LABELLING OF *DICTYOSTELIUM* AND *POLYSPHONDYLIUM* PLASMA MEMBRANES WITH A PHOTOSENSITIVE HYDROPHOBIC PROBE

Roger W. PARISH, Rudolf HINTERMANN, Elisabeth BANZ, Tuvia BERCOVICI* and Carlos GITLER*

Cytology, Plant Biology Institute, University of Zürich, Zollikerstrasse 107, 8008 Zürich, Switzerland and **Department of Membrane Research, Weizmann Institute of Science, Rehovot, Israel*

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1. Introduction

Changes in the surface properties of cells accompany the development of the cellular slime molds [1–17]. These changes are potentially involved in cellular adhesion, chemotaxis, aggregation and the induction of developmentally-regulated enzymes [18,19]. We have examined the plasma membrane proteins synthesized during development of the two species *Dictyostelium discoideum* [4–6] and *Polysphondylium pallidum* [8]. A number of proteins were synthesized at specific morphological stages. The majority of these proteins were glycoproteins, incorporating fucose and glucosamine [5,8], and hence presumably exposed at the cell surface. Monovalent antibodies have been used to show that some of these developmentally regulated proteins are involved in cell adhesion [7,9–11,20].

We have also been studying the arrangement of various protein species in the slime mold plasma membrane [21]. Here, we describe results obtained with a photosensitive hydrophobic probe, [5-^{125}I]iodonaphthyl-1-azide (INA).

Aromatic azides have a high partition coefficient into membrane lipids and generate a reactive species (nitrene) within the bilayer when exposed to UV radiation [22–30]. The nitrene is inserted into the membrane and covalently bound, with high efficiency, to both membrane proteins and membrane lipids. Such probes preferentially label the transmembrane region of intrinsic proteins without disrupting the structural and functional integrity of the membrane. Iodination of the aromatic azides improves their selectivity for labelling hydrophobic regions [28] as well as avoiding the formation of artefactual polymeric products [27]. The high specific activity of INA allows subsequent detection of proteins following SDS-gel electrophoresis.

Plasma membranes isolated from various developmental stages of the cellular slime molds *D. discoideum* and *P. pallidum* were incubated with the photosensitive hydrophobic reagent [5-^{125}I]iodonaphthyl-1-azide and the mixture was irradiated. High incorporation of [5-^{125}I]iodonaphthyl-1-azide was found in low M_r proteins (18 000–41 000 M_r). Patterns of strongly labelled proteins were similar in both slime mold species and, except for a 25 000 M_r protein, did not change during differentiation. One of the labelled proteins (41 000 M_r) is a phosphoglycoprotein and may be the ecto-ATPase. Myosin heavy chains and actin were not labelled, indicating that they do not penetrate the bilayer. No labelling of the developmentally regulated proteins could be detected in either species, suggesting that they are not intrinsic proteins. However, the selectivities of the naphthyl azide products may be responsible for the lack of labelling.

2. Materials and methods

We used *Dictyostelium discoideum* (strain NC-4) and *Polysphondylium pallidum* (strain Ti-1) cells grown in *Escherichia coli* medium or on 0.1% lactose–peptone agar, respectively [31,32]. Cells were harvested, washed in 20 mM KCl, 25 mM $MgSO_4$, 50 mM Sørensen's phosphate buffer (pH 6.5) (buffer A), plated onto millipore filters over filter pads and allowed to differentiate [5,8]. Cells were labelled with [1-^{14}C]acetate, D-[1-^{14}C]glucosamine and L-[6-^{3}H]-fucose as in [5]. Plasma membrane isolation, SDS-gel electrophoresis, gel staining and autoradiography were as in [4,5,21].

