DETECTION OF DEVELOPMENTALLY CONTROLLED PLASMA MEMBRANE ANTIGENS OF *DICTYOSTELIUM DISCOIDEUM* CELLS IN SDS–POLYACRYLAMIDE GELS

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Received 25 September 1978

1. Introduction

Starvation triggers *Dictyostelium discoideum* amoebae to form aggregates which differentiate into fruiting bodies [1]. Continuous cell contact is required and mechanical disruption of aggregates prevents further development until the contacts are restored [2,3]. A number of new plasma membrane proteins are synthesized at specific stages of development [4,5]. Labelling experiments with glucosamine and fucose showed the majority of these proteins are glycoproteins [5]. One of these glycoproteins (contact sites A), which appears during the acquisition of aggregation competence, is involved in the specific cell adhesion required for cell aggregation [6,7]. Hence, Fab fragments against this glycoprotein block end-to-end adhesion of aggregation competent cells [6–8].

We sought a simple method for detecting changes in membrane antigens during development. Such a method would be a useful adjunct to isotopic labelling. Moreover, specific antibodies could be used to examine the function of developmentally controlled surface antigens and their location within the differentiating aggregates.

The method chosen resembles the concanavalin A-peroxidase technique we use for detecting glycoproteins in SDS–gels [9]. Antisera against plasma membranes was added to gels of plasma membrane proteins; antibodies binding to specific proteins were detected using anti-IgG coupled to peroxidase [10]. We found 4 major developmentally controlled antigens in the plasma membranes. One corresponded with contact sites A, appearing in aggregation competent cells but disappearing during later stages of development. A second antigen corresponded with a glycoprotein whose synthesis begins during late aggregation and continues throughout the remainder of differentiation.

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2. Materials and methods

Dictyostelium discoideum NC-4 (wild-type) cells were grown in liquid medium with *E. coli* as in [11]. Vegetative amoebae were separated from bacteria by centrifugation and washing in PDF solution [12]. Amoebae were then plated out on millipore filters over filter pads containing PDF solution [11]. Cells were labelled by adding isotopes in 20 μl dist. water via a Hamilton syringe to the top of the millipore filters. The isotopes (Amersham) used were [1-^{14}C]-acetate (60 mCi/mol, 20 μCi/filter) and D-[1-^{14}C]-glucosamine hydrochloride (60 mCi/mmole, 4 μCi/filter).

Plasma membrane isolation, SDS–gel electrophoresis, amido black protein staining, concanavalin A-peroxidase glycoprotein staining and autoradiography of gels were carried out as in [4,5,9,13,14].

Plasma membranes from vegetative cells, aggregation competent cells and cells obtained from pseudoplasmodia (slugs) were lyophilized and used as antigens. Adult New Zealand rabbits (3–6 month and of either sex) were immunized with the different plasma membrane antigens as follows. Antigen preparations were dissolved in 0.02 M phosphate buffer (pH 7.0), 0.12 M NaCl solution to ~200 μg protein/ml. Each

rabbit received 1.0 ml antigen emulsified in 1.0 ml Freund's Complete Adjuvant injected into 4 subcutaneous sites. After 4 weeks the rabbits were challenged in a similar manner with 1.0 ml antigen emulsified in 1.0 ml Freud's Incomplete Adjuvant. The rabbits were bled 14 days after this secondary challenge and the serum harvested and stored at −20°C.

Plasma membrane antigens were detected in SDS–gels by first removing SDS with 25% isopropanol/10% acetic acid solution for ~20 h, the solution being once changed. The gels were then washed in 15 mM Na phosphate buffer (pH 6.8) for 2 h with one change of buffer. Gels were incubated for 2 h with antiserum diluted 1:5 with Na phosphate buffer. (Note: the same diluted antiserum could be used at least 3 times.) Gels were washed for 20 h in phosphate buffer with one change of buffer. They were next incubated with 0.1 mg/ml peroxidase-conjugated goat anti-rabbit IgG (Miles) in phosphate buffer for 24 h and subsequently washed for 24 h in phosphate buffer. Finally, staining was carried out for 10–30 min in phosphate buffer containing 0.5 mg/ml 3,3′-diaminobenzidine tetrahydrochloride and 0.2 μl/ml of 30% hydrogen peroxide. The reaction was stopped with 7% acetic acid.

Slab gels were cut into strips and shaken with the various solutions in wells fashioned in a polystyrene block. Smaller volumes (e.g., 10 ml) could then be used and antiserum and immunoglobulin conserved.

