PHOSPHORYLATION OF PLASMA MEMBRANE PROTEINS IN *DICTYOSTELIUM DISCOIDEUM*

Roger W. PARISH+, Ursula MÜLLER* and Sylvia SCHMIDLIN
Cytologie, Institut für Pflanzenbiologie, Universität Zürich, Zollikerstrasse 107, 8008 Zürich, Switzerland

Received 26 May 1977

1. Introduction

Starved cells of *Dictyostelium discoideum* aggregate to form multicellular organisms [1]. The aggregation occurs in response to cAMP which binds to cell surface receptors [2,3]. A delay of approx. 10 h occurs between starvation and the beginning of aggregation, presumably related partly to the appearance of cAMP binding sites [3,4]. When starved cells are incubated with ATP the delay is reduced by up to 50% [5] and is accompanied by activation of cAMP receptors [4]. Weinstein and Koritz [6] found intact cells were able to phosphorylate an exogenous protein substrate, while Mato and Konijn showed that incubation of cells with extracellular ATP resulted in the phosphorylation of two low molecular weight proteins [5]. Cyclic AMP had no effect on either endogenous or exogenous phosphorylation, [4,6].

We are currently examining the constituents of the plasma membrane and their changes during differentiation [7–9]. In this communication we describe the phosphorylation of certain membrane proteins following incubation of cells in [γ-^{32}P]ATP or growth in ^{32}P.

2. Materials and methods

D. discoideum cells (Ax 3) were grown in HL-5 medium [10]. Plasma membranes were isolated as previously described [8] except the washing buffer (0.1 M Tris–HCl, pH 8.5) contained 0.1 M EDTA.

Intact cells (8×10^6/ml) or plasma membranes were incubated with 0.016 mCi/ml [γ-^{32}P]ATP (3 mCi/mmol; Amersham) in 1% Bonner's Salt Solution [11] containing 10 mM $MgSO_4$, 10 mM NaF and 2 mM dithiothreotal for 10 min (cf. [4,6]). Cells were washed and plasma membranes isolated.

Cells (5×10^6/ml) were also incubated with ^{32}P (0.06 mCi/ml; 13.6 mCi/mg) in HL-5 medium or PDF salt solution [12].

The proteins of plasma membranes were separated on 10% acrylamide gels by the method of Laemmli [13]. Gels were stained with amido black or with the concanavalin A (con A)-peroxidase method [14] to detect glycoproteins. Gels were dried and autoradiographed.

3. Results

3.1. *Plasma membrane proteins and glycoproteins*

Figure 1 shows SDS gels of plasma membrane preparations stained for proteins (amido black) and glycoproteins (con A-peroxidase). A detailed discussion of the membrane proteins will be presented elsewhere. The presence of the two myosin heavy chains (one band on the gels shown) [15], actin, con A (used in the plasma membrane isolation) and a protein which migrates with the lectin 'discoidin' [16] should be noted. The majority of glycoproteins are not readily detectable with amido black.