[5-^{125}I]Iodonaphthyl-1-azide was prepared according to [27]. Membranes to be labelled were suspended

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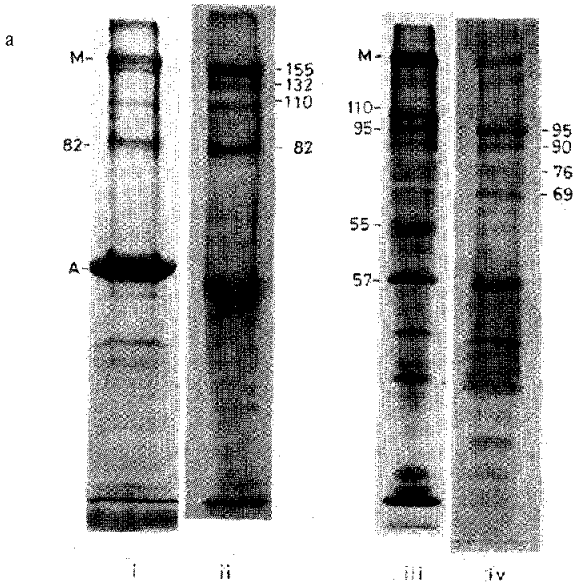
(1–2 mg/ml) in 1.0 ml buffer A in a cylindrical glass cuvette. INA was added and the solution irradiated for 2 min as in [27,30]. The membranes were then washed in PDF solution and dissolved in SDS–sample buffer.

