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Spore Coat Is Altered in *mod*B Glycosylation Mutants of *Dictyostelium discoideum*

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The modB mutation eliminates specific carbohydrate epitopes from glycoproteins which are expressed primarily in prespore and spore cells of differentiating *Dictyostelium discoideum*. Spores formed by the mutant show several phenotypes. Whereas mutant spores germinate efficiently after heat activation, they germinate poorly after urea activation. Following germination, at least one glycosylation-defective glycoprotein is cleaved, and the larger fragment is released in soluble form from the spore coat. However, an earlier difference in the spore coat can be traced back to the nongerminated spore coat, as detected by the elutability of protein from intact spores by chemical extraction. An altered character of the pregermination spore coat is also suggested by increased labeling by a fluorescent lectin which binds to its interior. The findings are consistent with a change in the character of certain molecular contacts leading to altered characteristics of the mutant spore coat, which are specific because they are distinctive from changes observed in another glycosylation mutant which affects a different epitope.

Key words: cellular slime mold, spore germination

The *mod*B mutation results in the loss of two (or more) carbohydrate epitopes which are predominantly expressed during and after cell aggregation in *Dictyostelium discoideum* [1,2]. The epitopes are expressed on numerous proteins in both prespore and spore cells. In aggregating cells, one of the *mod*B-dependent epitopes indirectly supports intercellular adhesion. In its absence, gp80, a homophilic cell adhesion molecule, fails to accumulate at the cell surface [3] and instead is proteolytically digested intracellularly and the fragments secreted [4]. As a result, intercellular adhesion is reduced, which

Abbreviations used: FITC-, conjugated with fluorescein isothiocyanate; GA, glycoantigen; gal/galNAc polysaccharide, a polysaccharide consisting of galactose and N-acetyl-galactosamine as primary components; mAb, monoclonal antibody; RCA-I an isoform of *Ricinus communis* agglutinin.

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explains, presumably, the observed smaller size of fruiting bodies in *mod*B mutants [5]. In slugs, carbohydrate associated with the epitope may be involved in generating traction relative to the slime sheath, because slug migration is deficient in *mod*B mutants [6]. In association with prespore cells, the epitope may be involved in cell positioning [7]. Cells which lack the epitope as a result of mutation sort to the anterior end of chimeric slugs where prestalk cells normally reside. Prestalk cells express the epitope only weakly.

These two *mod*B-dependent epitopes (glycoantigens-XI and -XX), together with a previously described third epitope referred to as the fucose epitope (GA-X), are also expressed in the spore coat matrix which surrounds spores [8,9]. Through study of a mutant which does not express the fucose epitope, fucose has been shown to contribute to the structural integrity of the spore coat [10]. Mutant spores fail to germinate after aging, underfucosylated proteins are proteolytically cleaved, and the spore coat is more accessible to labeling by a fluorescent lectin.

The association of *mod*B-dependent epitopes with glycoproteins of the spore coat led us to consider whether a functional role for protein-linked carbohydrate might be ascertained from analysis of *mod*B mutants. Several independent isolates of this mutation have been recovered, and two diploid strains have been constructed from noncomplementing alleles [5]. These diploids are useful because recessive mutations which may have accrued in these stocks should not be expressed.

MATERIALS AND METHODS

Spores

The diploid strains DL118 (*mod*B501/*mod*B503) and DL119 (*mod*B502/*mod*B503), which are homozygous for recessive *mod*B mutations, were compared with the heterozygous DL117 strain (*mod*B503/+) in these experiments. We refer to the heterozygous strain (DL117) as normal, though we have not ruled out the possibility of subtle differences between DL117 (which is genetically the most closely related 'normal' strain available) and a homozygous diploid as a result of a gene dosage effect. We have determined, however, that DL117 and HL100, a haploid (*mod*B$^+$) parental normal strain, have similar quantities per spore coat of spore coat protein (determined by dye-binding as in Table II) and similar levels of the proteins SP96, SP85 (detected by means of its *mod*B-dependent epitope), SP75 and SP70, as determined by immunoprobing of Western blots (not shown). These strains have been previously described [5]. Sorocarps were allowed to form after exhaustion of the food source *Klebsiella aerogenes* on SM agar. Spores were harvested from 1- or 7-day-old sorocarps either mechanically by abrupt inversion of petri plates on the counter or by suspending the entire culture in 0.02% NP-40 in 10 mM KH_2PO_4 adjusted to pH 6.5 with NaOH (KP/NP-40) for 15 min at 22°C. Spores were recovered from the KP/NP-40 solution by filtration through a 120 mesh screen and two successive centrifugations at 800*g* for 1 min in KP. Since no differences were detected between the two types of spores in this study, the detergent method was most often used owing to higher yields of recovery and reduced contamination by amoebae.

