The EMBO Journal Vol.2 No.6 pp.935–940, 1983

Monoclonal antibody characterisation of slime sheath: the extracellular matrix of *Dictyostelium discoideum*

Warwick N.Grant* and Keith L.Williams

Max-Planck-Institut für Biochemie, D-8033 Martinsried bei München, FRG

Communicated by D.Oesterhelt
Received on 14 March 1983

Proteins can be extracted from the slime sheath of *Dictyostelium discoideum* slugs by denaturing agents. A subset of these proteins is also released by cellulase digestion of the sheath, implying that protein-protein and protein-cellulose interactions are involved in sheath protein retention. It seems probable that the cellulose-associated sheath proteins are also associated with the cellulose of mature stalk cells. Monoclonal antibodies directed against sheath demonstrate extensive sharing of antigenic determinants between sheath proteins and a limited degree of antigenic sharing between sheath and slug cell proteins. All of the proteins recognised by these monoclonal antibodies are developmentally regulated. These results are discussed in terms of the structure of the sheath and its possible role(s) in *D. discoideum* development.

Key words: Dictyostelium discoideum/extracellular matrix/monoclonal antibody/protein modification/slime sheath

Introduction

In response to starvation, the vegetative amoebae of the cellular slime mould *Dictyostelium discoideum* form aggregates composed of 10^3-10^5 cells. Depending upon environmental conditions (Newell *et al.*, 1969; Bonner *et al.*, 1982) and genotype (Sussman *et al.*, 1978; Smith and Williams, 1980) these aggregates may either immediately form a fruiting body consisting of spore, stalk and basal disc cells or embark upon a period of migration. The cells of the migratory stage (pseudoplasmodium or slug) are partially differentiated into pre-stalk, pre-spore and pre-disc cells, arranged in that order in a linear pattern (Raper, 1940).

From late in the aggregation phase the aggregate is covered by a thin cellulose-protein layer: the slime sheath (Shaffer, 1965). Cells are unable to penetrate the sheath, so it acts as a physical barrier against the loss of cells and helps to determine the size of the aggregate by preventing the entry of new cells once the entire aggregate is covered (Shaffer, 1965). Sheath is continuously synthesised, so that during migration the slug may be envisaged as a cohesive group of cells moving through a pipe of their own making which is then left behind as a trail marking the slug's progress (Shaffer, 1965). Consequently, slug cells manufacture their own substratum for migration, a common feature of many migratory cells in higher organisms. The sheath may also act as a barrier to diffusion (Farnsworth and Loomis, 1974, 1975) i.e., as a primitive homeostatic organ, particularly to endogenously produced ammonia, a compound implicated in the switch from migration to fruiting body formation (Schindler and Sussman, 1977). One further possible role for the sheath is that a proposed gradient of modification of sheath components may provide positional information to the cells of the slug, which may be used in decisions regarding polarity and patterning (Ashworth, 1971; Loomis, 1972; Watts and Treffry, 1975).

The structure of the sheath is largely undetermined. It has been shown to consist of randomly arranged cellulose fibrils of 10–15 nm diameter embedded in an amorphous protein matrix (Hohl and Jehli, 1973). Freeze and Loomis (1977) have isolated and characterised an urea-SDS insoluble component which was largely cellulose with a small amount of low mol. wt. peptides. The use of urea-SDS would, however, remove most of the protein present and more recent work (Smith and Williams, 1979) has shown the association of a large number of proteins with the sheath. These proteins comprise ~50% of the dry weight of the sheath. In terms of its composition then, the slime sheath of *D. discoideum* is not truly 'slime'. It is biochemically and physically dissimilar to bacterial slimes and the mucous of acellular slime moulds, which are largely mucopolysaccharide (Wolf *et al.*, 1981), and may be more analogous to the covering sheaths of animal

