SOME PHYSIOLOGICAL AND MORPHOLOGICAL EFFECTS OF POLYLYSINE ON AMOEBA PROTEUS

E. J. SANDERS* AND L. G. E. BELL
Department of Zoology, University of Southampton, England

SUMMARY

The effect of polylysine on pinocytosis and the cell surface of Amoeba proteus was investigated. The intensity of the pinocytotic response was evaluated by a channel counting method, and the surface was examined by means of transmission and scanning electron microscopy.

Cells treated with polylysine and then immersed in a protein pinocytosis inducer showed a higher intensity of channel formation during the initial stages of the cycle than cells not pre-treated. This was considered to be an additive effect of the protein and the polymer, since the latter alone was demonstrated to induce pinocytosis. No influence of molecular weight of the basic polymer on this reaction could be demonstrated.

As well as normal pinocytotic channels electron micrographs revealed the presence of fine channels, 20–50 nm in width, in the cytoplasm as a result of polylysine treatment. The polymer was visualized as dense surface aggregates, about 250 nm in diameter, associated with the extracellular material. This deposit was not seen on Amoeba dubia, which lacked a well-developed extracellular covering, and micro-channels failed to develop in this case. It has been demonstrated that when polylysine and a protein pinocytosis inducer were applied to the cell sequentially both compounds occupied the same channels. In view of the lack of effect of metabolic inhibitors on the formation of channels by polylysine it was concluded that this phenomenon is distinct from pinocytosis. It is proposed that this kind of channel formation can be explained in terms of the bridging of areas of membrane by the polymer–polysaccharide complex.

INTRODUCTION

The relationship between cell surface charge and various cellular activities and interactions such as pinocytosis, phagocytosis, locomotion and adhesion has been the centre of much interest in recent years. The use of polyionic compounds to alter the charge characteristics of cell surfaces has been of considerable value (see Katchalsky, Danon, Nevo & De Vries, 1959; Kornguth, Stahmann & Anderson, 1961; Gingell & Palmer, 1968).

Ryser & Hancock (1965) and Ryser (1967) reported that treatment of sarcoma cells with basic polymers increased the uptake of albumin, and Smith, Witt & Brown (1968) demonstrated increased intestinal transfer of globulin in the presence of poly-L-arginine. In view of the considerable data now available in relation to the phenomenon of induced pinocytosis in amoebae (Brandt, 1958; Brandt & Pappas, 1962, 1962; Chapman–Andresen, 1962, 1964, 1965a; Holter, 1965; Nachmias & Marshall,

* Present address: Department of Zoology, University of Alberta, Edmonton, Alberta, Canada.
the present investigation was designed to determine whether a similar synergistic effect could be demonstrated in these cells.

Electrophoretic measurements on erythrocytes (Katchalsky et al. 1959) have shown that polybase adsorption first reduces the surface potential to zero and then, by continued adsorption, produced a positive value of potential. Although it is established that all pinocytosis inducers so far examined also cause a depolarization of the cell surface (Josefsson, 1966) there are no reports of pinocytosis induction by polycationic compounds. This study demonstrates that this effect does occur in amoebae and that there is a combined effect when polylysine and protein inducers are applied together. In addition, the reaction of polylysine with the cell surface is examined by means of electron microscopy.

MATERIALS AND METHODS

Amoeba proteus was used (P. duX 67 strain), fed with Tetrahymena by the method of Prescott & James (1955) and Griffin (1960), and cultured in modified Chalkley's medium pH 6.1 (Brewer & Bell, 1969). Pinocytosis was quantitated by a modification of the channel counting method of Chapman-Andresen (1962). Amoebae, starved for 1 or 2 days before use, were washed in clean medium then immersed in inducing solution in the depression of a cavity slide and covered with a coverslip. The cells were observed for a period of up to 40 min using plain or phase-contrast illumination and the numbers of channels occurring at various pre-arranged times after immersion determined. Only the channels occurring on the visible parts of the amoeba were therefore counted and no attempt was made to count the total number of channels. Forming and disappearing channels were counted as well as fully formed ones, and the procedure was repeated on up to 40 specimens. The mean channel number of any given time after immersion was used as a measure of the intensity of pinocytosis. In order to compare the overall pinocytic response in different experiments the total number of channels observed was taken as an index of relative activity. The pinocytosis-inducing solutions used were either 1% bovine gamma globulin (Fraction 2 from bovine plasma, Armour) in 0.01 M acetate buffer pH 5.6, or 0.125 M sodium chloride in 0.01 M phosphate buffer pH 6.2. To investigate the influence of polylysine (mol. wt. 50000, Koch-Light Ltd.) on the uptake of inducers treatment with a $5 \times 10^{-6}$ M solution in Chalkley's medium was used for 3 min, followed by washing in clean medium and immersion in inducer.

