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A monoclonal antibody that interferes with the post-aggregation adhesion of *Dictyostelium discoideum* cells

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Abstract

A monoclonal antibody that interferes with the EDTA-resistant adhesion of *Dictyostelium discoideum* slug cells recognised a carbohydrate epitope on four major antigens (95, 90, 35 and 30 kDa) in slug cells. The 35 and 30 kDa antigens were specific for stalks and spores, respectively. The 30 kDa antigen was identified as the cell surface glycoprotein, PsA. Cyclic AMP, acting via cell surface receptors, induced only the 90 kDa slug cell antigen. Slug cell adhesion proteins may be involved in cell-sorting and the glycosylation of the 95 and 90 kDa antigens appeared to be abnormal in a mutant defective in cell-sorting. Previously, a 150 kDa glycoprotein has been strongly implicated in slug cell adhesion and the present work suggests that additional glycoprotein(s) are involved.

Key words: Dictyostelium; Cell adhesion; Glycoprotein; Monoclonal antibody; Cell differentiation

1. Introduction

Cell–cell interactions underlie a variety of differentiation phenomena including adhesion, tissue patterning and specific gene regulation. *Dictyostelium discoideum* is a useful model system with which to study these interactions, as during its simple life cycle specific cell–cell adhesion occurs along with the formation of different cell types. Chemoattractants and morphogens are involved in the formation of cell aggregates and the generation of cell patterns. However, the role of cell surface components in these processes is not well understood [1–3].

A variety of experiments including metabolic labelling, radioiodination, monoclonal antibodies and lectins have shown that cell surface components are developmentally regulated [4–12]. Serological studies using adhesion-blocking antibodies indicate at least three different developmentally regulated adhesion systems exist. A 126 kDa glycoprotein has been implicated in the EDTA-sensitive adhesion between growth phase (vegetative) amoebae [13] and a 24 kDa glycoprotein during the first 8 h of development [14]. An EDTA-stable adhesion appears during aggregation and an 80 kDa glycoprotein (contact site A) is responsible [15–17]. This glycoprotein is modified by a phospholipid anchor at its C-terminus and two types of oligosaccharide residue, one of which is sulphated [18,19]. The adhesion is mediated by homophilic interaction of contact site A (csA) molecules via peptide regions near the amino terminus [20–22]. The N-carbohydrate residues are also essential for adhesion [16,23,24].

Antibodies that block aggregation-stage adhesion are not effective at later developmental stages when cell patterning is occurring. Studies using polyclonal antibodies implicated a 95 kDa glycoprotein in the adhesion of post-aggregation amoebae [25] and this was supported by work on a temperature-sensitive mutant that labeled gp95 at the restrictive temperature [26,27]. Specific Fab directed against a 150 kDa glycoprotein that rapidly accumulates during the first half of the developmental cycle prevents disaggregated slug cells from reaggregating [6]. Prestalk and prespore cell sorting is blocked when the two cell types are coaggregated in the presence of anti-gp150 Fab [28]. Recently, gp150 has been purified and when added to disaggregated preculmination cells, significantly inhibited cell–cell adhesion [29].

Using partially purified gp95 as an immunogen we have obtained a monoclonal antibody (TK1) that interferes with the EDTA-stable adhesion of slug cells [30]. This paper determines the antigens recognised by TK1.

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2. Materials and methods

Dictyostelium discoideum strains Ax2-B and HG220, a *modB* mutant provided by Professor G. Gerisch [16], were grown axenically in HL-5 medium with shaking at 150 rpm. Growth phase cells were harvested by washing free of medium in P buffer (20 mM KH_2/Na_2H phosphate, pH 6.1) using centrifugation at $200 \times g$ for 2 min. After three such washes cells were suspended at 10^8 cells · ml^{-1} P buffer, spread on 2% water agar in Petri dishes and allowed to dry until moist before being incubated in the dark at 22°C. In order to obtain large numbers of cells for wheat germ agglutinin (WGA)-affinity chromatography the water agar was poured into 30×50 cm stainless-steel trays. Following the spreading and drying, the lid was taped on, the tray inverted in a black plastic bag and kept at 4°C for 4–6 h to facilitate synchronization of differentiation. The trays were then kept at 22°C until the desired differentiation stage had been reached.