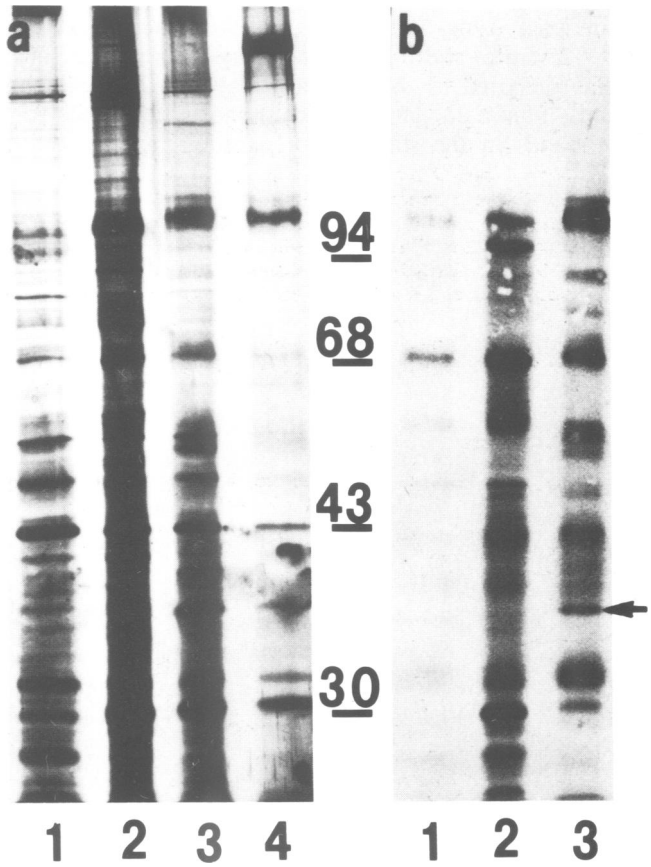

Fig. 1. (a) Silver staining of SDS-PAGE of: **(1)** SDS-extracted slug cell proteins, **(2)** slug cell crude membrane proteins, **(3)** SDS-extracted sheath proteins, **(4)** SDS-extracted proteins from the trails of 10 individually transferred slugs. **(b)** ConA-peroxidase staining of: **(1)** SDS-extracted slug cell proteins, **(2)** slug cell crude membrane proteins, **(3)** SDS-extracted sheath proteins.

*To whom reprint requests should be sent.

embryos (e.g., sea urchin hyaline, McClay and Fink, 1982). In this paper we present data relating to the extraction of the sheath-associated proteins and a characterisation of them using monoclonal antibodies. The results are discussed in terms of the possible roles of the sheath, its associated proteins and modifications to them in *D. discoideum* development.

Results

Release of sheath proteins

Comparison of proteins released by SDS from the sheath and slug cells using SDS-polyacrylamide gel electrophoresis (SDS-PAGE) shows many quantitative and some qualitative differences (Figure 1a, lanes 1 – 3). Many cellular proteins are greatly reduced or absent in sheath while others are apparently present in large amounts. A comparison of Concanavalin A (ConA)-binding sheath and slug cellular proteins (Figure 1b) also shows qualitative and quantitative differences between sheath and slug cells. In particular, there is a ConA-binding sheath protein of ~38 kd (arrow, Figure 1b) which has no counterpart in the slug cellular ConA-binding proteins. A comparison of sheath proteins released by 8.0 M urea and 6.0 M guanidine chloride with SDS-released sheath proteins showed no differences (data not shown), although release by urea is much less efficient than by SDS.

It was possible that the many similarities between sheath and slug cell extracts may be due to cellular contamination of the sheath, so a means was sought to reduce this possibility. The sheath from young, individually transferred NP84 slugs is essentially free from cells (Smith and Williams, 1979, 1981). Comparison, using SDS-PAGE, of the proteins extracted from ~25 cm of sheath from such slugs with that of sheath proteins prepared in the standard way (see Materials and methods, Figure 1a, lanes 4 and 3) shows that while some minor bands in the standard preparation may be due to cellular contamination, the two preparations are essentially the same.

Cellulase release of sheath proteins

Given that the sheath is a complex of cellulose and proteins (Hohl and Jehli, 1973), enzymatic degradation of the

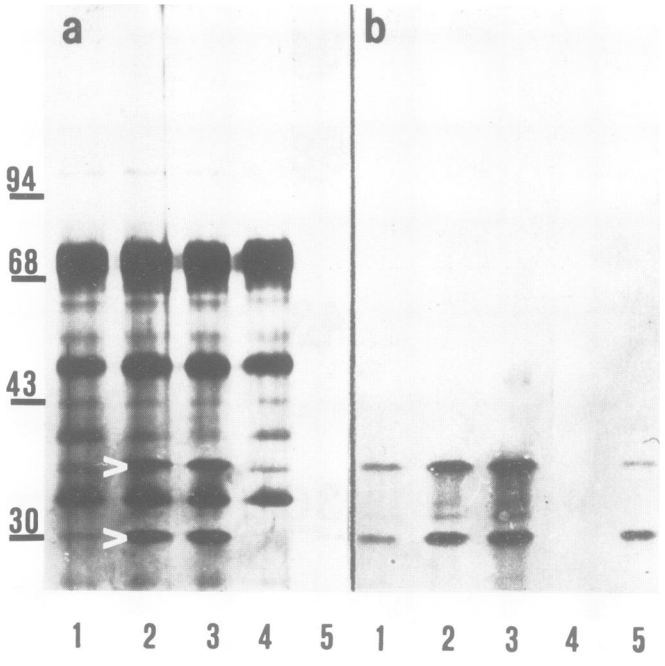

Fig. 2. Cellulase release of sheath proteins. **(a)** Silver staining. Sheath proteins are arrowed. **(b)** mAb (MUD50) staining of immunoblotted material from **(a)**. **(1)** Sheath + enzyme, 0 time, **(2)** sheath + enzyme, 30 min incubation, **(3)** sheath + enzyme, 60 min incubation, **(4)** enzyme alone, 60 min incubation, **(5)** sheath alone, 60 min incubation. Note: mAb staining is much more sensitive than silver staining and shows a low level of release of 38-kd and 30-kd proteins without enzyme **(b, lane 5)**.