Preparation for electron microscopy

Amoebae were processed for electron microscopy without centrifugation between steps and solutions were changed using an all-glass syringe. Glassware was siliconized to prevent the cells from sticking during preparation. Unless otherwise stated, fixation was carried out at room temperature for 30–60 min using 2% osmium tetroxide buffered to pH 6.9 with 0.01 M Sorensen's phosphate buffer. Following fixation the cells were washed with 2 changes of distilled water and dehydrated in graded concentrations of ethanol, allowing 15–30 min in each. Dehydration was completed in propylene oxide for 30 min, which also served as solvent for the embedding medium, Araldite (Ciba, HY 212). Sections were flattened with xylene vapour, collected on uncoated copper grids, stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined in an AEI 6B or Philips 300 electron microscope. Amoebae intended for examination with a scanning electron microscope were allowed to settle on aluminium foil, frozen in isopentane cooled with liquid nitrogen and vacuum dried.
RESULTS

The effect of polylysine on pinocytosis

The general effects of polylysine (mol. wt. 50,000) on amoebae may be summarized as follows. At concentrations from $10^{-8}$ M to $10^{-10}$ M prolonged treatment resulted in impaired adhesion to glass and consequently a cessation of locomotion, although the effect was frequently transitory and cells sometimes recovered while still immersed in the solution. At higher concentrations the amoebae became increasingly sticky, often adhering irreversibly to glass with prolonged exposure. Thirty-min treatment with concentrations greater than $10^{-6}$ M was always lethal.

![Fig. 1. Effect of polylysine (5 x 10^{-6} M) on pinocytosis. Amoebae 1 h after division. Vertical lines indicate the standard error of the mean. Relative activity: polylysine, 151.1 ± 6.5; control, 115.3 ± 5.3.](image)

Fig. 1 shows the effect of 3-min polylysine pre-treatment (5 x 10^{-6} M) on the pinocytosis of globulin by amoebae approximately 24 h after feeding and 1 h post-division, a time by which maximal response to pinocytosis inducers has developed. The results indicated some enhancement of uptake during the first 5 min immersion in inducer, but thereafter the effect was not significant. The figures for overall activity indicate some stimulation. Although Ryser (1967) demonstrated an increased stimulation of
uptake with increasing molecular weight of the polymer, the present experiments did not indicate this difference when polylysine of mol. wt. 195000 or polyornithine of mol. wt. 150000 was used. These experiments were all performed using globulin as the inducer; when sodium chloride was used, however, the channel counting technique indicated no enhancement of pinocytosis.

High-power observation (×400 NA 0.75) of amoebae during polylysine treatment revealed the presence of relatively wide (about 1 μm) pinocytosis channels identical to those produced by proteins or inorganic salts. Further investigation showed that the most effective concentration was 10⁻⁶ M, although channels were observed over the range 5×10⁻⁸ M to 10⁻⁶ M. The pH of the solutions was 6.5 in Chalkley's medium. The number of channels that developed with this treatment was very low in comparison with that produced by the standard inducers, being invariably less than 10 (Fig. 2). By the end of a 30-min cycle of pinocytosis the cells were irrecoverable. Considerable wrinkling and folding of the surface occurred during immersion in polylysine and the pinocytosis channels were distinguished by their conspicuous funnel-shaped opening and the characteristic channel-bearing protuberances.

**Electron-microscopical examination**

Fig. 3 shows a typical sublight-microscopical channel formed as a result of polylysine treatment. These are quite distinct from normal 1-μm wide pinocytosis channels. Channel widths varied from 20-50 nm, frequently with dilations at the inner end,
Effects of polylysine on *Amoeba proteus*

and channel lengths of up to 15 \( \mu m \) had developed after this time. The typical blunt pseudopodia of normal pinocytosis were not present, instead the channels invaginated directly from the surface. There was no evidence to suggest that vesicles were pinched off from the cytoplasmic end of the invagination as in the case of pinocytosis. The polymer was visualized as electron-dense material (Fig. 4), which formed clumps 25 nm in diameter and obliterated the normally filamentous layer on the amoeba surface (Fig. 5). The groups of dense clumps, which were separated by areas of membrane that were noticeably bare of filamentous covering, frequently filled the width of the channel on internalization and in many places the channels were dilated in order to accommodate the aggregates. The electron-dense deposit presumably represented a complex of polylysine with the polysaccharide-rich cell surface material and was demonstrated with both osmium and glutaraldehyde fixation. Evidence that the extent of adsorption was dependent on the nature of the cell surface was provided by the polylysine treatment of *Amoeba dubia* (A13 strain) (Fig. 6). This species possesses no distinct filaments on the surface comparable to those *A. proteus*, instead the extracellular material is much more diffuse. Fig. 6 shows that hardly any electron-dense material was attached to the surface and thorough examination did not reveal channels less than 0.1 \( \mu m \) in width. Such dimensions are within the range of normal pinocytotic channel size. Some polylysine-treated amoebae were washed and examined 24 h later, when recovery of normal locomotion appeared complete. It was seen that the cells now possessed an intact filamentous coat. The polylysine was identifiable as highly electron-dense material in cytoplasmic vesicles, concentrated in a similar way to pinocytotically ingested ferritin (Nachmias & Marshall, 1961).

