SYNTHESIS OF PLASMA MEMBRANE PROTEINS DURING DEVELOPMENT OF *DICTYOSTELIUM DISCOIDEUM*

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1. Introduction

The development of *Dictyostelium discoideum* cells is associated with changes in surface properties which are potentially involved in cellular cohesion, chemotaxis, aggregation and the induction of developmentally-coupled enzymes [1–4]. The changes in plasma membrane proteins have been studied using surface radioiodination [5–7], [^{35}S]-methionine labelling [7], radioimmunoassay [3] and affinity chromatography [6], all in association with dodecylsulfate gel electrophoresis.

We are studying the components of the plasma membrane [8–14] and this paper examines the developmentally-controlled synthesis of proteins using radioactive acetate, amino acids, fucose and glucosamine. Autoradiography of deodecylsulfate gels showed 13 labelled proteins, 11 of them glycoproteins, whose synthesis was developmentally controlled. The morphological stages at which these proteins were synthesized could be determined. One of the glycoproteins corresponded to the 'contact sites A' which are required for cell adhesion at the beginning of development. Synthesis of 'contact sites A' had ceased by late aggregation. Long-term labelling experiments suggested some of the proteins, including 'contact sites A', are lost from the plasma membrane at later stages of development. Some new glycoproteins which failed to incorporate radioactivity appeared during differentiation.

2. Materials and methods

Dictyostelium discoideum NC-4 (wild-type) amoebae were grown in liquid medium with *E. coli* according to [15]. Amoebae were used from cultures still containing bacteria by centrifugation and washing in PDF solution [15]. Amoebae were then plated out on millipore filters over filter pads containing PDF solution [16].

Labelling of cells was carried out by adding the isotope in 20 μl dist. water via a Hamilton syringe to the top of the millipore filters. The isotopes (Amersham) used and the amount per filter were: [1-^{14}C]acetate, 20 μCi (60 mCi/mmole); D-[1-^{14}C]glucosamine hydrochloride, 4 μCi (60 mCi/mmol); [U-^{14}C]protein hydrolysate, 4 μCi (55 Ci/matom); L-[1-^{14}C]fucose, 5 μCi (50 mCi/mmol).

Plasma membranes were usually isolated using the con A–Triton X-100 method [10], however, similar results were obtained using the digitonin and 2-phase separation method ([7,17,18], in preparation). SDS–gel electrophoresis, autoradiography and staining for proteins with amido black and glycoproteins with con A/peroxidase were carried out as in [10–14]. Early aggregation, the very first aggregation centres appearing; mid aggregation, many aggregation centres as indicated by 'bumpiness' of the culture surface; late aggregation, the bumps beginning to separate; tip, the aggregates form a nipple at the apex. The other stages are as usually described [10].

3. Results

No dramatic changes associated with development were detected in plasma membrane proteins when gels were stained with amido black. Con A-peroxidase staining showed changes in glycoproteins, however,