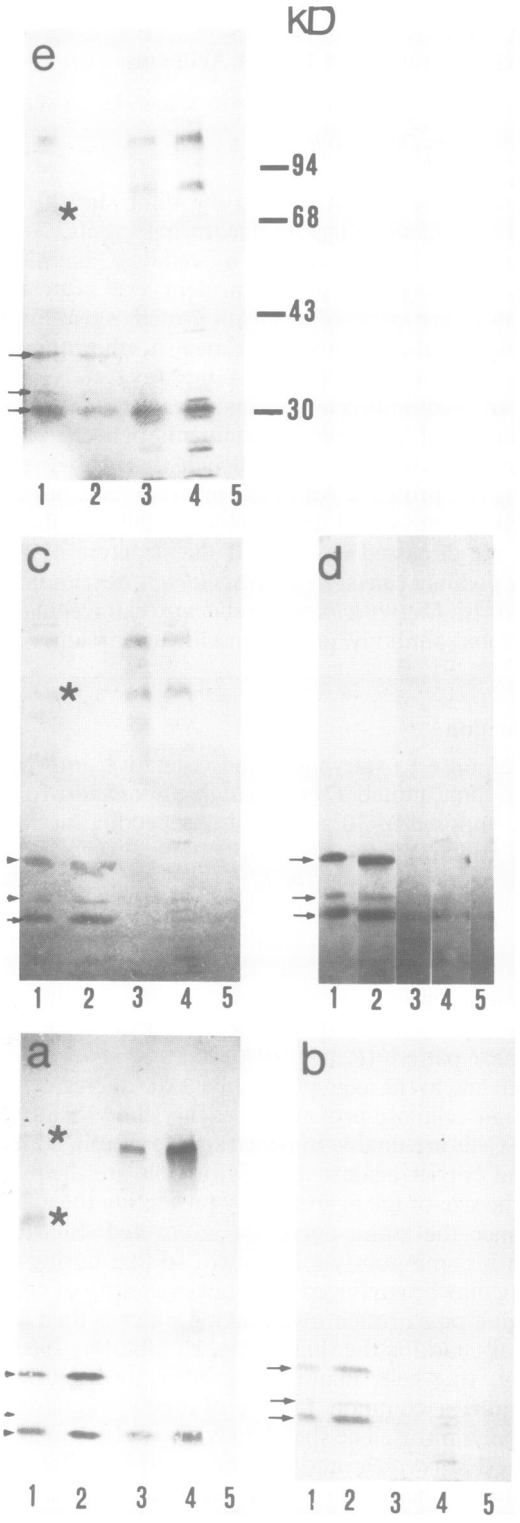

Fig. 3. Staining of immunoblots with mAbs **(a)** MUD50, **(b)** MUD51, **(c)** MUD52, **(d)** MUD53, **(e)** MUD54. **(1)** SDS-extracted sheath proteins, **(2)** cellulase-extracted sheath proteins, **(3)** SDS-extracted slug cell proteins, **(4)** slug cell crude membrane proteins, **(5)** SDS-extracted vegetative amoebae proteins. Arrows indicate proteins of 30 kd, 32 kd and 38 kd. Asterisks denote proteins not released by cellulase.

cellulose component of the sheath with cellulase enzymes should release any proteins which are retained by cellulose-protein interactions. Figure 2 shows that such proteins do exist and are released by cellulase treatment of the sheath. Comparison of lane 5 (sheath alone), lane 4 (enzyme alone) and lanes 1–3 (sheath plus enzyme) in Figure 2a shows that at least two proteins (at ~30 kd and 38 kd, arrows lane 2) are released in a time- and enzyme-dependent fashion. This is made clearer in Figure 2b, which shows monoclonal antibody staining of the same material (see also below). In addition to the 30-kd and 38-kd proteins, other sheath proteins are also released by cellulase. The pattern of protein release was reproducible using cellulase enzymes from a wide variety of sources and an extensively purified cellulase (data not shown). Pre-incubation of enzyme and sheath with the protease inhibitors pepstatin (at 25 μg/ml) and phenylmethylsulphonyl fluoride (PMSF, 2 mM) did not alter the pattern of protein release by either cellulase or denaturing agents.

The apparent carbohydrate association of cellulase-released sheath proteins raised the possibility that they may be related to discoidin (a family of endogenous *D. discoideum* lectins, Bartles and Frazier, 1982). Purified discoidin (a gift from G. Gerisch) neither co-migrated with the cellulase-released proteins in PAGE nor did it react with a range of monoclonal antibodies against sheath proteins (see below).

Monoclonal antibodies identifying sheath proteins

The differences between sheath and cellular extracts shown in Figure 1 suggest the association of a number of proteins with the slime sheath. Unequivocal demonstration of sheath specificity requires, however, a specific probe. Monoclonal antibodies (mAbs) provide such a probe.

