SYNTHESIS OF PLASMA MEMBRANE PROTEINS AND ANTIGENS DURING DEVELOPMENT OF THE CELLULAR SLIME MOLD *POLYSPHONDYLIUM PALLIDUM*

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1. Introduction

Changes in the surface properties of cells occur during the differentiation of slime molds [1–8]. These changes are potentially involved in chemotaxis, cell adhesion and the induction of developmentally-coupled enzymes [9–11]. The synthesis of certain plasma membrane proteins in *Dictyostelium discoideum* is developmentally regulated [6,7]. The function of most of these proteins is unknown, although two are apparently required for cell adhesion [6,8,12].

Polysphondylium pallidum and *D. discoideum* form common aggregates, however, the two cell types sort out within the aggregates [13]. This occurs because the adhesion sites are species specific [13].

Here we examine the synthesis of *P. pallidum* plasma membrane proteins during development using radioactive acetate, fucose and glucosamine. We also describe changes in plasma membrane antigens. We found 32 plasma membrane proteins, largely glycoproteins, were synthesized during development. The synthesis of 17 of these was initiated at specific stages of development. Four developmentally regulated antigens were detected and their possible role in cell adhesion is discussed.

2. Materials and methods

Polysphondylium pallidum (strain Ti-1) amoebae were grown on 0.1% lactose–peptone agar [14] in sterile aluminium trays (30 × 50 × 2.5 cm). Each tray contained 400 ml agar and was inoculated with $2–3 \times 10^5$ spores mixed with *E. coli* (strain B/r in 4.0 ml 17 mM phosphate buffer (pH 7.0)). A second tray was inverted over the first and incubated for 44–48 h at 23°C. Amoebae were harvested in buffer and separated from bacteria by low speed centrifugation. Two such trays provided $1–2 \times 10^9$ cells. Washed amoebae were plated out on millipore filters (10^8 cells in 0.2 ml phosphate buffer) over filter pads containing 17 mM phosphate buffer (pH 7.0) [15]. (It was necessary to wash the filters with dist. water before use.) The cultures were incubated in the light at 23°C.

Labelling of cells was carried out by adding the isotope in 50 μl dist. water via a Hamilton syringe to the top of the filters. The isotopes used and the amount per filter were: [1-^{14}C]acetate, 10 μCi (60 mCi/mmol, Amersham); D-[1-^{14}C]glucosamine hydrochloride, 6 μCi (10 mCi/mmol, NEN); L-[6-^{3}H]fucose, 10 μCi (15 Ci/mmol, NEN). Cultures were labelled for 1.5 or 2 h at 6 different developmental stages: vegetative amoebae; early aggregation (0–2 h after plating out); mid-aggregation (2–4 h); late aggregation (4–6 h); tip formation (6–7.5 h); culmination (7.5–9 h).

Plasma membranes were isolated using either the con A–Triton X-100 [16] or digitonin [17] methods, both giving similar results. SDS–gel electrophoresis, staining for proteins with Coomassie blue and glycoproteins with con A/peroxidase were carried out as in [18].

A membrane fraction was isolated from vegetative and mid-aggregation cells by homogenizing in phosphate buffer (Potter teflon homogenizer), centrifuging at 3000 × *g* (10 min) and then at 170 000 × *g* (60 min). The 170 000 × *g* sediments were lyophilized and used as antigens. The immunization of rabbits and the detection of plasma membrane antigens in SDS–gels were carried out as in [8].

Plasma membranes were treated with *n*-butanol. A

membrane suspension (5 mg protein/ml) in 10 mM citrate buffer (pH 5.5) was shaken with *n*-butanol (1:0.75, v/v) for 5 min at 4°C. The solution was centrifuged for 5 min at 1900 × *g* and the lower water-phase then dialysed against 5000 vol. 0.5% Triton X-100. Following a centrifugation for 10 min at 10 000 × *g* the supernatant was concentrated by vacuum dialysis and mixed with electrophoresis sample buffer.