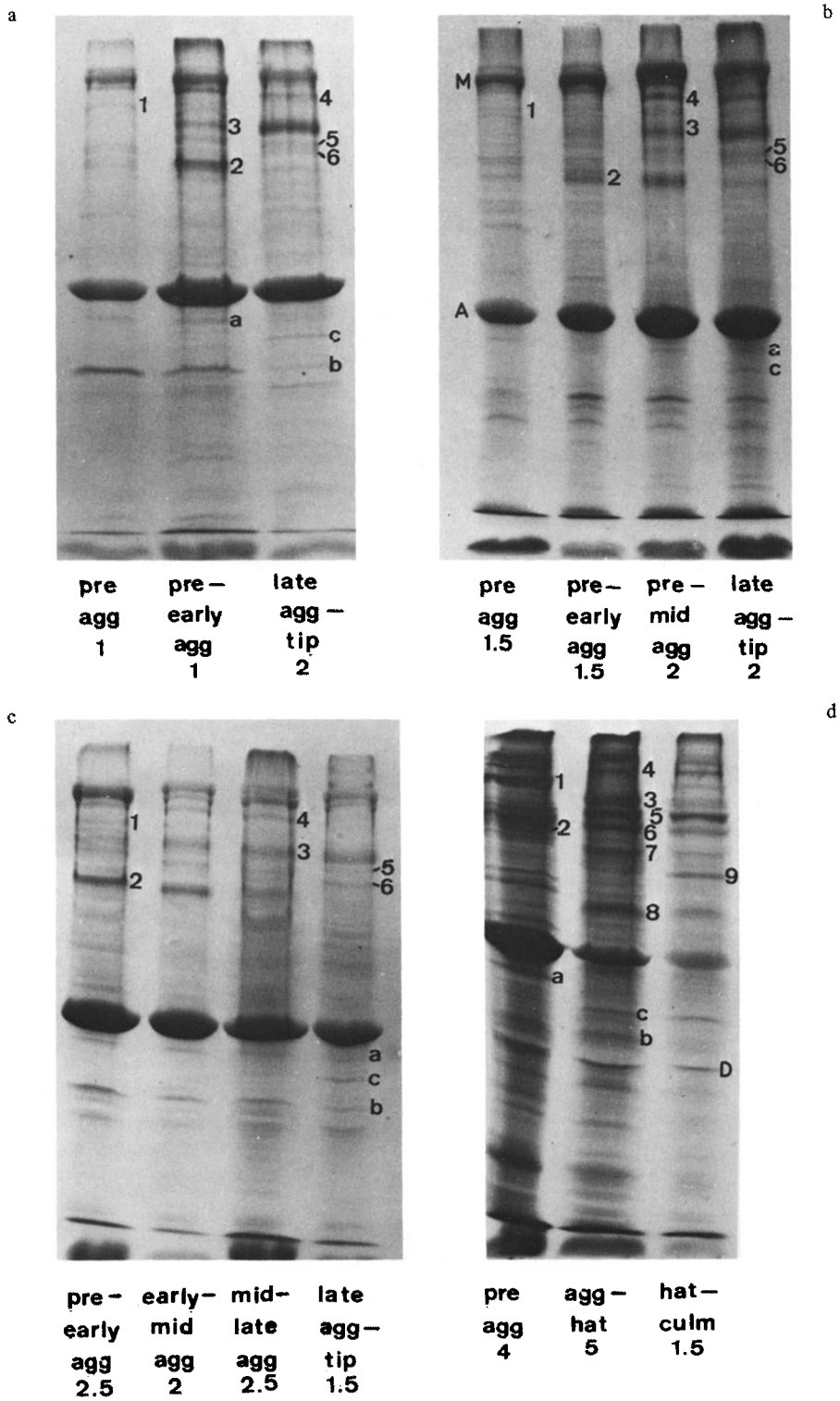
a
1
3
2
4
5
6
a
c
b
pre agg 1
pre – early agg 1
late agg – tip 2
b
M
1
2
4
3
5
6
A
a
c
pre agg 1.5
pre – early agg 1.5
pre – mid agg 2
late agg – tip 2
c
1
2
4
3
5
6
a
c
b
pre – early agg 2.5
early – mid agg 2
mid – late agg 2.5
late agg – tip 1.5
d
1
2
4
3
5
6
7
8
9
a
c
b
D
pre agg 4
agg – hat 5
hat – culm 1.5

Fig.1

the large number of glycoproteins [12] made quantitative studies difficult. (We have as yet been unable to separate these glycoproteins or two-dimensional gels.) Hence, we preferred isotopic labelling.

3.1. *[^{14}C]Acetate*

A variety of experiments, 4 of which are depicted in fig.1, showed a consistent pattern of developmentally-regulated protein synthesis. The proteins with mol. wt above actin (>42 K) were numbered in order of appearance. A large number of labelled proteins were present in the 'supernatants' (homogenate remaining after removal of plasma membranes) and gave a gel pattern completely different from the plasma membranes (not shown).

3.2. *[^{14}C]Protein hydrolysate*

Synthesis patterns resembled those obtained with acetate (e.g., fig.2), although labelling was weaker, possibly due in part to poor amino acid uptake. The 'supernatants' contained many labelled proteins and, until tip formation, none of plasma membrane proteins could be detected among them (not shown).

3.3. *[^{14}C]Glucosamine and [^{14}C]fucose*

The majority of developmentally-controlled proteins were glycoproteins, with the exception of bands 8 and 'a' (fig.3). Band 10 was not observed with acetate labelling, presumably because it was masked by myosin. Very few labelled glycoproteins were detected in the 'supernatant' fractions and the most prominent bands corresponded to plasma membrane glucoproteins (fig.3).

3.4. *Unlabelled glycoproteins*

A number of low mol. wt glycoproteins (arrows) appeared during aggregation and reached their highest concentrations in the slug (fig.4). These glycoproteins did not incorporate any of the 4 isotopes.