Figure 3 shows the pattern of recognition by five mAbs raised against urea-extracted sheath proteins and reacted against immunoblots of SDS-PAGE of proteins from a variety of sources. Sheath-associated proteins are recognised by all of the antibodies (Figure 3a–e, lane 1). In particular, proteins of ~30 kd, 32 kd and 38 kd (arrowed) are recognised by all of the antibodies.

Table I. Summary of the mAb recognition of proteins on immunoblots of sheath and cellular extracts

mAb	Sheath[a]	Slug cells	Vegetative cells
MUD50	106*, 85, 75–79 50*, 38, 32, 30*, 26*	115, 107*, 95–100 88, 73, 52, 50* 45, 42, 30*, 26*	no reaction
MUD51	38, 32, 30	only extremely weak reaction	no reaction
MUD52	124, 105, 97–100* 78*, 60, 38, 32, 30	97–100*, 78–80* 70, 46, 38, 32, 30	no reaction
MUD53	38, 32, 30	no reaction	no reaction
MUD54	107*, 103*, 73–76, 38, 32, 30*	108*, 103*, 79–82 70, 43, 30*, 27, 26	no reaction

The numbers refer to the apparent mol. wt. (kd) of each protein and are calculated from Figure 3. Proteins marked with * are possibly common to both sheath and slug cells (see text).

[a]The 38-kd, 32-kd and 30-kd proteins recognised by all mAbs in sheath extracts are the same for each antibody. When sheath proteins bound to a MUD50 affinity chromatography column were immunoblotted against MUD51, MUD52, MUD53 or MUD54, proteins of 38 kd, 32 kd and 30 kd were stained in all cases. This implies that the same proteins are recognised by all mAbs (Grant and Williams, in preparation). It also implies that the 38-kd, 32-kd and 30-kd proteins share multiple antigenic determinants.

When reacted with immunoblots of cellulase-released sheath proteins, all of the antibodies again recognise the 30-kd, 32-kd and 38-kd proteins (Figure 3a–e, lane 2). However, not all SDS-released proteins are released by cellulase (Figure 3a,c,e; lanes 1 and 2). In particular, proteins in Figure 3a, c and e, lane 1 marked with an asterisk are not released by cellulase.

Slug cell proteins are also recognised by the mAbs (especially MUD50, MUD52, MUD54, Figure 3a, c and e, lane 3) despite the fact that they were raised in response to sheath extracts (see Materials and methods). Virtually all of these proteins are membrane associated (Figure 3a–e, lane 4) and MUD50 is strongly positive against intact slug cells in a FACS-IV cell sorter (data not shown), implying that at least some of the membrane proteins are at the cell surface.

Of particular interest is that both MUD50 and MUD54 strongly recognise a 30-kd protein in sheath (Figure 3a,e; lane 1) and slug cell (Figure 3a,e; lanes 3,4) extracts while the other mAbs show no or very faint recognition of a 30-kd protein in cellular extracts. This is a clear instance of mAb recognition of proteins of the same apparent mol. wt. in both sheath and slug cells. There are other cases of mAb recognition of proteins with similar mol. wts. in both extracts (see Table I) but,

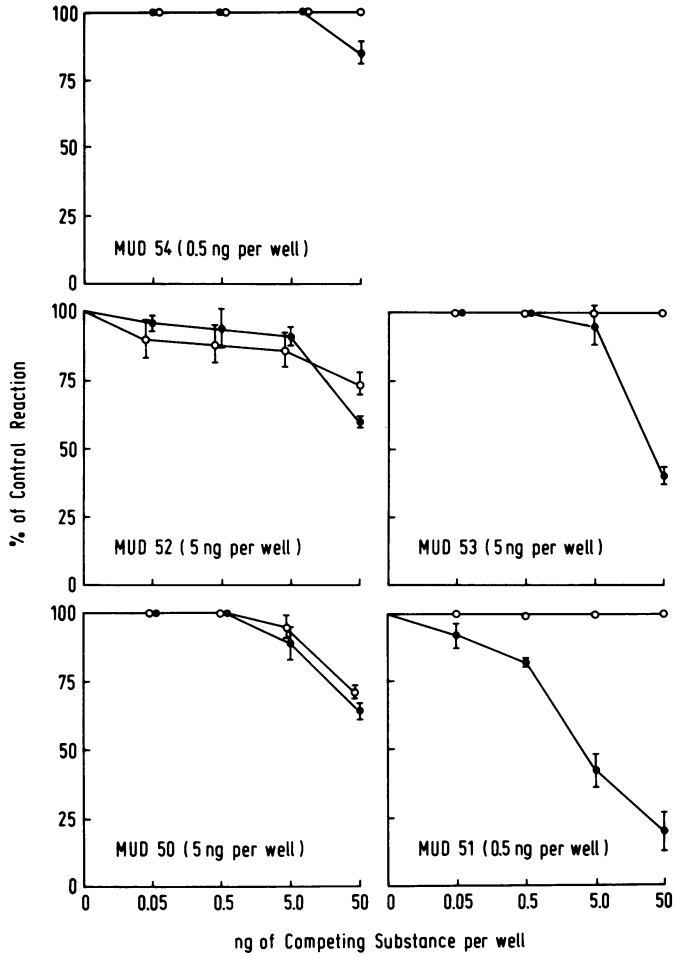

Fig. 4. Competition ELISA. The control (no competing antigen) absorbance was taken as 100% and absorbance of wells with competing antigens expressed as a percentage of this value. For competing antigens, the amounts added refer to: (i) the amount of protein for undigested mateial; or (ii) the amount of protein before Pronase digestion for the glycopeptide fraction. Closed circles: sheath proteins as competing antigen; open circles: sheath glycopeptides as competing antigen. Bars represent standard error of mean of 12 determinations.