Germination was induced by immersion of a tube of spores in a 45°C water bath for 30 min or by exposure of spores to an 8 M urea solution for 30 min at 22° [11] followed by return to KP and gyration at a rate sufficient to keep the spores in suspension. Germination efficiency was measured by calculating the ratio of (spore coats): (total spores + spore coats) at each time point in the presence of Calcofluor White ST in a

hemacytometer viewed epifluorescently through a Nikon V filter kit. As previously noted [10], this method yields a 50–100% higher value for germination efficiency compared to the ratio of spore coats:input spore number.

Spore Coat Purification

Spores were suspended at 5×10^7/ml in KP, heat activated in a 45°C water bath for 30 min, diluted 2.5-fold in KP, and shaken at 22°C until germination was maximal as observed by calcofluor-induced fluorescence (ca. 12 h). Most amoebae and spores were removed by centrifugation at 800*g* for 40 s. Spore coats were pelleted at 21,000*g* for 18 min, resuspended in KP, and then centrifuged at up to 10^9 spore coats on a 16 ml linear gradient of Renografin-76 (Squibb), ranging from 40% (v/v in H_2O) to 100%, at 23,000*g* for 20 min. These conditions were isopycnic as determined by recentrifugation. Early experiments were performed by loading the sample on the top of the gradient, but later it was found that yields were nearly quantitative if the sample was premixed with the renografin solutions prior to pouring the gradient. The spore coat layer was visualized about ⅔ of the distance down the gradient by light scattering, removed by a syringe, diluted and recovered by centrifugation in KP, and stored at −80°C. Mutant spore coats were always slightly more dense than normal spore coats. Contamination by any spores or other cells as assessed microscopically was unusual, but was always less than 0.1%.

Biochemical Analyses

Protein was estimated either by a Coomassie blue dye-binding assay [12] or the method of Lowry et al. [13], using bovine serum albumin as a standard. Total acid-insoluble polysaccharide was assayed as cellulose as previously described [14] on samples consisting of $1–5 \times 10^8$ spore coats. Whatman CC31 cellulose powder was used as a standard. For examination of individual protein species, spores and/or spore coats were boiled for 3 min in 6 M urea, 1% (v/v) 2-mercaptoethanol, diluted 1:1 with 2×SDS-sample buffer, electrophoresed on discontinuous SDS- polyacrylamide gradient gels, and were visualized by Coomassie blue dye binding [2]. In some cases protein was electrophoretically transferred to nitrocellulose and immunoprobed by an indirect technique using an alkaline phosphatase-coupled second antibody [2].

Electron Microscopy

Spores were fixed in a mixture containing 1% glutaraldehyde, 4% freshly prepared paraformaldehyde, and 50 mM L-lysine (free base) in 0.1 M Na cacodylate buffer (pH 7.2). Following dehydration in ethanol they were embedded in Lowicryl K4M at room temperature according to reference 15. Thin sections mounted on Formvar-coated nickel grids were immunolabeled as previously described [2]. Labeling with RCA-I-gold complexes and enzyme affinity labeling with cellulase-gold were as described in reference 9.