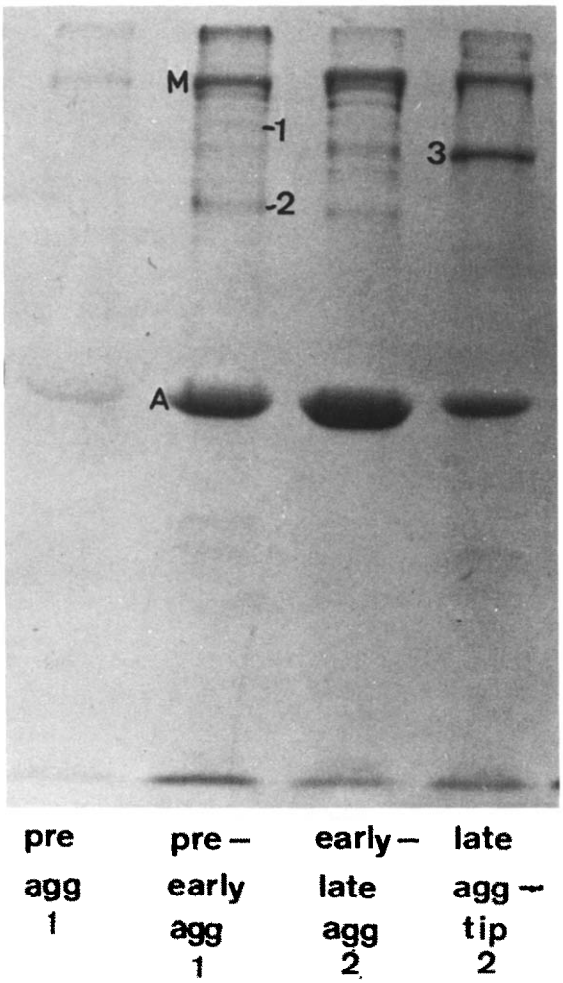

Fig.2. Autoradiograph of dodecylsulfate gel of plasma membranes from 4 developmental stages. The cultures were labelled with ^{14}C-labelled amino acids (protein hydrolysate) for times (h) indicated.

Fig.1. Autoradiographs of dodecylsulfate gels of plasma membranes isolated from different developmental stages. The cultures were labelled with [^{14}C]acetate and labelling times (h) are indicated beneath the morphological descriptions. The major developmentally regulated bands are numbered in order of appearance. Four different experiments are shown. A, actin; M, myosin heavy chains; D, discoidin (pre-agg, preaggregation stage; agg, aggregation; culm, culmination stage).

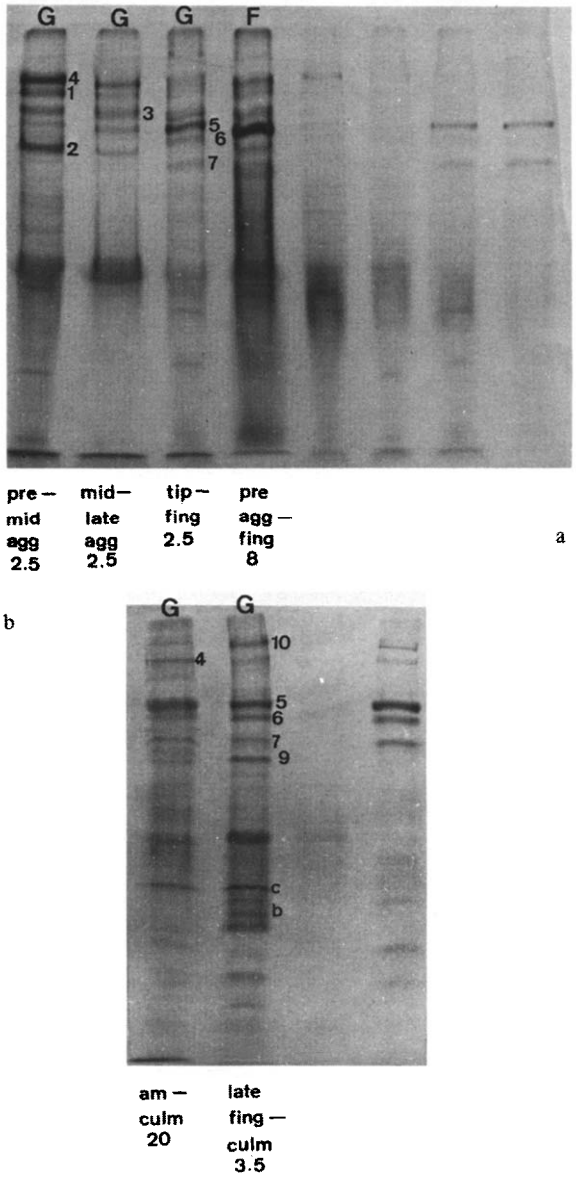

Fig.3. Autoradiographs of dodecylsulfate gels of plasma membranes and 'supernatants' of cultures labelled with [^{14}C]glucosamine (G) and [^{14}C]fucose (F). Labelling times (h) are indicated. (a) Three developmental stages labelled with [^{14}C]glucosamine and an 8 h labelling with ^{14}C-fucose; the 4 samples on the left are plasma membranes, the 4 samples on the right the equivalent 'supernatants' loaded in the same order as the plasma membrane fractions. (b) [^{14}C]-Glucosamine labelling during almost the entire development (20 h) and during late development; plasma membranes on the left, equivalent 'supernatants' on the right. fing, finger stage.