in general, such overlap is not seen despite the apparent similarity of sheath and slug cell proteins seen in Figure 1. This lack of generally overlapping mAb recognition shows clearly that the sheath has its own protein complement and is not contaminated with cellular proteins.

One further important point is that all of the antigenic determinants recognised by the mAbs reported here are developmentally regulated, i.e., they are not detected in extracts of vegetative amoebae (Figure 3a–e, lane 5).

Nature of the antigenic determinants recognised

The nature of the antigenic determinants was examined by comparing the ability of urea-extracted sheath proteins and Pronase digests of them to inhibit the enzyme-linked immunosorbent assay (ELISA) of each mAb. The results are shown in Figure 4. Pronase digestion destroys the ability of sheath proteins to inhibit the ELISAs of MUD51 and MUD53, so these mAbs recognise Pronase-sensitive determinants. MUD50 and MUD52 are inhibited by both undigested and digested material and so recognise Pronase-insensitive determinants. MUD54 is poorly inhibited by both fractions but when higher concentrations of inhibiting material are used it also appears to recognise Pronase-insensitive determinants. To test whether inhibition by Pronase-insensitive material was due to the presence of low concentrations of undigested protein, up to 100 times the amount of Pronase-digested material required for inhibition of the ELISA was subjected to SDS-PAGE and immunoblotted. No bands were detected (data not shown). This observation, coupled with the fact that Pronase digestion does not affect the inhibitory activity of antigens in the ELISAs of MUD50 and MUD54, is consistent with the Pronase-insensitive material being glycopeptide and the antibodies inhibited by it (i.e., MUD50, MUD52 and MUD54) as recognising carbohydrate determinants. The remaining antibodies (i.e., MUD51 and MUD53) probably recognise protein determinants.

Mature stalk cells contain sheath proteins

Figure 5 shows that the pattern of recognition of the mAbs when they are reacted against immunoblots of stalk cell proteins is strikingly similar to that obtained against sheath proteins. Some new proteins are present but, more importantly, some sheath proteins are missing. In general, proteins of the same mol. wt. as cellulase-sensitive sheath proteins are found in the stalk and proteins corresponding in mol. wt. to cellulase-insensitive sheath proteins are not observed. Cellulase does not release detectable amounts of protein from stalks (data not shown).

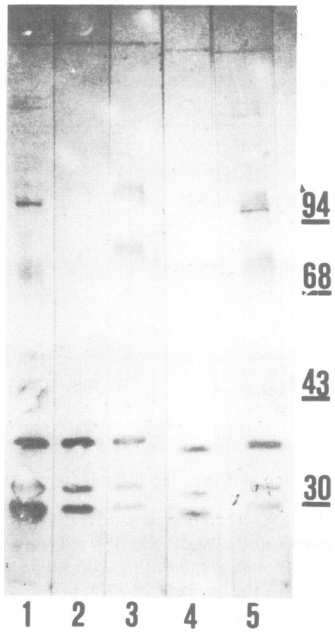

Fig. 5. Immunoblots of mAbs reacted against SDS-extracted stalk cell proteins. **(1)** MUD50, **(2)** MUD51, **(3)** MUD52, **(4)** MUD53, **(5)** MUD54.

Discussion

Hohl and Jehli (1973) have demonstrated that the sheath of *D. discoideum* slugs consists of a complex of cellulose and protein. The cellulose component of the sheath has been previously characterised (Freeze and Loomis, 1977) and work by Smith and Williams (1979) suggested that the sheath has its own complement of proteins. We have demonstrated that this is indeed the case. The ability of SDS and urea to release the sheath proteins implies that they are retained by non-covalent, conformation-dependent interactions. The release of a subset of these proteins by mild cellulase digestion divides the sheath proteins into two distinct classes: those associated with cellulose (cellulase-sensitive), and those which are not (cellulase-resistant). The 'amorphous protein matrix' noted by Hohl and Jehli (1973) which remains after cellulase digestion is presumably the cellulase-resistant fraction (marked with an asterisk in Figure 3, lane 1).