Scanning electron microscopy was used in order to determine more precisely the three-dimensional configuration of the invaginations. Fig. 7 clearly demonstrates numerous surface pits of comparable diameter to those observed in thin sections, thus confirming that the invaginations were actually channels and not merely folds in the membrane.

Identical channels to those described were produced by the higher molecular weight polylysine and by polyornithine. The cationic protein salmine is considerably less basic than polylysine and electron microscopy of cells treated with the former showed that no fine channels were induced; an observation discussed later in connexion with the work of Ryser (1967). Similarly, anionic compounds such as polyglutamic acid and heparin also failed to cause micro-channel formation.

Clearly it was of importance to determine the influence of these micro-channels on the uptake of normal pinocytosis inducers in the type of experiment reported in the previous section. Amoebae were treated with polylysine, washed, immersed in globulin for a further 12 min, and then fixed. Fig. 8 shows a section through a channel resulting from this treatment. The dense polylysine complex can be seen attached to the surface while the globulin is taken into the same channel and overlies the polymer as a regular less dense layer.

There is now considerable evidence to suggest that the ultrastructural localization of acid mucopolysaccharides in tissues is reflected in the distribution of an electron-dense iron deposit resulting from treatment with colloidal iron hydroxide (CIH)
In view of the presumed change in surface of the amoebae after polylysine treatment, cells fixed with 4% neutral formol or 3% glutaraldehyde were taken through the CIH procedure according to Mowry (1963) and Curran, Clark & Lovell (1965). Subsequent electron-microscopical examination revealed a particulate deposit associated with the filamentous coat (Fig. 9). Treatment with polylysine before fixation produced the effect shown in Fig. 10, where particulate matter is still visible but in association with aggregates 80-0-90-0 nm in diameter. It would appear, therefore, that the colloidal iron is able to attach to the cell surface despite the presence of polylysine, perhaps indicating that the reduction of surface charge is not as great as in erythrocytes (Katchalsky et al. 1959), or isolated rat liver membranes, where polylysine completely blocked the CIH reaction (Benedetti & Emmelot, 1967). It is noticeable that the aggregates shown in Fig. 10 are very similar to those produced by the CIH reaction alone on serosal cells (Curran et al. 1965).

In order to determine whether the mechanism of polylysine channel formation was related to normal pinocytotic mechanisms as understood at present, the influence of several metabolic inhibitors was determined. Chapman-Andresen (1965b, c, 1967) demonstrated that pinocytosis was prevented by inhibitors of glycolysis and respiration. In the experiments described here amoebae were treated for 20 min with solutions of the following compounds: sodium fluoride (10^{-3} M), iodoacetate (5 \times 10^{-4} M), and potassium cyanide (10^{-5} M), and after subsequent immersion in polylysine were fixed and examined. There was no apparent reduction in the length or number of channels observed. A similar result was obtained when polylysine treatment and fixation were carried out at 3 °C, conditions which markedly inhibit normal pinocytosis (de Terra & Rustad, 1959).

**DISCUSSION**

It seems likely that the enhanced pinocytotic activity observed in the present experiments during the first 5-10 min of immersion in globulin after polylysine pretreatment (Fig. 1) could be due to normal pinocytosis channels formed by the polymer itself. Thus there was a simultaneous response to both stimulators. The markedly different results obtained when sodium chloride was used instead of globulin as an inducer could be explained on the basis of the suggestion by Smith, Witty & Brown (1968) that enhancement of globulin uptake in the presence of polyarginine was a result of globulin-polyelectrolyte complex formation. The complexing of polylysine with albumin has been demonstrated by Rice, Stahmann & Alberty (1954). The lack of a measurable effect when sodium chloride was used could, therefore, have been due to the absence of such interaction and the effect of counterions around the polymer molecule which altered the inducing capabilities of the latter.