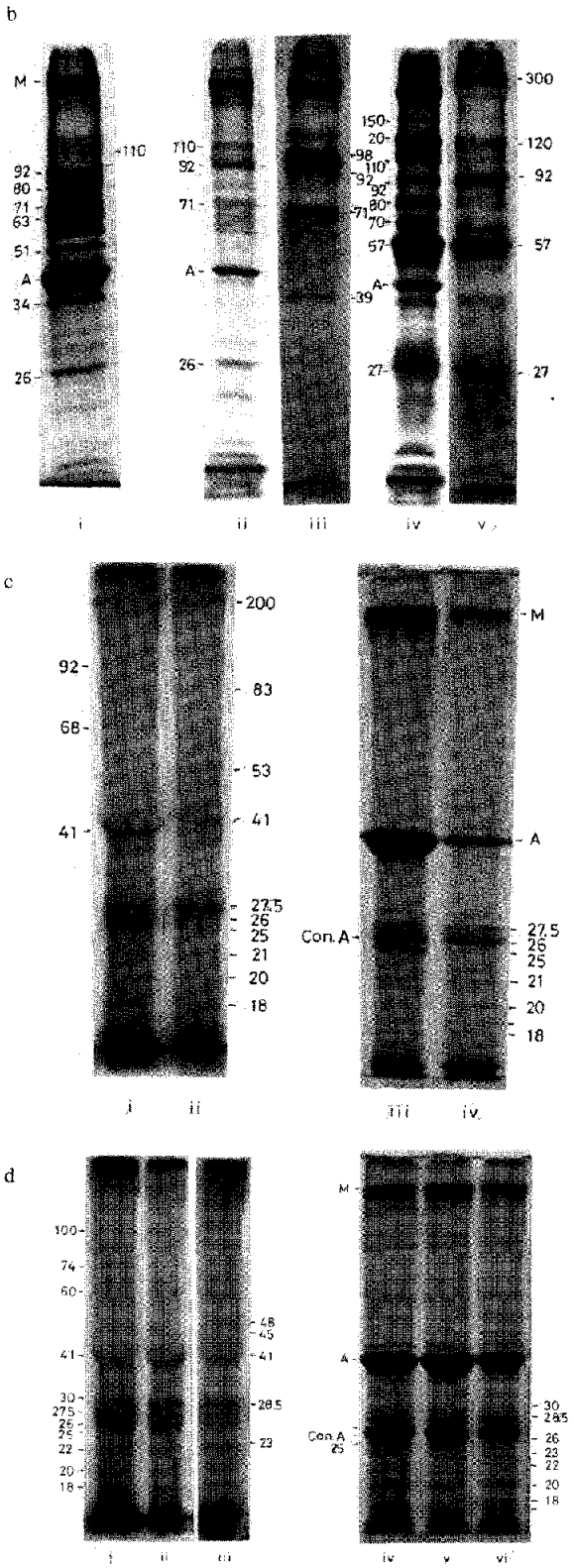
3. Results and discussion

Dictyostelium discoideum plasma membranes isolated from two different stages of differentiation following labelling with either acetate or glucosamine were electrophoresed on SDS gels. The major developmentally regulated proteins and glycoproteins [5] were detected by autoradiography (fig.1a). Synthesis of the two antigenic glycoproteins (82 000 and 95 000 M_r) believed to be involved in cell adhesion [7,20] at the two different developmental stages was readily detectable (fig.1a).

Plasma membrane proteins and glycoproteins being synthesized during vegetative, early and late aggregation stages of the development of *P. pallidum* [8] are shown in fig.1b. Two antigenic glycoproteins (71 000 and 57 000 M_r) are thought to be involved in cell adhesion [8,9]. Synthesis of the 57 000 M_r antigen commences during mid-aggregation [8].

Plasma membranes isolated from the developmental stages shown in fig.1a,b were photo-labelled with INA. SDS gel electrophoresis indicated that INA was attached primarily to lower M_r bands. Labelling patterns were similar in both slime mold species. The





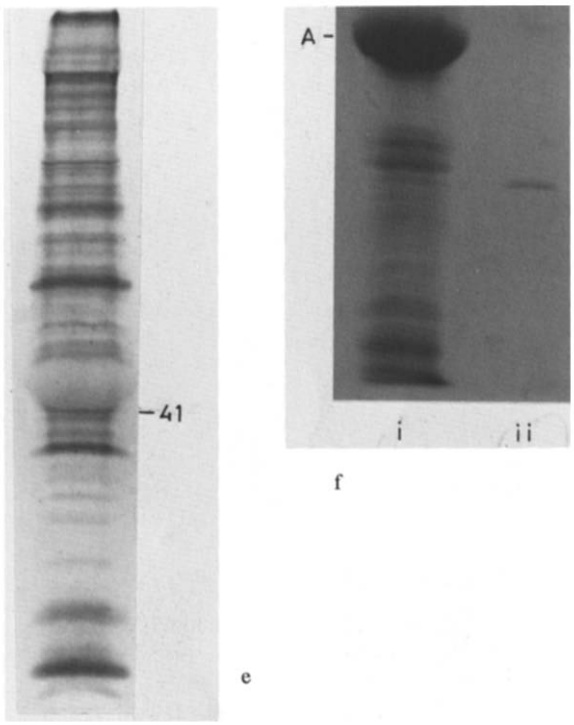


Fig.1. SDS–polyacrylamide (10%) gels of *D. discoideum* and *P. pallidum* plasma membranes. Apparent M_r-values ($\times 10^{-3}$) are indicated: A, actin; M, myosin heavy chains; con A, concanavalin A, added to stabilize plasma membranes during isolation.

(a) *D. discoideum*, autoradiographs: (i) [^{14}C]acetate labelling (1 h) between pre- and early-aggregation; (ii) [^{14}C]glucosamine labelling (2.5 h) between pre- and mid-aggregation; (iii) [^{14}C]acetate labelling (2.5 h) between finger and slug stages; (iv) [^{14}C]glucosamine labelling (3.5 h) between late finger and culmination stages.

(b) *P. pallidum*, autoradiographs: (i) [^{14}C]acetate labelling (2 h) of vegetative cells; (ii) [^{14}C]acetate and (iii) [^{3}H]fucose labelling (2 h) of early aggregation stage; (iv) [^{14}C]acetate and (v) [^{3}H]fucose labelling (2 h) of late aggregation stage (4–6 h after plating out).

(c) *D. discoideum*; autoradiographs of INA-treated plasma membranes from (i) early aggregates and (ii) slugs; (iii) and (iv) Coomassie blue-stained gels of samples (i) and (ii), respectively.