3.2. *Phosphorylation with [γ-^{32}P]ATP*

When cells were incubated with [γ-^{32}P]ATP a

+ To whom correspondence should be addressed

* Present address: Biochemisches Institut, ETH Zürich, Switzerland

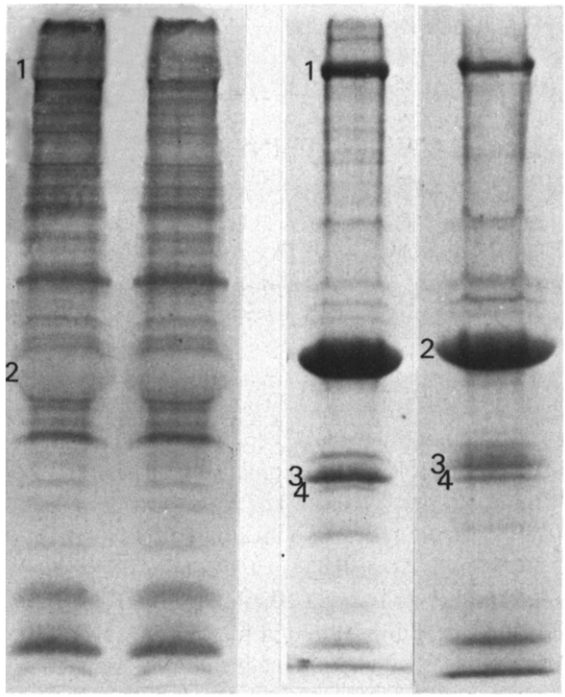

Fig.1. SDS polyacrylamide gels of plasma membranes isolated from four different batches of cells. (A) Proteins stained with amido black. (B) Glycoproteins stained with con A-peroxidase. Myosin heavy chains (1), actin (2), con A (3) and a protein which may be 'discoidin' (4) indicated. Where these proteins are not visible their region on the gel is nevertheless sometimes numbered.

number of plasma membrane proteins were phosphorylated. Figure 2 shows four such experiments. At least seven proteins are phosphorylated and there is some difference between experiments, mainly in relative activities of label. The myosin heavy chains, or proteins migrating with them, were clearly labelled in three experiments, while labelling is less obvious in the fourth. A protein corresponding to 'discoidin' on gels is also phosphorylated. The two phosphorylated proteins immediately below actin and the most strongly labelled protein in the upper half of the gels are probably glycoproteins.

When isolated plasma membranes were incubated with [γ-^{32}P]ATP similar phosphorylation patterns were observed. No phosphorylation occurred during incubation with ^{32}P.

Cyclic AMP had no effect on phosphorylation of plasma membrane proteins.

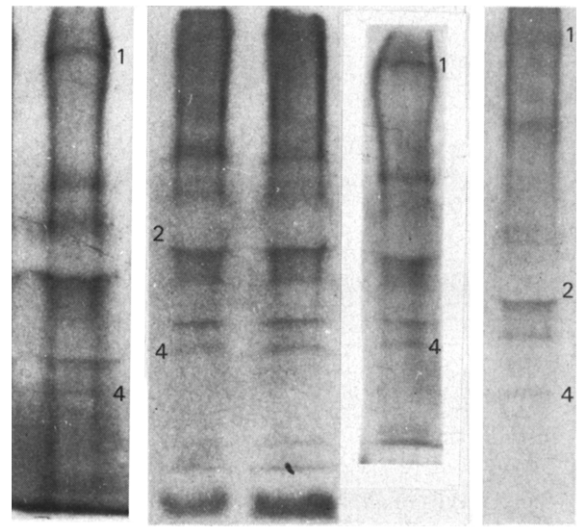

Fig.2. Autoradiograph of gels of plasma membranes isolated from cells incubated with [γ-^{32}P]ATP. Four experiments are shown. Numbering as in fig.1.

3.3. *Phosphorylation of plasma membrane proteins in cells incubated with ^{32}P*

An incubation period of 1–2 h with ^{32}P in HL-5 medium or PDF salt solution was necessary before label could be detected in plasma membrane proteins. Figure 3 shows two different experiments which involved incubation in HL-5 medium for 6 h. A large number of proteins are phosphorylated including the proteins phosphorylated by the surface protein kinase (cf. fig.2). However, the majority of strongly labelled proteins are not labelled during the [γ-^{32}P] ATP experiments. They appear to be glycoproteins. The region corresponding to myosin heavy chains also showed labelling, the bands being clearly visible on autoradiographs exposed for shorter times.