Although, under the relatively mild conditions employed here, the cellulase-mediated release of protein is not quantitative (Grant and Williams, unpublished data) the contention that cellulase-sensitive proteins are indeed cellulose-associated is supported by two further observations. Firstly, cellulase-sensitive but not cellulase-resistant proteins are associated with mature stalk cells (Figure 5). These cells have cellulose walls, the cellulose of which is chemically very similar to that of the sheath (Freeze and Loomis, 1978). The sharing of cellulose-associated proteins between these two cellulose-containing 'tissues' is consistent with and extends this similarity. Secondly, on the basis of their patterns of protein recognition, the mAbs described here recognise at least three antigenic sites. (MUD50 and MUD54 are very similar, as are MUD51 and MUD53. These mAbs may define only two antigenic sites, and MUD52 defines a third; see Figure 3 and Table I.) The 30-kd, 32-kd and 38-kd sheath proteins, which are all cellulase-sensitive, are all recognised by all mAbs (Figure 3) and so probably share multiple antigenic determinants. It has been shown in other systems (e.g., brain fodrin, Glenney *et al.*, 1982; intermediate filaments, Pruss *et al.*, 1981) that the sharing of mAb determinants is often correlated with structural and functional similarities. The shared function in this case is cellulose association.

Whether the subdivision of sheath proteins into cellulose-resistant and sensitive classes has any functional significance is unclear. Migration of slugs on cellulase-containing agar results in a switch from the slugging to the fruiting mode which is dependent upon slug size and cellulase concentration. Proteins isolated from the trails of such slugs appear antigenically altered, raising the possibility that they and/or their association with cellulose may be involved in the slugger-fruiter switch (Grant and Williams, in preparation; Smith and Williams, 1980).

Three of the mAbs described here react with slug cell as well as sheath proteins (MUD50/54 and MUD52) while two

react only with sheath (MUD51/53). It appears, therefore, that sheath proteins carry carbohydrate determinants (defined by MUD50/54 and MUD52) which are also carried by slug cell proteins and proteinaceous determinants (defined by MUD51/53) which are restricted to the sheath. However, despite this antigenic overlap and the apparent overall similarity between sheath and slug cells noted in Figure 1, there is generally little overlap in the mAb recognition of specific proteins of defined mol. wt. between sheath and slug cells (Table I). The most striking exception involves a 30-kd protein. A protein of this mol. wt. is strongly recognised by all mAbs in the sheath and by MUD50/54 but not MUD51/53 and MUD52 in slug cells (Figure 3, lanes 1 and 3). If the proteins of the sheath and cells recognised by MUD50/54 are essentially the same, then the antigenic determinants defined by MUD51/53 and MUD52 on the sheath 30-kd protein represent modifications made to the cellular 30-kd protein which are associated with its location in the sheath. It has recently been suggested (Rougon *et al.*, 1982) that in developmental systems the same polypeptide may be differentially modified according to its location. Alternatively, the sheath and cellular 30-kd proteins may be closely related but genetically distinct proteins which are differently modified according to their locations. Peptide mapping studies aimed at resolving these hypotheses are under way.

In functional and molecular terms, then, the slime sheath of *D. discoideum* slugs has many of the properties of a primitive embryonic extracellular matrix. In functional terms it may be analogous to the hyaline layer which covers sea urchin embryos from fertilisation until metamorphosis (McClay and Fink, 1982) and helps maintain the structural integrity of the embryo while providing a substratum for developmentally important cell movements. In molecular terms, the proposed modification of sheath proteins may be analogous to the extracellular processing of pro-collagen (Ramachandran and Reddi, 1976) i.e., processing may be an essential part of sheath assembly. In this case, *D. discoideum* provides a simple model system in which the role of protein modification in the assembly of an extracellular matrix can be studied. Alternatively, the possible production of extracellular and cellular forms of a protein which share many structural features is reminiscent of the occurrence of distinct cellular and extracellular forms of proteins in other systems, (e.g., fibronectin, a protein proposed to play a developmental role in a wide range of tissues and organisms, Yamada *et al.*, 1980). In either case, it is clear that the sheath is a potentially useful model system for the study of the developmental role of extracellular matrix proteins and their modification.

Materials and methods

Materials

ConA and horseradish peroxidase (R.Z. 1.6) were from Sigma, cellulase (EC 3.2.1.4, 20 mU/mg from Basidiomycetes) and Pronase from Merck, horseradish peroxidase-conjugated goat anti-mouse IgG (HRP-anti-mouse) from Tago Immunodiagnostics Inc., USA, nitrocellulose (0.45 μm pore size) from Schleicher und Schüll, Titertek 96-well ELISA plates from Linbro and mol. wt. standards [phosphorylase B, 94 kd; bovine serum albumin (BSA), 68 kd, ovalbumin, 43 kd, carbonic anhydrase, 30 kd] were from BioRad, diaminobenzidine (DAB) was from Sigma.

Preparation of sheath

Slugs of *D. discoideum* strain NP84 (North and Williams, 1978) were prepared as previously described (Smith and Williams, 1979) and allowed to migrate across the Petri plate towards a point light source for 6–7 days. By this time virtually all slugs had completely traversed the plate, leaving it covered with a layer of sheath essentially free from cells. Sheath was scraped into distilled water, thoroughly homogenised, then washed three times in distilled water by centrifugation at 1000 *g*. Following washing, the sheath was lyophilised and stored desiccated at 4°C.