Slugs were harvested by scraping them off the agar with a sterile spatula and transferring them to 10–20 ml of 20 mM phosphate buffer. Cells were dissociated by passing them through a 0.71 mm needle several times. The cells were washed in 50 ml of 20 mM phosphate buffer and centrifuged at $600 \times g$ for 5 min. The cells were then frozen at −20°C. Frozen dissociated slug cells were thawed at room temperature and washed twice in 20 mM Tris-HCl pH 7.8 by spinning at $20,000 \times g$ for 15 min. The sediment was resuspended in 10 ml 20 mM Tris-HCl pH 7.8 containing 1% (w/v) deoxycholate and gently rocked for 40 min at room temperature. The preparation was then spun at $100,000 \times g$ for 45 min. 10–15 μl of wheat germ agglutinin Sepharose 6MB (Pharmacia) beads were added to the clear supernatant. After shaking gently at 4°C for several hours or overnight (no stirrer was used to avoid breaking the Sepharose beads) the WGA-Sepharose beads were collected by a brief centrifugation at low speed. The beads were given three 5-min washes in 5 ml of 20 mM Tris-HCl pH 7.8 containing 0.5% Triton X-100 (v/v) and 1 M NaCl, followed by three 5-min washes in 20 mM Tris-HCl pH 7.8 containing 0.1% Triton X-100 (v/v). The beads were stored at 4°C in 50–100 μl of 20 mM Tris-HCl pH 7.8 containing 0.1% Triton X-100 until required and used within a week of preparation.

The WGA-Sepharose 6MB beads with the slug glycoproteins bound were incubated in 100 μl SDS buffer (62.5 mM Tris-HCl, pH 6.8, 10% glycerine, 3% SDS) at 60°C for 15 min. We found that a 95 kDa glycoprotein was preferentially released from the beads by this treatment, although some of the other proteins were released to a lesser extent. The beads were then removed by centrifugation and the supernatant used as the immunogen. Monoclonal antibodies were obtained in the conventional way. TK1 was selected by its ability to bind WGA-binding proteins from slug cells.

One-dimentional polyacrylamide gel electrophoresis and Western blotting were carried out as previously described [7,31]. Rainbow molecular weight markers (Amersham), which are conjugated to dyes, were used as they could be seen on nitrocellulose filters and allowed more precise estimates of antigen molecular weights.

Partially purified plasma membrane fractions were obtained using the Concanavalin A/Triton X-100 method which includes washing cell ghosts in 0.1 M Tris-HCl, pH 8.5, containing 0.1 M EDTA [32]. Cells were disaggregated prior to membrane isolation.

Stalks and spores were obtained by washing mature fruiting bodies from agar plates with 20 mM KK2 buffer and separated by passing a number of times through a nylon filter (mesh size 60 μm, Nybolt). The stalks retained on the filter were washed thoroughly; spores were washed by low speed centrifugation. Washing and filtering was continued until microscopic examination indicated pure spores or stalks were present. Spores and stalks were ruptured in a glass bead mill at 0°C. Equal volumes of 62.5 mM Tris-HCl, 10% glycerine, 3% SDS and 5% 2-mercaptoethanol were added and boiled for 3 min.

3. Results

Fab fragments of the TK1 antibody (100 μg/ml) have been shown to strongly inhibit the reaggregation of disaggregated slug cells in the presence of 15 mM EDTA [28]. Maximum inhibition was obtained using 30 μg · ml^{-1} TK1 Fab (10^7 cells · ml^{-1}) (not shown).