RESULTS

Spore Germination

DL118 and DL119 (mutant) spores germinated to the same extent as did DL117 (normal) spores after heat activation (Table I). Thus mutant spores can germinate normally. When mutant spores were instead exposed to 8 M urea for 30 min, however,

TABLE I. Germination of Mutant and Normal Spores*

Strain	Time (h) after activation	% germination	
		Heat activation	Urea activation
DL117 (normal)	7	48	22
	10	52	—
	12	—[a]	32
	16	59	—
	22	—	51
DL118 (mutant)	7	—	9
	12	53	8
	16	51	—
	22	—	9
DL119 (mutant)	7	38	4
	10	52	—
	12	—	11
	16	61	—

*One-day-old spores were harvested as described, activated as indicated, and allowed to germinate in suspension culture. % germination was determined as the No. of spore coats divided by the total No. of spores and spore coats counted at each time point. The sample was diluted in 0.2% calcofluor white ST and viewed under epifluorescence optics to facilitate detection.

[a]—, not determined.

germination was reduced to less than 20% of normal levels. Activation or swelling, the first step in germination [16], was not blocked as determined in the phase microscope. In contrast, germination efficiency of DL117 was unaffected though it was slightly slower. Inhibition of mutant spore germination was observed even if spores were washed free of urea and then heat-activated (not shown). Thus mutant spores were not simply non-responsive to urea activation. These results suggested that mutant spores are sensitive to toxic effects of urea.

Effect of the *mod*B Mutation on Spore Coat Proteins of Intact Spores

As a first step in exploring the basis for the urea sensitivity of mutant spores, we examined how protein glycosylation was altered in mutant spores. Two epitopes, GA-XI (mAb 16.1) and GA-XX (mAb 54.2), are missing from the spore as a result of the *mod*B mutation [2,3 and Fig. 1]. Missing epitopes reported by other authors are most likely

TABLE II. Protein and Cellulose Content of Mutant and Normal Spore Coats*

Strain	Protein (pg/spore coat)			Cellulose (pg/spore coat)
	Dye-binding	Dye-binding (after extraction)	Lowry	
DL117 (normal)	0.58	1.2	3.4	2.7
DL118 (mutant)	0.64	0.90	3.3	2.0
DL119 (mutant)	0.62	1.2		2.6

*Protein content of spore coats was determined using either a Coomassie blue dye-binding method [12] or the method of Lowry et al. [13]. Bovine serum albumin served as the standard. In one trial, the spore coats were extracted, as indicated, by boiling for 3 min in 6 M urea, 6% (v/v) 2-mercaptoethanol. The entire mixture was then assayed. Other values are the means of 2 or more trials. The range of values was less than 20% of the mean value.

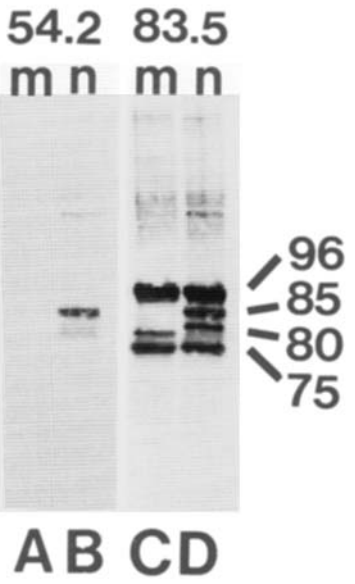

Fig. 1. Spore coat proteins of the *mod*B mutant. Spores were boiled in SDS-sample buffer under reducing conditions, electrophoresed, and Western blotted. The blot was probed first with mAb 54.2 (**left**) and secondly with mAb 83.5 (**right**). DL119 (m = mutant) is shown on the left and DL117 (n = normal) on the right of each pair. Proteins are identified by their apparent MW in kD as indicated in the margins. SP85, SP80, and an unidentified smaller species are labeled with mAb 54.2, though only SP85 produces a strong reaction. This protein also reacts specifically with mAb 16.1 (not shown). SP96, SP80, and SP75 are labeled by mAb 83.5, as observed by post-labeling of the same blot with mAb 83.5. Labeling with mAb 54.2 is not observed in DL119 because this antibody recognizes a *mod*B-dependent epitope. SP80, which carries epitopes recognized by both antibodies, can be observed to be reduced in apparent MW by 2–3 kD in DL119 as determined by labeling with mAb 83.5.