In some experiments, the trails from 10 individually transferred 18–20 h old NP84 slugs (Smith and Williams, 1980) which had migrated for 15–18 h were collected. This amount of material (~25 cm of sheath) was sufficient for one electrophoresis lane.

Extraction of sheath proteins

SDS extraction was carried out in 1% w/v SDS in 125 mM Tris-HCl (pH 6.5). Urea extraction was carried out in freshly made 8.0 M urea, followed by desalting over a Bio-Gel P6 column and concentration using a Millipore PTGC ultrafiltration membrane (nominal mol. wt. cut-off, 10 kd). Both extractions were performed at 40°C for 60 min. Cellulase extraction was carried out using 0.25 mg/ml enzyme in 0.1 M sodium acetate (pH 4.5) at 40°C for 2 h unless otherwise noted (see Figure 2).

In all cases, the sheath concentration was 5 mg/ml and, following incubation, insoluble material was removed by centrifugation for 10 min in an Eppendorf benchtop centrifuge. Extraction of slug cells (from 18 h old NP84 slugs), vegetative amoebae and stalk cells (prepared according to Orlowski and Loomis, 1979) was carried out as for sheath, using 2 x 10^6 cells/ml. Crude slug cell membranes were a gift from M.Krefft.

Electrophoresis

Discontinuous SDS-PAGE was performed according to Laemmli (1970), employing 10% resolving and 4% stacking gels cast in 11.5 x 13.5 x 0.1 cm slabs. Samples were of 20 μl, containing ~5 x 10^4 cells or 20 μg sheath. Gels were run at 7.5 mA for 1 h, followed by 17.5 mA until the tracking dye was 1–2 cm from the end.

Staining was performed using a modified silver stain for protein (Morrissey, 1981) or a modification of the ConA-peroxidase procedure (Wood and Sarinana, 1975) for ConA-binding glycoproteins. For ConA-peroxidase staining, proteins were electrophoretically transferred to nitrocellulose sheets following SDS-PAGE (Towbin *et al.*, 1979), washed for 40 min in four changes (10 min per wash) of Tris-NaCl buffer (10 mM Tris-HCl, 0.9% NaCl, 0.05% w/v Tween 20) then incubated overnight in a moist chamber with 20 μg/ml ConA in Tris-NaCl containing 3% w/v BSA and 2% w/v sodium azide. The sheets were then washed for 4 x 10 min in Tris-NaCl and incubated for 2 h with 100 μg/ml horseradish peroxidase in Tris-NaCl containing 3% w/v BSA. Following four further 10 min washes in Tris-NaCl, ConA-binding proteins were visualised by incubation with DAB (in Na_2HPO_4/NaH_2PO_4 buffer, 15 mM with respect to phosphate, pH 6.5, containing 0.5 mg/ml DAB and 0.015% H_2O_2).

Immunoblotting of proteins separated by SDS-PAGE was identical to ConA-peroxidase staining except that purified hybridoma IgG at 0.2–0.5 μg/ml replaced ConA in the first step and HRP-anti-mouse replaced peroxidase in the second.

mAb production

Female Balb/C mice (6–18 weeks) were injected i.p. with 100–150 μg of urea-extracted sheath proteins in complete Freunds adjuvant, followed by a second identical immunisation 4–6 weeks later. Three days before fusion, mice were boosted i.v. with 100 μg of urea-extracted proteins in normal saline. For each fusion the spleens from two mice were pooled and the number of spleen cells kept constant at 10^7 cells per fusion with 10^7–2 x 10^7 myeloma cells (cell line Ag8-653, a non-secreting NS1 derivative obtained from T.Meo, Institut für Immunologie, Universität München). Fusion and subsequent cloning were carried out according to de St. Groth and Scheidegger (1980) and all hybrids were cloned at limiting dilution at least three times before use. Production of ascites in Balb/C mice and protein-A affinity chromatographic purification of IgG were according to Goding (1980).

ELISA of anti-sheath mAbs

Hybrids were screened for activity against sheath proteins by an ELISA. Urea-extracted sheath proteins were coupled to ELISA plates at 5–10 μg/ml in carbonate/bicarbonate buffer (pH 9.6), and the plates washed and stored according to Voller *et al.* (1979). For assay, 50 μl of culture supernatant was pipetted into duplicate wells, incubated for 1–2 h at 37°C (or overnight at 4°C) then washed three times in PBS-T (8.0 g NaCl, 0.2 g KH_2PO_4, 2.9 g Na_2HPO_4, 2 g KCl/l plus 0.05% w/v Tween 20, pH 7.2). Then, 50 μl of HRP-anti-mouse was added to each well and incubated for 1 h at 37°C, after which three further PBS-T washes were conducted. Antibody was detected using *p*-orthophenylenediamine as a substrate (Voller *et al.*, 1979) and the reaction stopped with H_2SO_4 when positive controls reached an absorbance of ~1.0. Absorbances were read using a MicroELISA reader (Dynatech) at 488 nm. Background absorbance was generally 0.01–0.02 absorbance units and wells with an absorbance >0.1 were considered positive.