3. Results

3.1. *Synthesis of plasma membrane proteins*

A large number of labelling experiments were carried out using the three isotopes. Three of these are shown (fig.1–3) and the results summarized in table 1. Plasma membranes isolated with con A–Triton X-100 or digitonin gave identical results (e.g., fig.2). The majority of newly synthesized proteins are present in low concentrations and not readily detected on gels stained with Coomassie blue (fig.1).

The strong labelling of actin and myosin heavy chains with acetate prevented the detection of proteins with similar molecular weights. However, the synthesis of four such proteins (no. 15–17,30) was detected using labelled sugars.

Pallidin is a carbohydrate binding protein thought to be involved in cell adhesion [19] (see section 4) and we found isotope incorporation into a protein with the relevant molecular weight. However, pallidin is not a glycoprotein and comparison of acetate and sugar incorporation patterns indicated two different proteins (no. 7,8) with similar molecular weights were being synthesized (table 1).

Five proteins which were relatively strongly labelled with acetate and glucosamine either failed to incorporate fucose (no. 20,21,28) or incorporated it only weakly (no. 13,19). Fucose but no glucosamine was incorporated into protein 16. Proteins 8 and 9 were unusual in exhibiting different times for maximal fucose and glucosamine incorporation. (Protein 8 incorporated fucose from early aggregation until culmination, glucosamine only until mid-aggregation; protein 9 incorporated glucosamine during early aggregation, fucose mainly between tip and culmination.)

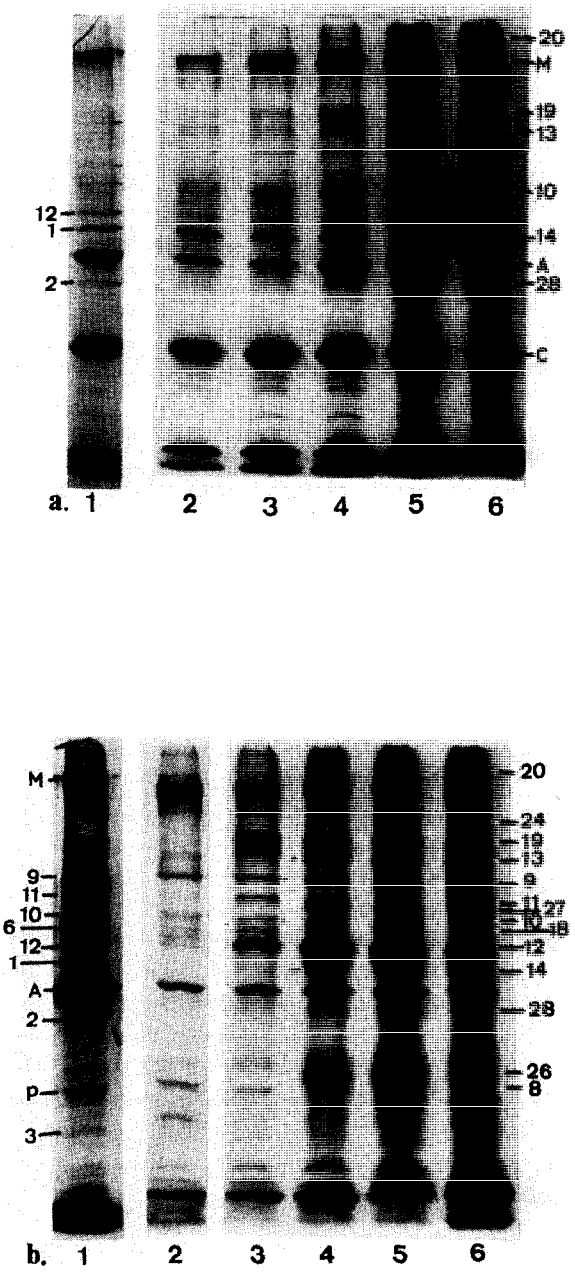

Fig.1. SDS–gels of plasma membranes isolated (using con A–Triton X-100) from 6 developmental stages following labelling with [^{14}C]acetate. The stages were: (1) vegetative; (2) early aggregation; (3) mid-aggregation; (4) late aggregation; (5) tip formation; (6) culmination. (a) Stained for proteins. A, actin; M, myosin heavy chains; C, concanavalin A; p, pallidin (?). (b) Autoradiograph of the same samples as shown in (a). The numbers of the major bands are indicated.