Ryser (1967) interpreted the stimulation of albumin uptake by polylysine as indicating an interaction between the basic polymer and the membrane protein causing conformational changes in the latter. However, since this worker determined the uptake of isotopically labelled albumin by measuring the total radioactivity within the
Effects of polylysine on *Amoeba proteus*

cell, it is possible that some protein penetrated the cell by means of fine polylysine-induced channels of the type described here, and not by the normal pinocytotic mechanisms. It is significant that Ryser (1967) reported that highly charged molecules such as spermine were ineffective in stimulating uptake, since it has been shown by the present study that a similar molecule, salmine, is also ineffective in inducing the formation of micro-channels.

The agglutination of erythrocytes by polyelectrolytes was studied in depth by Katchalsky et al. (1959) who concluded that the width of the seam between the clumped and deformed cells was dependent on the molecular weight of the polybase. The seam was presumed to consist entirely of a polymer film in which the polylysine molecules were anchored at each end to anionic groups on the adjacent cell membranes. The maximum width of the film was nearly equal to the length of the fully extended molecules and the overall seam width was 60–60 nm. Similar agglutinates were produced with a variety of polybases by Easty & Mercer (1962) who obtained an intercellular gap of 20–30 nm. Both of these groups of workers showed that in the erythrocyte aggregates the cells became very distorted and assumed a shape which allowed maximum surface contact. Amoebae appeared to behave in a similar way, since they also became highly adhesive and easily deformable. The work on erythrocytes and also that on *Xenopus* eggs (Gingell & Palmer, 1968) showed no trace of distinct surface aggregates of the type demonstrated here with *A. proteus*. This is presumably correlated with the absence of elaborate filaments on these cells, a conclusion supported by the present data in which aggregates were absent from the surface of *A. dubia*.

The conclusion of Katchalsky et al. (1959) that agglutination of cells was caused by macromolecular bridging was used in order to explain the formation of micro-channels described in the present study. It is, therefore, proposed that the polylysine bridges different areas of membrane on the same amoeba, causing the surface to ‘zip’ together into channels in order to maximize the area of contact, as in the case of individual erythrocytes. The channel width of 10–20 nm corresponds satisfactorily with the size calculated by Katchalsky et al. (1959) for the length of an extended polymer molecule, although the formation of dense complexes in amoebae precludes a precise correlation of channel width with molecular weight. It is significant that microchannels were absent in *A. dubia* where there was no surface clumping. These aggregates have been shown to be separated from one another by patches of bare plasma membrane and it is considered that the surface coat could have been removed from these areas as a result of the aggregate formation. The degree of dissociation of the ionogenic groups, and hence the configuration, of polyelectrolytes can be altered by changes in the concentration of hydrogen ions and counterions around the molecule (Katchalski & Sela, 1958; Katchalsky, 1964; Gill & Omenn, 1965; Mandel, 1965). In this way a transition can occur from an extended coil configuration to a contracted helix by neutralization of the electrostatic forces of repulsion within the molecule. Whether conditions at the cell surface would favour such a change is not clear, but a contraction of this kind could be responsible for the removal of the surface coat, in the manner suggested by Kornguth et al. (1961).
The possibility that micro-channel formation is a cytoplasmic response to the application of polylysine to the surface, analogous to the contraction of the *Xenopus* egg (Gingell & Palmer, 1968) is rendered unlikely in view of the lack of effect of metabolic inhibitors. When the latter are used to inhibit pinocytosis they exert their action on ‘the active process of channel formation’ (Chapman-Andresen, 1967) and it is therefore concluded that the occurrence of invaginations in amoebae as a result of polylysine treatment is not mediated through cellular mechanisms directly involved in pinocytosis.

One of us (E. J. S.) was in receipt of an M.R.C. scholarship throughout this work.

REFERENCES


Effects of polylysine on Amoeba proteus


(Received 21 February 1970)
Fig. 3A. Effect of polylysine (5 x 10^{-6} M) on the surface of *A. proteus*. x 13 500.

Fig. 3B. Higher magnification of part of the channel shown in Fig. 3A. x 24 300.

Fig. 4. Dense aggregates formed on the surface of *A. proteus* by polylysine. x 31 600.
Effects of polylysine on *Amoeba proteus*

Fig. 5. The untreated surface of *A. proteus*. ×72500.

Fig. 6. The surface of *A. dubia* after treatment with polylysine. ×31900.
Fig. 7. A scanning electron micrograph of the surface of *A. proteus* after polylysine treatment, showing the presence of pit-like depressions which are absent from the surface of untreated controls. \( \times \) 46,100.

Fig. 8. A section through a channel lined with dense polylysine aggregates (arrows) and containing protein. \( \times \) 16,100.
Effects of polylysine on Amoeba proteus
Fig. 9. The result of the colloidal iron hydroxide (CIH) reaction on the surface of A. proteus. × 38200.

Fig. 10. Aggregates on the surface of A. proteus as a result of polylysine treatment followed by the CIH reaction. × 31600.
Effects of polylysine on Amoeba proteus