(d) *P. pallidum*; autoradiographs of INA-treated plasma membranes from: (i) vegetative cells; (ii) early aggregation (2 h after plating out); (iii) late aggregation (6 h after plating out); (iv–vi) Coomassie blue-stained gels of samples (i–iii), respectively.

(e) *D. discoideum* plasma membranes from vegetative cells; gels stained for glycoproteins using the concanavalin A/peroxidase method [15].

(f) Coomassie blue-stained gel of (i) plasma membranes of aggregating *D. discoideum* cells and (ii) 'discoidin' isolated from the same cells. Only the lower half of the gel is shown.

most strongly labelled bands were 41 000, 27 500, 26 000 and 25 000 M_r in *D. discoideum* and 41 000, 28 500, 27 500, 26 000 and 25 000 M_r in *P. pallidum*. Less strongly labelled bands (18 000–23 000 M_r) were also detected in both species. The labelling of these proteins did not change significantly during differentiation. The 25 000 M_r protein of both species was an exception and appeared to be lost from the membrane during development (fig.1c,d).

The strong labelling of these proteins relates them to transmembrane function.

The 41 000 M_r protein is a glycoprotein (fig.1e) and is phosphorylated when [γ-^{32}P]ATP is added to cells or when they are incubated with $^{32}P_i$ [33]. It is a candidate for the ecto-ATPase [34] or ecto-protein kinase [33,35,36].

Wallace and Frazier [37], using photoaffinity labelling, have identified the chemotactic receptor for cyclic AMP on the surface of *D. discoideum* cells as a protein with M_r 40 000 in SDS. (However, in [38] this protein was found to be soluble and not membrane bound.) The 41 000 M_r protein labelled by INA was presumably not the cyclic AMP receptor, since its concentration did not increase during early development.

The remaining proteins which were strongly labelled with INA do not appear to be glycoproteins. Concanavalin A, added to plasma membranes as part of the isolation procedure, was not labelled. Since the cells were bacterially grown the possibility that some of the labelled proteins are of bacterial origin must be taken into account. However, this is unlikely since labelling patterns of membranes from slugs (where bacterial digestion has long been completed) were almost identical. Furthermore, no differences in labelling were found between plasma membranes from *D. discoideum* amoebae grown with bacteria or in defined medium.

The carbohydrate binding proteins from the two species ('discoidin' and 'pallidin') have $M_r \sim 26\,000$ and are thought to be involved in cell adhesion [39,40] (however, see [7,11]). Strong INA labelling was found in a 26 000 M_r protein in both *D. discoideum* and *P. pallidum* plasma membranes (fig.1c,d). The location of purified 'discoidin' (isolated according to [39]) in our gel system is shown in fig.1f. However, treatment of intact cells with *N*-acetylgalactosamine elutes discoidin from the membranes [52] and the lectin may only be loosely associated with the outer surface of the plasma membrane.

A number of very weakly labelled bands were also detected (fig.1d). These did not change during the differentiation of *P. pallidum* (fig.1d) although some differences were found between the two *D. discoideum* developmental stages. The significance of these changes is not known.

Myosin heavy chains and actin represent a large amount of the total protein present in the plasma membrane fraction. They were not labelled by INA, suggesting that they do not penetrate the bilayer (but see below), although myosin [41,42] and actin [43] have been reported to be exposed on the outer surface of fibroblasts and lymphocytes, respectively. Microfilaments may be attached to the inner surface of the plasma membrane by one of the INA-labelled proteins [21,44].

Bordier et al. [12] have studied an amphiphilic protein complex associated with *D. discoideum* plasma membranes which accumulates dramatically as a result of increased synthesis during early development. In SDS the complex dissociates to proteins of 32 000, 18 000 and 15 000 M_r and components of <12 000 M_r, respectively. However, these were probably not the proteins labelled by INA since the labelled bands did not increase in concentration during development (fig.1c).