Partially purified plasma membranes were isolated at various stages of development and Western blotting indicated that TK1 recognizes a number of developmentally regulated antigens (Fig. 1). The two major antigens have molecular weights of 95 and 90 kDa, appearing in the plasma membrane fraction during cell aggregation and reaching a maximum level at the finger stage (Fig. 1a). The 95 kDa antigen is initially present at higher levels than the 90 kDa antigen. Both proteins are also found in the detergent-solubilized fraction (Fig. 1b). The molecular weights vary somewhat depending on the acrylamide concentrations used. The 95 kDa antigen migrated at up to 105 kDa on gels with acrylamide concentrations above 10%. The 90 kDa antigen ran at up to 95 kDa on these latter gels. A 28 kDa antigen was present only in the aggregation stage while 35 kDa and 30 kDa antigens appeared at the finger and slug stages, respectively (Fig. 1a).

Stalk cells retained the 95 and 90 kDa proteins but three stalk-specific TK1-antigens were also present, 35, 31 and 29 kDa (Fig. 2). The 30 kDa antigen was specific

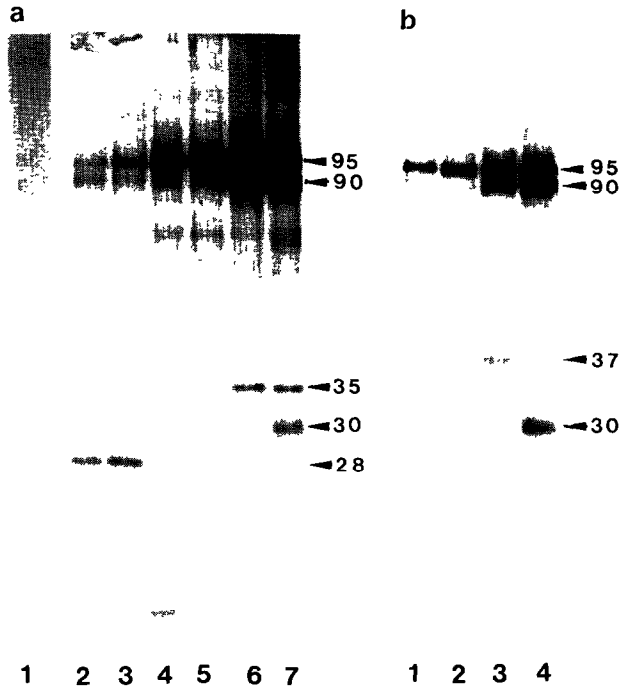

Fig. 1. Developmental regulation of TK1 antigens. Following SDS-PAGE (10%), proteins from different developmental stages were Western blotted using the monoclonal antibody TK1. Numbers represent apparent molecular weights. (a) Partially purified plasma membranes, (1) vegetative cells, (2) aggregates, (3) late aggregates, (4) tipped aggregates, (5) early fingers, (6) fingers, (7) slugs. (b) The soluble material remaining after removal of plasma membranes; (1) aggregates, (2) late aggregates, (3) fingers, (4) slugs.

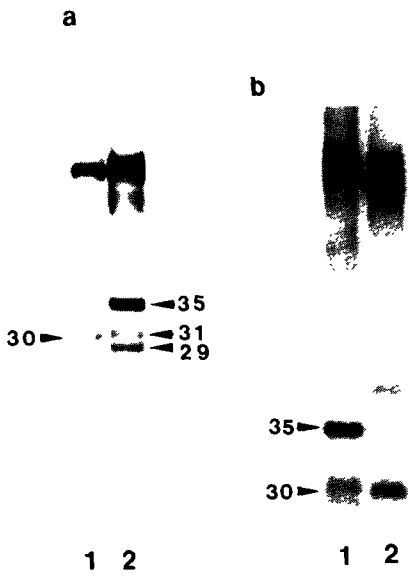

Fig. 2. TK1 antigens in mature stalks and spores. Proteins from pure preparations of stalks and spores were Western blotted using the monoclonal antibody TK1. (a) 15% polyacrylamide gels; (1) spores, (2) stalks. (b) 10% polyacrylamide gels; (1) stalks, (2) spores.

for spore cells. While the 90 kDa antigen was present on spore cells the 95 kDa antigen was weaker and a (relatively weak) 82 kDa antigen was present.