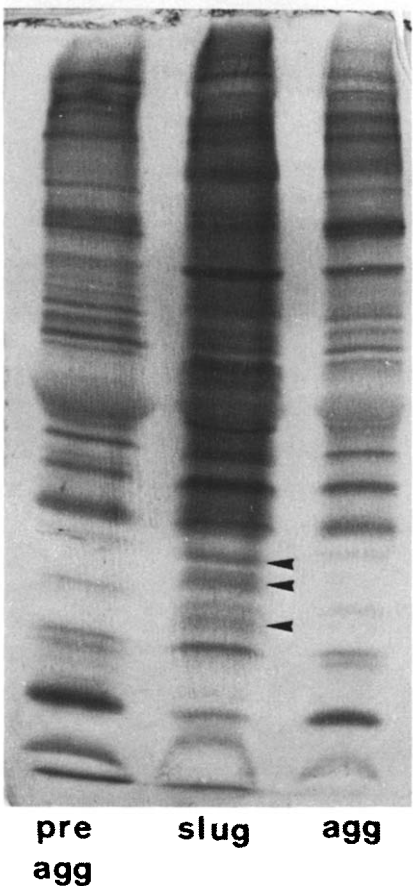

Fig.4. Dodecylsulfate gels of plasma membranes isolated from 3 developmental stages and stained for glycoproteins. The arrows indicate low mol. wt glycoproteins which first appear during late aggregation.

4. Discussion

The results of the labelling experiments are summarized in table 1. The majority of developmentally-regulated proteins are glycoproteins and at later stages of differentiation they represent the major glycoproteins being synthesized in the cell. The function of these glycoproteins, with the exception of band 2 is unknown. Band 2 is identifiable as the glycoprotein corresponding to 'contact sites A' [18]. This glycoprotein is required for cell adhesion during aggregation [18] and its synthesis ceases during late aggregation (table 1). Long-term labelling experiments (e.g., fig.3) indicate the 'contact sites A' are sub-

Table 1
The major developmentally-regulated proteins and glycoproteins of purified plasma membranes in *D. discoideum*

Protein	Apparent mol. wt.	Glycoprotein	Stage of development when synthesised
			Pre agg. / Early agg. / Mid agg. / Late agg. / Tip / Finger / Slug / Hat / Culm.
1	132 000	yes	
2	82 000	yes	
3	110 000	yes	
4	155 000	yes	
5	95 000	yes	
6	90 000	yes	
7	76 000	yes	
8	55 000	no	
9	69 000	yes	
10	210 000	yes	
a	37 000	no	
b	28 500	yes	
c	31 000	yes	
Myosin h.c.	210 000	no	
Actin	42 000	no	
Discoidin	26 000	no	
'X'	57 000	yes	No incorporation of radioactivity; appears at tip stage

Thin lines indicate synthesis is occurring, thick lines depict times of maximum synthesis. The molecular weights were determined using 10% acrylamide gels and may be an overestimate for the glycoproteins. Some low mol. wt glycoproteins which did not incorporate radioactivity and appeared during aggregation (cf. fig.4) and others which incorporated [^{14}C]glucosamine later in development (cf. fig.3) are not included. Protein 2 corresponds to contact sites A [18], identification being based on sugar incorporation, mol. wt, time of appearance, high antigenicity [13] and butanol extraction. The identification of discoidin is tentative, being based on mol. wt alone

sequently lost from the plasma membrane and we have confirmed this using immunological techniques [13]. Like 'contact sites A' the 95 K glycoprotein (band 5) is strongly antigenic and may replace the former as a mediator of cell adhesion. We are using antibodies to test this possibility.

The 132 K and 110 K glycoproteins (band 1,3) also appear to be lost from the membrane when their synthesis ceases.

As reported [7,21], very active synthesis of actin, but also of myosin heavy chains, continued up to tip formation. We did not, however, observe any major changes in concentration of these proteins in the plasma membrane preparations during differentiation. The release and reattachment of actin fibres to the plasma membrane, which possibly accompanies cell movement, may depend on the synthesis of new actin (and myosin) molecules.

Labelling with ^{35}S-methionine, 6 plasma membrane proteins were found synthesized during the latter half of the developmental cycle [7]. The molecular weights reported [7] are somewhat different from ours, presumably because they used gradient gels and glycoprotein migration was changed. The probable identification is: 103 K (our band 3); 96 K (band 5); 85 K (band 6); 63 K (band 7); 51 K (band 8); 280 K (band 10). The synthesis of low mol. wt proteins during pre-aggregation was also described [7]. Although we found similar proteins in cell 'ghosts' isolated by the method in [19] (not shown) they were not detectable in plasma membranes. (Note: the 'ghosts' are contaminated by intracellular membranes.) These proteins may be peripherally associated with the cytoplasmic surface of the membrane (not labelled by surface iodination [7]) and lost during digitonin or Triton X-100 treatments.

Acknowledgements

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