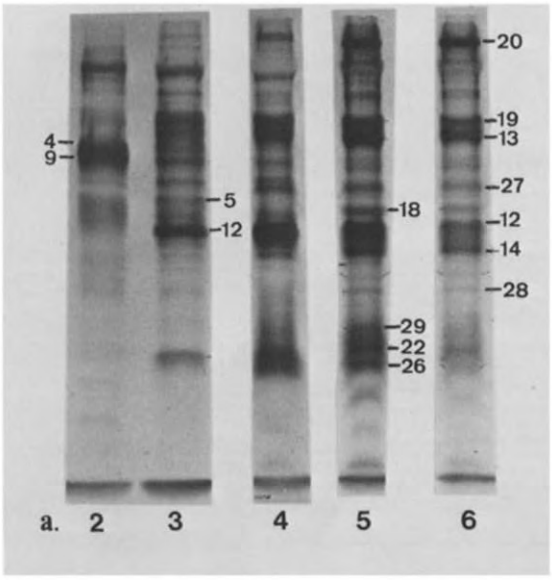

Fig.2. Autoradiographs of SDS–gels of plasma membranes isolated from different developmental stages following labelling with [^{14}C]glucosamine. The stages are numbered as in fig.1. (a) Plasma membranes isolated with con A–Triton X-100. (b) Plasma membranes isolated with digitonin.

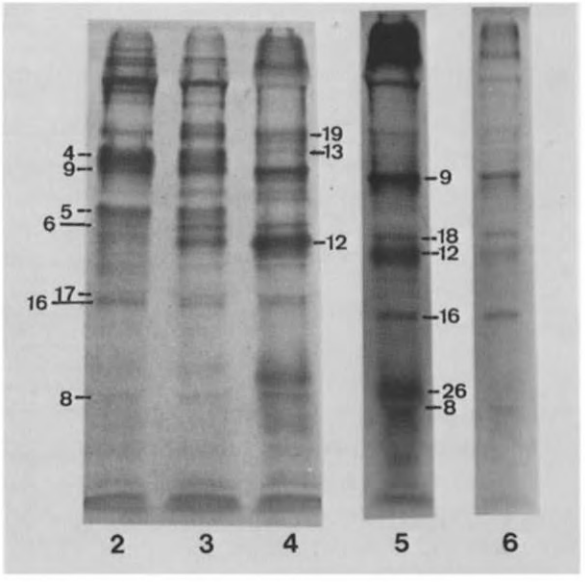

Fig.3. Autoradiographs of SDS–gels of plasma membranes isolated from different developmental stages (see fig.1) following labelling with [^{14}C]fucose.

3.2. *Plasma membrane antigens*

Myosin heavy chains, actin and a protein (often a double band) which may have been pallidin were antigenic and present in plasma membranes from both developmental stages examined (fig.4).

When antiserum against membranes from mid-aggregation cells (antiserum B) was incubated with plasma membranes from the same stage, a number of additional antigen–antibody binding regions could be detected on the gels (fig.4b). Some minor bands and three major bands were present. The major antigens corresponded to proteins 5 (71 kilodaltons), 12 (57 kilodaltons) and 13 (110 kilodaltons). The 110 kilodalton antigen was often, but not always, a broad band and more than one protein may be present. The 110 and 71 kilodalton antigens were the major antigens extracted from mid-aggregation plasma membranes by butanol (fig.4). The 57 kilodalton antigen was not always extractable with butanol (fig.4b). Using antiserum B, low concentrations of the 110 and 71 kilodalton antigens were detected in plasma membranes from vegetative cells (fig.4b). The 57 kilodalton antigen was not present in vegetative cells. A 180 kilodalton antigen was a major band in vegeta-

Table 1 Proteins and glycoproteins of the P. pallidum plasma membrane synthesized during development