Plasma membranes were partially purified from the different developmental stages of the glycosylation-deficient mutant HG220. When Western blots were probed with TK1 no bands were detected (not shown).

Exponentially growing Ax2-B cells were washed in buffer and plated onto agar. When aggregation had commenced, the cells were washed from the agar and shaken in 20 mM phosphate buffer. Cyclic AMP or cAMP analogues (0.5 mM) were added to the suspensions. After 16 h the cells were isolated and immunoblotting used to assay for the presence of TK1 antigens (Fig. 3). The control cells contained barely detectable amounts of the 90 and 95 kDa antigens (Fig. 3, lane 1). Cyclic AMP induced the synthesis of the 90 kDa antigen and an 82 kDa antigen (Fig. 3, lane 2). The latter was not readily detectable in cells developing on agar (Fig. 1). Synthesis of the 95 kDa antigen did not appear to be induced. The 35 kDa antigen did not appear. 2′-Deoxy-cAMP gave results identical to cAMP (Fig. 3, lane 3). N^6-(aminohexyl) cAMP also induced the 95 and 82 kDa antigens, but only relatively weakly (Fig. 3, lane 4).

The oligosaccharide that is absent in *modB* mutants carries a structure that binds WGA [15,33]. Since TK1 also recognizes a structure on this oligosaccharide we used WGA-affinity chromatography to isolate putative TK1-antigens from slug cell plasma membranes.

The MUD102 monoclonal antibody recognizes an oligosaccharide epitope in a prespore-specific glycoprotein, PsB [34]. This epitope is absent in strains carrying a mutation at the *modB* locus. PsB first appears after tip formation, reaches maximum levels at the late slug stage and remains high throughout the remainder of differentiation. PsB shows size polymorphisms in the range 80–89 kDa. The properties of PsB suggested it may be one of the antigens recognized by TK1. The MUD102 antibody recognised a 90 kDa glycoprotein on Western blots of slug cell WGA-binding proteins that migrated to the same position as the 90 kDa antigen on the gels (Fig. 4a).

PsA, originally defined as a 30 kDa prespore-specific cell surface protein [35], also lacks a glycoconjugate(s) in *modB* mutants [36]. We used the PsA-specific monoclonal antibody MUD1 and the 30 kDa WGA-binding glycoprotein was recognized by this antibody as well as TK1 (Fig. 4b). MUD1 also recognised a second antigen with a lower molecular weight whose identity is unknown but could be a product of PsA proteolysis (K.L. Williams, personal communication).

Since slug cell adhesion proteins have been postulated to be involved in cell sorting we examined the TK1 antigens in a cell-sorting mutant. KYH-13 is a temperature-sensitive aggregateless and stalkless mutant [37]. If development is allowed to occur normally at 21°C to the finger stage and then the temperature raised to 27°C, during subsequent slug migration the distribution pattern of prestalk and prespore cells is disturbed. The number of prestalk cells in the anterior region of the slug is greatly reduced while prestalk cells are accumulated at the foot or posterior region of the slugs. Since these pattern changes reflect alterations in cell sorting we examined the TK1 antigens in slugs migrating at 21°C and 27°C as well as in the respective fruiting bodies (Fig.