We did not detect INA labelling of the developmentally regulated plasma membrane proteins in either species (fig.1c,d). However, the conclusion that they are extrinsic proteins is not necessarily valid. In fact, the ratio of polar and hydrophobic amino acids is ~1:1 in the 82 000 M_r 'contact sites' of *D. discoideum* [45]. The lack of labelling may be related to the selectivities of the naphthyl azide products [47–51]. Recent results indicate that aryl azide photolabels do not insert to a significant extent into C–H bonds of membrane polypeptides [47,48,50,51]. The photoproducts exhibit a general preference for nucleophiles and consequently certain polypeptide segments in the lipid bilayer may not be reactive. Although all the known intrinsic proteins in human erythrocyte membranes are labelled by INA [27,46], not much activity was found on gels in the regions of PAS 1 and band 3 [51]. This could reflect the different susceptibilities to labelling of various amino acid side chains.

When synthesis of some developmentally regulated proteins ceases (e.g., 82 000 and 100 000 M_r in *D. discoideum*, 71 000 M_r in *P. pallidum*) they are subsequently lost from the plasma membrane [4,5,9]. The means by which these glycoproteins are selectively removed is unknown, although shedding or endocytosis followed by degradation may be involved. Proteins embedded in or spanning the lipid bilayer may be more difficult to remove selectively.

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References

[1] Smart, J. E. and Hynes, R. A. (1974) Nature 251, 319–321.
[2] Geltosky, J. E., Siu, C. H. and Lerner, R. A. (1976) Cell 8, 311–321.
[3] Siu, C. H., Lerner, R. A. and Loomis, W. F. jr (1977) J. Mol. Biol. 116, 469–488.
[4] Parish, R. W., Schmidlin, S. and Parish, C. R. (1978) FEBS Lett. 95, 366–370.
[5] Parish, R. W. and Schmidlin, S. (1979) FEBS Lett. 98, 251–256.
[6] Parish, R. W. and Schmidlin, S. (1979) FEBS Lett. 99, 270–274.
[7] Müller, K. and Gerisch, G. (1978) Nature 274, 445–449.
[8] Hintermann, R. and Parish, R. W. (1979) FEBS Lett. 108, 219–225.
[9] Steinemann, C., Hintermann, R. and Parish, R. W. (1979) FEBS Lett. 108, 379–384.
[10] Geltosky, J. E., Weseman, J., Bakke, A. and Lerner, R. A. (1979) Cell 18, 391–398.
[11] Bozzaro, S. and Gerisch, G. (1978) J. Mol. Biol. 120, 265–279.
[12] Bordier, C., Loomis, W. F., Elder, J. and Lerner, R. (1978) J. Biol. Chem. 253, 5133–5139.
[13] Hoffman, S. and McMahon, D. (1977) Biochim. Biophys. Acta 465, 242–259.
[14] West, C. M. and McMahon, D. (1977) J. Cell Biol. 74, 264–273.
[15] Hoffman, S. and McMahon, D. (1978) J. Biol. Chem. 253, 278–287.
[16] West, C. M., McMahon, D. and Molday, R. S. (1978) J. Biol. Chem. 253, 1716–1724.
[17] Hoffman, S. and McMahon, D. (1978) Arch. Biochem. Biophys. 187, 12–24.
[18] Newell, P. C., Longlands, M. and Sussman, M. (1971) J. Mol. Biol. 58, 541–554.
[19] Gerisch, G., Malchow, D., Huesgen, A., Nanjundiak, V., Roos, W., Wick, U. and Hülser, D. (1975) in: ICN–UCLA Symp. Mol. Cell. Biol. (McMahon, D. and Fox, C. F. eds) vol. 2, pp. 76–88, W. A. Benjamin, San Francisco CA.
[20] Steinemann, C. and Parish, R. W. (1980) Nature 286, 621–623.