Protein	Apparent mol. wt. (kd)	Fucose labelled	Glucosamine labelled	Stage of development when synthesized: Vegetative / Pre-early agg. / Mid agg. / Late agg. / Tip / Culmination
1	51	?	?	
2	34	?	?	
3	24	?	+	
4	98	+	+	
5	71	+	+	
6	63	+	+	
Myosin h.c.	210	-	-	
Actin	42	-	-	
7 (Pallidin?)	26	-	-	?
8	26	+	+	
9	92	+	+	
10	70	-	-	
11	80	+	+	
12	57	+	+	
13	110	+	+	
14	50	+	+	
15	190-200	+	+	
16	39	+	-	
17	41	+	+	
18	66	+	+	
19	120	+	+	
20	300	-	+	
21	140	-	+	
22	29	?	+	
23	53	+	+	
24	150	+	+	
25	115	+	+	
26	27	+	+	
27	74	+	+	
28	36	-	+	
29	30	?	+	
30	220-230	+	+	

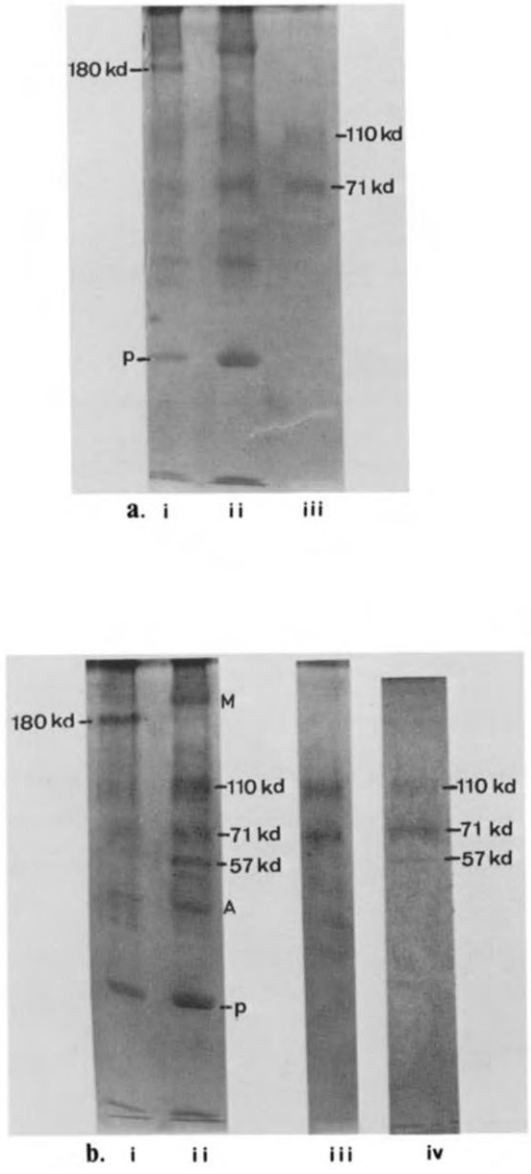

Fig.4. SDS–gels of plasma membranes isolated from two stages of differentiation and incubated with antisera raised against membranes from the same two stages. Antibodies binding to specific proteins were detected using IgG coupled to peroxidase [8]. (a) Incubated with antisera raised against membranes from vegetative cells. (i) Plasma membrane from vegetative cells. (ii) Plasma membrane from mid-aggregation cells. (iii) Butanol extract from plasma membranes isolated from mid-aggregation cells. (b) Incubated with antisera raised against membranes from mid-aggregation cells. (i), (ii), (iii) as in (a): (iv) A second butanol extract which included the 57 kilodalton antigen.

tive cells but only weakly detected in mid-aggregation cells (fig.4).

The addition of antiserum against vegetative cell membranes (antiserum A) (fig.4a) confirmed the results obtained with antiserum B. No antibodies against the 57 kilodalton antigen were present. Some antibodies against the 110 and 71 kilodalton antigens were detected, again indicating these antigens do occur on vegetative cells.