To determine whether the monoclonal antibodies recognised a protein or the carbohydrate portion of a glycoprotein, a modification of the assay

described above was developed. A glycopeptide fraction of urea-extracted sheath proteins was prepared by exhaustive Pronase digestion (Spiro, 1972). Various amounts of either undigested protein or glycopeptides were then added at the same time as purified IgG in the first step of the assay, so that the antigens in solution would compete with those bound to the plate for antibody and thus decrease the amount of antibody bound and the final absorbance (see Figure 4).

Acknowledgements

We thank Dr.M.Krefft for assistance in obtaining the mAbs and for the FACS-IV data, and A.Kühnlein and S.Düthorn for typing the manuscript.

References

Ashworth,J.M. (1971) *Symp. Soc. Exp. Biol.,* **25**, 27-47.
Bartles,J.R. and Frazier,W.A. (1982) *Int. Rev. Cytol.,* **75**, 61-99.
Bonner,J.T., Davidowski,T.A., Hsu,W.-L., Lapeyrolerie,D.A. and Suthers, H.L.B. (1982) *Differentiation,* **21**, 123-126.
de St. Groth,S.F. and Scheidegger,D. (1980) *J. Immunol. Methods,* **35**, 1-21.
Farnsworth,P.A. and Loomis,W.F. (1974) *Dev. Biol.,* **41**, 77-83.
Farnsworth,P.A. and Loomis,W.F. (1975) *Dev. Biol.,* **46**, 349-357.
Freeze,H. and Loomis,W.F. (1977) *J. Biol. Chem.,* **252**, 820-824.
Freeze,H. and Loomis,W.F. (1978) *Biochim. Biophys. Acta,* **539**, 529-537.
Glenney,J.R., Glenney,P. and Weber,K. (1982) *Proc. Natl. Acad. Sci. USA,* **79**, 4002-4005.
Goding,W. (1980) *J. Immunol. Methods,* **39**, 285-308.
Hohl,H.R. and Jehli,J. (1973) *Arch. Mikrobiol.,* **92**, 179-187.
Laemmli,U.K. (1970) *Nature,* **227**, 680-685.
Loomis,W.F. (1972) *Nature New Biol.,* **240**, 6-9.
McClay,D.R. and Fink,R.D. (1982) *Dev. Biol.,* **92**, 285-293.
Morrissey,J. (1981) *Anal. Biochem.,* **117**, 307-310.
Newell,P.C., Telser,A. and Sussman,M. (1969) *J. Bacteriol.,* **100**, 763-768.
North,M.J. and Williams,K.L. (1978) *J. Gen. Microbiol.,* **107**, 223-230.
Orlowski,M. and Loomis,W.F. (1979) *Dev. Biol.,* **71**, 297-307.
Pruss,R.M., Mirsky,R., Raff,M.C., Thorpe,R., Dowding,A.J. and Anderton,B.H. (1981) *Cell,* **27**, 419-428.
Ramachandran,G.N. and Reddi,A.H. (1976) *Biochemistry of Collagen,* published by Plenum Press, NY.
Raper,K.B. (1940) *J. Elisha Mitchell Sci. Soc.,* **59**, 241-282.
Rougon,G., Deagostini-Bazin,H., Hirn,M. and Goridis,C. (1982) *EMBO J.,* **1**, 1239-1244.
Schindler,J. and Sussman,M. (1977) *J. Mol. Biol.,* **116**, 161-169.
Shaffer,B.M. (1965) *J. Embryol. Exp. Morphol.,* **13**, 97-117.
Smith,E. and Williams,K.L. (1979) *FEMS Microbiol. Lett.,* **6**, 119-122.
Smith,E. and Williams,K.L. (1980) *J. Embryol. Exp. Morphol.,* **57**, 233-240.
Smith,E. and Williams,K.L. (1981) *J. Embryol. Exp. Morphol.,* **61**, 61-67.
Spiro,R.G. (1972) *Methods Enzymol.,* **28**, 3-42.
Sussman,M., Schindler,J. and Kim,H. (1978) *Exp. Cell Res.,* **116**, 217-227.
Towbin,H., Stahelin,T. and Gordon,J. (1979) *Proc. Natl. Acad. Sci. USA,* **76**, 4350-4354.
Voller,A., Bidwell,D.E. and Bartlett,A. (1979) *The Enzyme Linked Immunosorbent Assay,* Available from Dynatech.
Watts,D.J. and Treffry,T.E. (1975) *FEBS Lett.,* **52**, 262-264.
Wolf,K.V., Hoffmann,H.-U. and Stockene,W. (1981) *Protoplasma,* **107**, 345-359.
Wood,J.G. and Sarinana,R.J. (1975) *Anal. Biochem.,* **69**, 320-322.
Yamada,K.M., Aden,K. and Hahn,L.-H.E. (1980) *Symp. Soc. Dev. Biol.,* **38**, 43-77.