3. Results

A number of antigen–antibody binding regions could be detected on the gels (fig.1). Minor bands

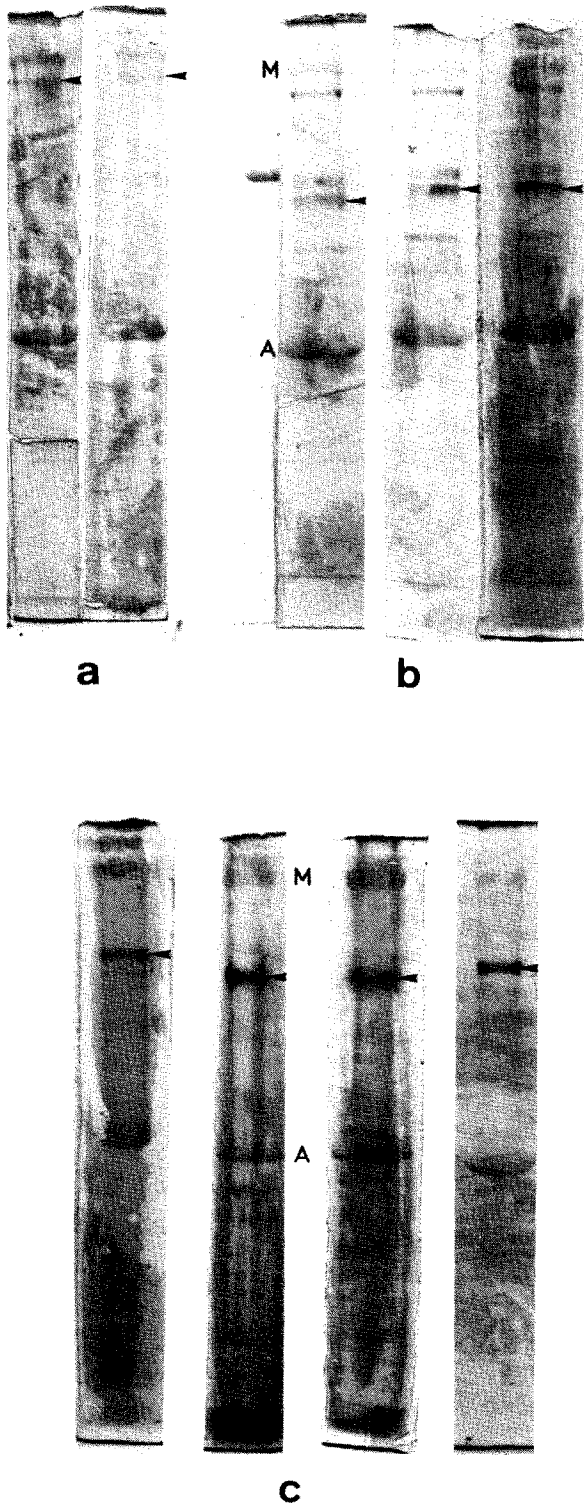

Fig.1. SDS–gels of plasma membranes isolated from various stages of differentiation. Gels were incubated with antisera raised against plasma membranes and the regions of antibody–antigen binding detected as in section 2. M, myosin heavy chains; A, actin. (a) Plasma membranes from late-vegetative cells, incubated with antisera raised against the same plasma membranes (2 experiments). (Antibodies raised against aggregation competent cells gave the same result.) Arrows indicate staining localized to the 130 kd protein. (b) Plasma membranes from aggregation competent cells incubated with antisera raised against plasma membranes from the same developmental stage (3 experiments). Arrows indicate staining localized to the contact sites A. Phosphorylase *b* (mol. wt 92 500) is shown in the left hand lane. (c) Plasma membrane from slugs incubated with antisera raised against slug plasma membranes (4 experiments). Arrows indicate staining localized to the 95 kd glycoprotein.

were also present, however, uneven background staining of gels often interfered with their detection. Antibodies binding myosin heavy chains and actin were present at all developmental stages examined (fig.1).

Antiserum against late-vegetative amoebae also contained a relatively high concentration of antibodies binding to a plasma membrane protein with app. mol. wt ~120 000 in 10% gels (fig.1a). Using the same antiserum this protein could also be detected in plasma membranes from aggregation competent cells but not from slugs.