The synthesis of the 110 and 71 kilodalton antigens was in accordance with the antisera results. Both proteins were synthesized in vegetative cells and their synthesis increased during aggregation (table 1). The 57 kilodalton antigen was mainly synthesized from mid-aggregation until the end of differentiation (table 1). However, synthesis also appeared to occur in vegetative cells, almost ceasing during pre-early aggregation. The antisera results show that in fact another and non-antigenic protein with the same molecular weight was synthesized in vegetative cells. The 180 kilodalton antigen was apparently not synthesized in differentiating cells and disappeared from the plasma membrane.

4. Discussion

The results of the labelling experiments are summarized in table 1. The synthesis of 32 plasma membrane proteins was detected during differentiation. The proteins may be divided into two major groups:

(1) Proteins already synthesized in vegetative cells.

(a) Synthesis ceased during development (6 proteins).
(b) Synthesis continued throughout development but at a decreasing rate (actin, myosin heavy chains, pallidin).
(c) Synthesis continued throughout development, the maximum being reached at a specific developmental stage (6 proteins).

(2) Proteins first synthesized during development (17 proteins).

The majority of the proteins were glycoproteins. The four exceptions were actin, myosin heavy chains and proteins 7 (pallidin?) and 10. Pallidin is the name

given to carbohydrate binding proteins on the surface of the cells which have been implicated in cell adhesion [13,19,20]. We tentatively identified protein 7 as pallidin based on molecular weight and failure to incorporate sugars. However, we could not say whether protein 7 was synthesized after aggregation because a glycoprotein with the same molecular weight was then also being synthesized (table 1). A similar protein (discoidin) to pallidin from *D. discoideum* [21] appeared to be synthesized throughout development [6].

The relative incorporation of fucose and glucosamine differed between glycoproteins. One glycoprotein incorporated only fucose while five incorporated little or no fucose whatever. The stages at which maximum incorporation of glucosamine and fucose occurred were more or less identical, except for two proteins where they did not coincide. This suggests either different proteins which comigrated were involved or a modification in the oligosaccharide composition took place.

Francis [22] also labelled developing *P. pallidum* cultures with [^{14}C]acetate and examined changes in total protein synthesis. He detected 24 proteins whose synthesis changed and, based on molecular weights and times of synthesis, we could correlate seven of these with the plasma membrane proteins of table 1 (no. 2,3,12,13,20, myosin heavy chains and actin).

Seven major antigens were detected in the plasma membranes. Three of these (actin, myosin heavy chains and possibly pallidin) were identifiable. One antigen (180 kilodalton) was associated only with vegetative cells and subsequently lost from developing cells. The remaining three antigens were developmentally controlled glycoproteins. The 57 kilodalton antigen (protein 12) was absent from vegetative cells and its synthesis reached a maximum during the late aggregation and tip stages (table 1). (A protein which comigrated with this antigen was synthesized in vegetative cells.) The 71 and 110 kilodalton antigens (proteins 5 and 13) were present on vegetative cells but, as expected from the labelling experiments (table 1), their concentration was higher on aggregating cells. The three antigens (proteins 5, 12 and 13) could be extracted from the plasma membrane with butanol, although protein 12 was not as readily extracted as the other two.

The contact sites A required for cell adhesion at the beginning of development in *D. discoideum* are also antigenic and may be extracted with butanol [12]. They are first synthesized during pre-aggregation, synthesis ceasing at mid-late aggregation after which they are lost from the plasma membrane [6,8]. Hence, the most likely candidate for a similar function in *P. pallidum* development is the 71 kilodalton antigen (protein 5). Synthesis of this antigen ceased during mid-late aggregation. Using adhesion-blocking Fab, two classes of target sites have been found [13] involved in adhesion during *P. pallidum* development. One class is already present on vegetative (growth phase) cells, the second appears during early development. We detected the 71 kilodalton antigen in vegetative as well as differentiating cells. The 57 kilodalton antigen would be a candidate for the second class of target sites. In *D. discoideum* a second antigen appears to replace contact sites A as a mediator of cell adhesion during later stages of development ([8], in preparation).

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