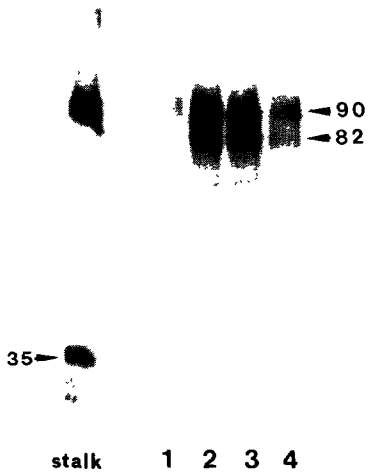

Fig. 3. Induction of TK1 antigens by cAMP. Aggregation competent cells (10^6 cells/ml) were shaken at 120 rpm in Erlenmeyer flasks for 16 h in the dark at 23°C. Proteins from partially purified plasma membranes were Western blotted using the monoclonal antibody TK1. (1) Controls, (2) 0.5 mM cAMP, (3) 0.5 mM 2′-deoxy-cAMP, (4) 0.5 mM N^6-(aminohexyl) cAMP. A lane containing stalk proteins is also shown.

5). No dramatic changes were observed in the TK1 antigens at the different temperatures. (A group of weakly staining 50–60 kDa antigens was absent from slug plasma membrane fractions at 27°C.) However, rather than two major antigens at 90 and 95 kDa, in the mutants a number of antigens were present in this region of the gels at both the permissive and restrictive temperatures.

4. Discussion

We have obtained a monoclonal antibody, TK1, that interferes with EDTA-stable post-aggregative (slug) cell adhesion. The TK1 immunodeterminant is absent from a strain with a mutation in the *modB* locus, which blocks post-translational glycosylation of certain membrane glycoproteins [38]. Hence, TK1 either recognizes a unique carbohydrate epitope on the *modB* sensitive glycoconjugate or the TK1 antigens are rapidly degraded in the absence of this glycoconjugate. Many glycoproteins carry the *modB* sensitive glycoconjugate and the TK1 epitope apparently delineates a small number of proteins within this class. Indirect evidence exists that surface carbohydrates participate in the cell-cell adhesion of *D. discoideum* cells [23,30,39–41]. However, inhibition of adhesion by TK1 does not necessarily implicate the TK1-epitope since the antibody might interfere sterically with protein moieties on the relevant antigen(s) that are involved in adhesion.

TK1 does not bind to any antigens with molecular weights around 150 kDa. Hence, gp150 is not the only cell adhesion molecule in slugs. The TK1 antigen(s) involved in adhesion might constitute an additional adhesion system or be part of a heterotypic adhesion complex involving gp150. Multiple cell–cell adhesion systems have been implicated in aggregation during early development [17,42].

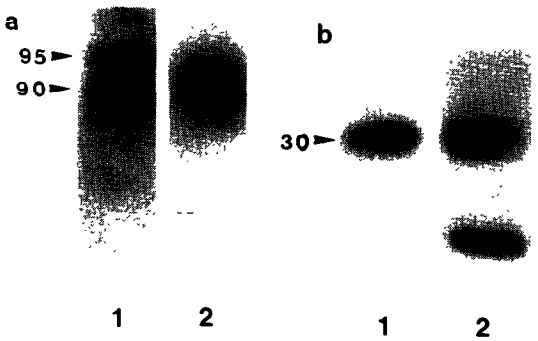

Fig. 4. Comparison of PsB and PsA with TK1 antigens. (a) Wheat germ agglutin binding proteins isolated from slug cell membranes, separated on polyacrylamide (7.5%) gels and Western blotted using TK1 (1) or PsB-specific MUD102 (2) monoclonal antibodies. (b) A deoxycholate extract of slug cell membranes separated on polyacrylamide (12%) gels and Western blotted using TK1 (1) or PsA-specific MUD1 (2) monoclonal antibodies. Only the region of the gel around 30 kDa is shown.