Four major bands (in addition to actin and myosin) were obtained when antiserum against plasma membranes from aggregation competent cells was incubated with gels of the equivalent plasma membranes (fig.1b). One of the bands corresponded to the 130 kd protein found in the plasma membranes of late-vegetative cells (fig.1a). The strongest band corresponded to a glycoprotein (app. mol. wt 82 000) essential for the end-to-end adhesion characteristic of aggregating cells (contact sites A) [6–8]. Labelling experiments showed that synthesis of this glycoprotein begins when cells are becoming aggregation competent and continues until mid-aggregation (fig.2) [5].

When gels of plasma membranes from slugs were incubated with the corresponding antiserum one strong band was detected (fig.1c). This band corresponded to a glycoprotein (app. mol. wt ~95 000 in 10% gels) whose synthesis begins during early aggregation, increases at tip formation and continues strongly throughout the remainder of development (figs.2,3). When antiserum against slug plasma membranes was incubated with gels of plasma membranes isolated from different developmental stages, the appearance of the 95 kd glycoprotein paralleled the changes in synthesis (not shown).

The developmentally controlled plasma membrane antigens were minor components of the plasma membrane, contact sites A and the 95 kd glycoprotein (fig.3), for example, being relatively weak bands on gels stained for glycoproteins.

Control experiments were also performed. When antiserum against slug plasma membranes was incubated with gels of plasma membranes from late-vegetative cells, for example, no band corresponding to the 95 kd glycoprotein was found. Moreover, incubation of the slug antiserum with cells derived from slugs removed the antibodies to the 95 kd glycoprotein, whereas incubation with vegetative cells did not.

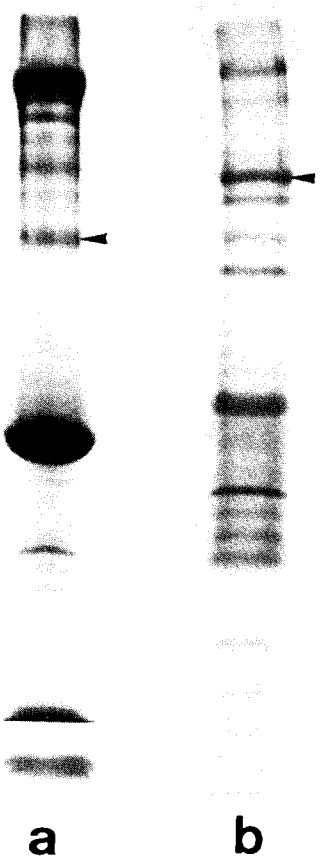

Fig.2. Autoradiographs of SDS–gels of plasma membranes isolated from differentiating cultures labelled with isotopes. (a) Cultures labelled with [^{14}C]acetate for 2.5 h between aggregation competence and mid-aggregation. The arrow indicates contact sites A. (b) Cultures labelled with [^{14}C]-glucosamine for 3.5 h between the finger and culmination stages of differentiation. The arrow indicates the 95 kd glycoprotein.

4. Discussion

Antisera could be used in combination with SDS–gel electrophoresis to detect the appearance and disappearance of plasma membrane antigens during development. Furthermore, the two major antigens corresponded with glycoproteins shown by isotopic