4. Discussion

A number of proteins and glycoproteins of the *D. discoideum* plasma membrane may be phosphorylated. Experiments with [γ-^{32}P]ATP indicated the presence of a surface protein kinase as already suggested by other workers [4,6]. Mato and Konijn [4] reported that two proteins with mol. wt 20 000 and 15 000 were preferentially phosphorylated. We found a

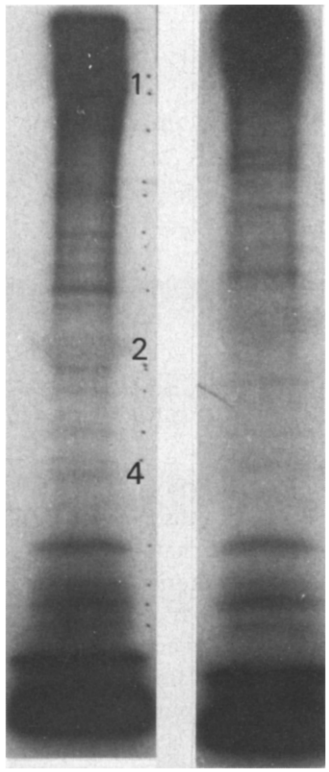

Fig.3. Autoradiography of gels of plasma membranes isolated from cells grown with ^{32}P in HL-5 medium for 6 h. Two experiments are shown. Numbering as in fig.1.

number of proteins were phosphorylated but none had these molecular weights. We are unable to explain this discrepancy, although Mato and Konijn ran total homogenates and not plasma membranes on SDS gels.

The role of phosphorylation in plasma membrane function and differentiation is unknown. However, it is pertinant that phosphorylation of a protein corresponding to 'discoidin' occurs, as 'discoidin' is thought to mediate intercellular recognition and adhesion during differentiation by interacting with specific cell-surface receptors [18]. Moreover, if the myosin heavy chains are phosphorylated, as the results suggest, this may be related to surface movement and cell migration.

The existence of a surface protein kinase suggests substrate may also be available outside the cell. We are examining the hypothesis that external cAMP pulses induce the release not only of cAMP (cf. [3]) but also of ATP. This could lead to local transitory phosphorylations of plasma membrane proteins which may be important, for example, in the chemotactic response or induction of the refractory period when the cells are insensitive to cAMP.

Acknowledgements

This work was supported by the 'Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung' (Grant No. 3.673.-0.75). Isotopes were provided by the 'Jubiläumsspende für die Universität Zürich'.

References

[1] Bonner, J. T. (1971) Ann. Rev. Microbiol. 25, 75–92.
[2] Malchow, D. and Gerisch, G. (1974) Proc. Natl. Acad. Sci. USA 71, 2423–2427.
[3] Gerisch, G. and Malchow, D. (1976) Adv. Cycl. Nucl. Res. 7, 49–68.
[4] Mato, J. M. and Konijn, T. M. (1975) Develop. Biol. 47, 233–235.
[5] Mato, J. M. and Konijn, T. M. (1976) Exp. Cell Res. 99, 328–332.
[6] Weinstein, B. I. and Koritz, S. B. (1973) Develop. Biol. 34, 159–162.
[7] Parish, R. W. and Pelli, C. (1974) FEBS Lett. 48, 293–296.
[8] Parish, R. W. and Müller, U. (1976) FEBS Lett. 63, 40–44.
[9] Parish, R. W. (1976) Biochim. Biophys. Acta 444, 802–809.
[10] Cocucci, S. M. and Sussman, M. (1970) J. Cell Biol. 45, 399–407.
[11] Bonner, J. T. (1949) J. Exptl. Zool. 110, 259–271.
[12] Newell, P. C. and Sussman, M. (1969) J. Biol. Chem. 244, 2990–2995.
[13] Laemmli, U. K. (1970) Nature 227, 680–685.
[14] Wood, J. G. and Sarinana, F. O. (1975) Anal. Biochem. 69, 320–322.
[15] Clark, M. and Spudich, J. A. (1974) J. Mol. Biol. 86, 209–222.
[16] Simpson, D. L., Rosen, S. D. and Barondes, S. H. (1974) Biochemistry 13, 3487–3493.
[17] Reitherman, R. W., Rosen, S. D., Frasier, W. A. and Barondes, S. H. (1975) Proc. Natl. Acad. Sci. USA 72, 3541–3546.