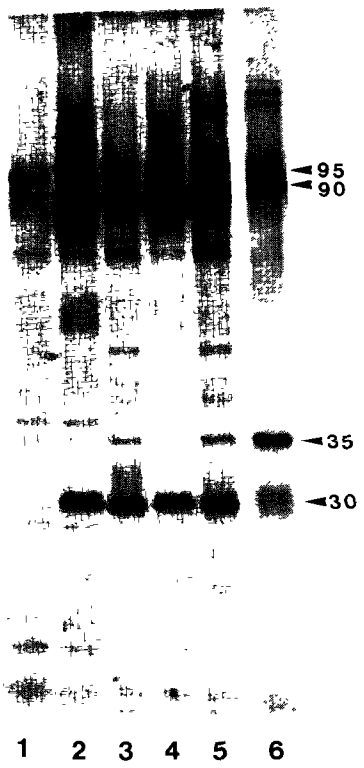

Fig. 5. Developmentally regulated TK1 antigens in the temperature sensitive mutant KYH-13. Development to the finger stage was at 21°C after which some cultures were transferred to 27°C. Total proteins, extracted using SDS, were Western blotted using the monoclonal antibody TK1. (1) Tipped aggregates; (2) slugs, 21°C; (3) fruiting bodies, 21°C; (4) slugs, 27°C; (5) fruiting bodies, 27°C; (6) stalks from Ax2-B fruiting bodies.

All of the antigens recognised by TK1 are developmentally regulated. Of the four major antigens in slug cells (95, 90, 35 and 30 kDa), the 35 kDa and 30 kDa antigens are specific for stalk cells and spores respectively. In stalks 31 and 29 kDa antigens appear, while in spores an 82 kDa antigen is first detected.

The 30 kDa antigen is almost certainly PsA since both glycoproteins migrate together on gels following partial purification from membranes and subsequent WGA-affinity chromatography. Both glycoproteins are identically regulated during development and associated with pre-spore and immature spore cells. PsA is actually lost during spore cell maturation [43] but low levels of the 30 kDa antigen were still present in our spore preparation.

WGA-purified 90 kDa antigen and PsB co-migrate on gels, however, PsB is thought to be a soluble internal pre-spore protein [34,44] whereas the 90 kDa antigen is present in crude plasma membrane fractions and both spores and stalk cells. The 35 kDa antigen is stalk-specific and probably the small st35 protein identified by others [45]. This latter protein first appears at the tipped aggregate stage in prestalk cells and is tightly bound to the cells. Whether it has a surface location is unknown.

Since cell adhesion proteins have been implicated in

cell-sorting we determined whether any of the TK1 antigens were absent or modified in a cell sorting-mutant. Slug cells of the KYH-13 temperature-sensitive mutant that is defective in cell-sorting gave a number of TK1-specific bands in the 85–100 kDa region, i.e. showed a much more complex pattern than NC4 wild type (not shown) or Ax2-B cells. The 35 kDa and PsA bands were unchanged. No dramatic differences were found between the banding patterns at permissive and restrictive temperatures in the mutant. However, if for example abnormal glycosylation of the 95 and/or 90 kDa antigens occurs, their adhesion properties may be slightly impaired at the higher temperature and cell sorting disturbed.

Cyclic AMP induced the appearance of only two TK1 antigens, 90 and 82 kDa, when aggregation-competent cells were shaken in buffer for 16 h. The genes induced by high cAMP levels in such suspension cultures generally code for prespore-specific proteins [46,47] and indeed we found 90 and 82 kDa TK1 antigens associates with spores. The two antigens were only weakly induced by N^6-aminohexyl cAMP which activates the cAMP-dependent protein kinase whereas 2′-deoxy-cAMP, which does not activate the kinase but binds to the cell surface receptor, induced the two antigens as strongly as cAMP. Hence, cAMP induction is apparently via binding to cell surface receptors [48]. Hence, cAMP alone is insufficient to induce the 95, 35 and 30 kDa TK1 antigens.

PsA is the only one of the four slug TK1 antigens so far known to be associated with the cell surface. Like csA, PsA is attached to the plasma membrane via a glycosyl-phosphatidylinositol link and is selectively expressed on pre-spore cells [49]. Hence, PsA is a likely candidate for a slug cell adhesion molecule.

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