[21] Parish, R. W., Schmidlin, S. and Müller, U. (1977) Exp. Cell Res. 110, 267–276.
[22] Klip, A. and Gitler, C. (1974) Biochem. Biophys. Res. Commun. 60, 1155–1162.
[23] Gupta, C. M., Radhakrishnan, R. and Khorana, W. G. (1977) Proc. Natl. Acad. Sci. USA 74, 4315–4319.
[24] Abu-Salah, K. M. and Findlay, J. B. C. (1977) Biochem. J. 161, 223–228.
[25] Sigrist-Nelson, K., Sigrist, H., Bercovici, T. and Gitler, C. (1977) Biochim. Biophys. Acta 468, 163–176.
[26] Karlish, S. J. D., Jorgensen, P. L. and Gitler, C. (1977) Nature 269, 715–717.
[27] Bercovici, T. and Gitler, C. (1978) Biochemistry 17, 1484–1489.
[28] Wells, E. and Findlay, J. B. C. (1979) Biochem. J. 179, 257–264.
[29] Wells, E. and Findlay, J. B. C. (1979) Biochem. J. 179, 265–272.
[30] Tarrab-Hazday, R., Bercovici, T., Goldfarb, V. and Gitler, C. (1980) J. Biol. Chem. 255, 1204–1209.
[31] Sussman, M. (1966) in: Methods in Cell Physiology (Prescott, D. ed) vol. 2, pp. 397–410, Academic Press, New York.
[32] Raper, K. B. (1951) Quart. Rev. Biol. 26, 169–190.
[33] Parish, R. W., Müller, U. and Schmidlin, S. (1977) FEBS Lett. 79, 393–395.
[34] Parish, R. W. and Weibel, M. (1980) FEBS Lett. 118, 263–266.
[35] Weinstein, B. I. and Koritz, S. B. (1973) Dev. Biol. 34, 159–162.
[36] Mato, J. and Konijn, T. M. (1976) Exp. Cell Res. 99, 328–332.
[37] Wallace, L. J. and Frazier, W. A. (1979) Proc. Natl. Acad. Sci. 76, 4250–4254.
[38] Cooper, S., Chambers, D. A. and Scanlon, S. (1980) Biochim. Biophys. Acta 629, 235–242.
[39] Simpson, D. L., Rosen, S. D. and Barondes, S. H. (1974) Biochemistry 13, 3487–3493.
[40] Chang, C.-H., Rosen, S. D. and Barondes, S. H. (1977) Exp. Cell Res. 104, 101–109.
[41] Olden, K., Willingham, M. and Pastoan, I. (1976) Cell 8, 383–390.
[42] Puskin, E. G., Maldonado, R., Spaet, T. H. and Zucker, M. B. (1977) J. Biol. Chem. 252, 4371–4378.
[43] Owen, M. J., Auger, J., Barber, B. H. Edwards, A. J., Walsh, F. S. and Crumpton, M. J. (1978) Proc. Natl. Acad. Sci. USA 75, 4484–4488.
[44] Clarke, M., Schatten, G., Mazia, D. and Spudich, J. A. (1975) Proc. Natl. Acad. Sci. USA 72, 1758–1762.
[45] Müller, K., Gerisch, G. Fromme, I., Mayer, M. and Tsugita, A. (1979) Eur. J. Biochem. 99, 419–426.
[46] Gitler, C. and Bercovici, T. (1979) Ann. NY Acad. Sci. 346, 199–211.
[47] Bayley, H. and Knowles, J. R. (1978) Biochemistry 17, 2414–2419.
[48] Hu, V. W. and Wisnieski, B. J. (1979) Proc. Natl. Acad. Sci. USA 76, 5460–5464.
[49] Cerletti, N. and Schatz, G. (1979) J. Biol. Chem. 254, 7746–7751.
[50] Brunner, J. and Richards, F. M. (1980) J. Biol. Chem. 255, 3319–3329.
[51] Bayley, H. and Knowles, J. R. (1980) Biochemistry 19, 3883–3892.
[52] Springer, W. R., Haywood, P. L. and Barondes, S. H. (1980) J. Cell Biol. 87, 682–690.