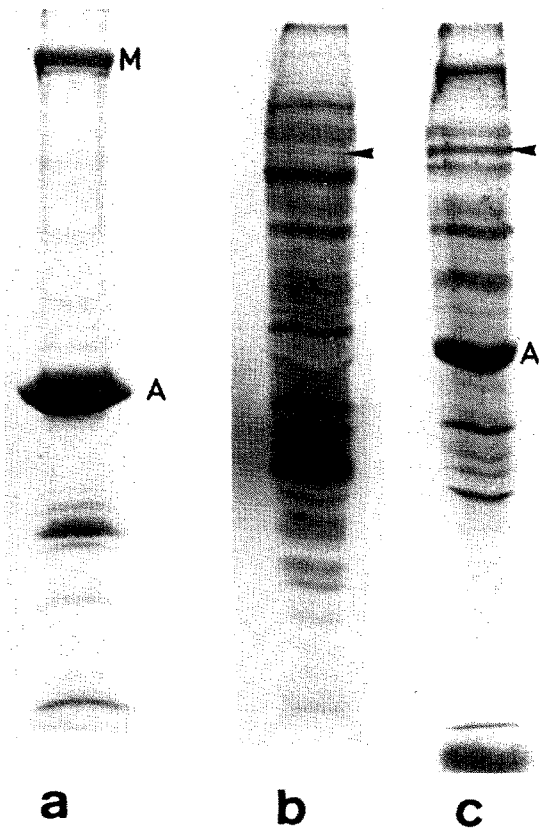

Fig.3. SDS–gels of plasma membranes. (a) Plasma membranes from vegetative cells; gels stained with amido black. (b) Plasma membranes from slugs; gels stained for glycoproteins. (c) Plasma membranes from slugs labelled for 2 h with [^{14}C]-acetate; autoradiograph of gel. (Sample identical to b.) Arrows indicate the 95 kd glycoprotein. M, myosin heavy chains; A, actin.

labelling to be developmentally controlled [5]. A number of weaker antigens were also present, however variable background staining of gels interfered with their detection. Greatly extended washing times may reduce this background [10]; we are also experimenting with fluorescein-labelled goat anti-rabbit IgG and protein A.

We have shown that the synthesis of contact sites A ceases during middle-to-late aggregation [5]. Long-term labelling experiments further suggested that contact sites A subsequently disappear from the plasma membrane [5]. This was confirmed by the antisera experiments described here; plasma membranes from slugs contained no antigen corresponding to contact sites A.

The strong immunogenicity of contact sites A, otherwise a relatively minor plasma membrane component, may be related to the properties of the glycoprotein. (The immunization procedure used, i.e., antigen in oil emulsions, may also be relevant.) These properties may in turn be related to the role of contact sites A in cell adhesion. It would then follow that the 95 kd glycoprotein is also important in cell adhesion, possibly replacing the contact sites A in this capacity during the later developmental stages.

Side-to-side adhesion of aggregation competent cells can also be blocked by Fab directed against membrane antigens [6–8]. The relevant antigen (contact sites B) is present in growth phase as well as aggregation competent cells [6]. The 130 kd protein (fig.1) would be a candidate for contact sites B, and is developmentally controlled as shown by its absence from the membrane of slug cells.

Using antibodies directed against the 95 kd glycoprotein we are now attempting to clarify its function and distribution between pre-spore and pre-stalk cells during development. Such a glycoprotein may, for example, be involved in the cell recognition and sorting out postulated to occur during differentiation [1,15–18].

Acknowledgements

This work was supported by the Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung (grant no. 3.673–0.75).

References

[1] Bonner, J. T. (1967) The Cellular Slime Molds, Princeton Univ. Press, Princeton, N.J.
[2] Newell, P. C., Longlands, M. and Sussman, M. (1971) J. Mol. Biol. 58, 541–554.
[3] Newell, P. C., Franke, J. and Sussman, M. (1972) J. Mol. Biol. 63, 373–382.
[4] Siu, C. H., Lerner, R. A. and Loomis, W. F. (1977) J. Mol. Biol. 116, 469–488.
[5] Parish, R. W. and Schmidlin, S. (1978) submitted.
[6] Beug, H., Katz, F. and Gerisch, G. (1973) J. Cell Biol. 56, 647–658.
[7] Müller, K. and Gerisch, G. (1978) Nature 274, 445–449.

[8] Gerisch, G. (1976–77) in: International Cell Biology (Brinkley, B. R. and Porter, K. R. eds) pp. 36–42, The Rockefeller University Press, New York.
[9] Parish, R. W., Schmidlin, S. and Müller, U. (1977) Exp. Cell Res. 110, 267–276.
[10] Olden, K. and Yamada, K. M. (1977) Anal. Biochem. 78, 483–490.
[11] Sussman, M. (1966) in: Methods in Cell Physiology (Prescott, D. ed.) vol. 2, pp. 397–410, Academic Press, New York.
[12] Newell, P. C., Telser, A. and Sussman, M. (1969) J. Bacteriol. 100, 763–768.
[13] Parish, R. W. and Müller, U. (1976) FEBS Lett. 63, 40–44.
[14] Parish, R. W., Müller, U. and Schmidlin, S. (1977) FEBS Lett. 79, 393–396.
[15] Takeuchi, I. (1963) Dev. Biol. 8, 1–26.
[16] Francis, D. and O'Day, D. (1971) J. Exp. Zool. 176, 265–272.
[17] Müller, U. and Hohl, H. R. (1973) Differentiation 1, 267–276.
[18] Forman, D. and Garrod, D. R. (1977) J. Embryol. Exp. Morph. 40, 229–243.