that closer approximation between surfaces in these ionic conditions had altered the nature of the association between them so as to make de-adhesion more difficult. Analysis of this change is not amenable to reflection interference microscopy, since this technique will not resolve separations of less than 20 nm. Comparison of cells and

Fig. 1. Light and electron micrographs of *Naegleria gruberi* amoebae on Con A-coated glass coverslips. (Bar marker represents 10 μ m for *a*, *b*, *c*, *f* and *g*.)

(*a*) These amoebae, whose locomotion across a Con A-coated coverslip has become arrested, are rounded in appearance and display no obvious polarity (bright field micrograph).

(*b*) The same field observed by reflection interference microscopy; the presence of copious amounts of sloughed material (previously undetected) is evident closely apposed to the protein substratum.

(*c*) Reflection interference micrograph revealing the dendritic appearance of the Con A-induced trail left by an advancing amoeba (arrow). The axial sheet is not as pronounced here as in (*b*).

(*d*) Scanning electron micrograph showing the thin folds, from which microprocesses emanate, drawn out from the cell body and held down on the lectin film. Such an extensive contact is not seen on uncoated glass (see Fig. 1*d*, Preston & King, 1978*a*). (Approximate magnification $\times 3500$.)

(*e*) Scanning electron micrograph demonstrating the branching without loss in diameter of the trail cylindrical microextensions. (Approximate magnification $\times 7000$.)

(*f*) Reflection interference micrograph showing part of an axial sheet portion of a trail formed on Con A in the presence of deionized water. Note the uniform dark appearance of the sheet.

(*g*) The same field after perfusion with 0.5 M-methyl- α -D-mannopyranoside. Much of the membranous sheet now lifted away from the lectin film produces a white interference image. Black regions of close contact – focal contacts – still persist, however, in the presence of the aqueous solution of hapten sugar.

trails observed by reflection interference microscopy is in itself helpful, as the images produced increase confidence that the images from cells are indeed from ventral surface structures, rather than from cytoplasmic features.

It is also noteworthy that amoebae halted on a Con A film continue to billow. Locomotion clearly involves functions of both the surface membrane and cytoplasm, but it does not seem that coordination is such that immobilization of the ventral surface leads to the switching off of activities above it. A parallel can be drawn here with the phenomenon of contact inhibition in various vertebrate cell types when a cell surface event – the collision between cells – leads to an almost immediate local paralysis but not to the abolition of locomotory activities elsewhere in the cell (Heaysman, 1978).

We thank A. Whiffen and L. Cooper for technical assistance and the Science Research Council for a grant. The S.E.M. Services were provided by the Zoology Board of Studies, University of London.

REFERENCES

- GOLDSTEIN, I. J., HOLLERMAN, C. E. & SMITH, E. E. (1965). Protein-carbohydrate interaction. II. Inhibition studies on the interaction of concanavalin A with polysaccharides. *Biochemistry* **4**, 876–883.
- HEAYSMAN, J. E. M. (1978). Contact inhibition of locomotion: a reappraisal. *International Review of Cytology* **55**, 49–65.
- KING, C. A., WESTWOOD, R., COOPER, L. & PRESTON, T. M. (1979). Speed of locomotion of the soil amoeba *Naegleria gruberi* in media of different ionic compositions with special reference to interactions with the substratum. *Protoplasma* **99**, 323–334.
- LIS, H. & SHARON, N. (1973). The biochemistry of plant lectins (phytohemagglutinins). *Annual Review of Biochemistry* **42**, 541–574.
- O'DELL, D. S., PRESTON, T. M., KING, C. A. & GARDINER, P. R. (1976). An approach to cell-surface receptors in an amoeba-flagellate protozoon. *Biochemical Society Transactions* **4**, 124–125.
- PRESTON, T. M. & KING, C. A. (1978*a*). Cell-substrate associations during the amoeboid locomotion of *Naegleria*. *Journal of General Microbiology* **104**, 347–351.
- PRESTON, T. M. & KING, C. A. (1978*b*). An experimental study of the interactions between the soil amoeba *Naegleria gruberi* and a glass substrate during amoeboid locomotion. *Journal of Cell Science* **34**, 145–158.
- PRESTON, T. M., O'DELL, D. S. & KING, C. A. (1975). Fluorescence microscope observations of some surface components of the amoeba-flagellate *Naegleria gruberi* during amoeboid locomotion. *Cytobios* **13**, 207–216.