related to GA-XI and GA-XX based on the similar proteins which react with the various antibodies [5,6]. GA-XI is found in the inner layer of the spore coat of spores, as determined by EM immunocytochemistry, on a single species, SP85, as determined by Western blotting of spores or spore coats [2,8,9]. GA-XX is found both in the outer layer of the spore coat and in association with intracellular vesicles [2,8,9]. The predominant species recognized by mAb 54.2 is SP85, a spore coat protein which is also recognized by mAb 16.1. A second reactive protein is SP80, a spore coat protein which also expresses GA-X (mAb 83.5) (Fig. 1). Consistent with its expression of a *mod*B-dependent epitope, SP80 is reduced in apparent MW by 2–3 kD in the mutant, as determined by subsequent labeling of the same Western blot with mAb 83.5 (Fig. 1). SP85 could not be observed owing to a lack of an antibody to recognize this protein in *mod*B mutants.

On a per spore basis as determined by Western blotting, SP70 (detected with Ab 2H3) [18] (not shown), SP75 (mAb 83.5), and SP96 (mAb 83.5 and mAb A6.2) were present at similar levels in spores of DL117, DL118, and DL119 (Fig. 1). SP80 was also detected at similar specific levels, though in the experiment shown (Fig. 1), this is concealed by prelabeling of the DL117 form with mAb 54.2 (Fig. 1). A polysaccharide containing galactose and N-acetyl-galactosamine (gal/galNAc polysaccharide) was found at similar specific levels as determined by overlay of SDS-gels with FITC-RCA-I (as performed in Fig. 1K of ref. 9; data not shown). DL117 and DL118 spores were also

compared by EM immunocytochemical labeling of thin sections [8,9]. mAb 83.5, RCA-I/gold and cellulase/gold labeled the spore coats indistinguishably. Hence not only were the glycoproteins and polysaccharides examined present at similar specific levels in spores, they were also associated with the proper laminae of the spore coat.

Composition of Spore Coats Released From Spores by Germination

The sensitivity of mutant spore germination to urea, coupled with the absence of *mod*B-dependent epitopes from proteins of the mutant spore coat, led to a closer examination of the mutant spore coat. Mutant and normal spore coats purified after germination showed no gross morphological differences by phase contrast microscopy (not shown). Total protein of mutant spore coats isolated after germination, measured by a Coomassie blue dye-binding assay [12], was within 10–20% of values for normal spore coats (Table II). The protein content of the spore coat estimated by the dye-binding assay was about fivefold smaller than that estimated by the method of Lowry et al. [13]. In an attempt to reconcile this difference, protein was extracted from the coats by boiling in 6 M urea and 6% 2-mercaptoethanol (see below). This treatment doubled the amount of protein measured using the dye-binding assay (the presence of reducing agent precluded utilization of the Lowry method for comparison). Regardless of the method used, protein values were found to be similar between normal and mutant coats. Similarly, total cellulose was only slightly reduced in the mutant (Table II). The value observed was about twice that previously reported [17], which may be a result of the diploid nature of these spores. The gal/galNAc polysaccharide appeared to be expressed normally as determined by probing dot blots of urea/SDS/2-mercaptoethanol-extracts of spores with biotinylated RCA-I and avidin alkaline phosphatase, and measuring lactose-sensitive optical density (data not shown). Spore coats were examined on SDS gels to determine whether any individual protein species were altered in level. The protein profile of normal spores was similar to previous reports [19,10] (data not shown). In contrast to findings from intact spores (Fig. 1), the level of SP80 was reduced in mutant spore coats isolated after germination, as determined by probing with antibody 83.5 (see below). A major Coomassie blue-binding protein [19,21], SP60, was similar between mutant and normal coats (not shown). Other major protein species (SP96, SP75, and SP70) were found at normal levels compared to DL117. The reduced amount of SP80 observed in the mutant spore coat after germination contrasted with the normal amount found in intact mutant spores, and with the normal of amounts of other proteins and polysaccharides found in mutant spore coats.

Disappearance of SP80

The expression of SP80 was examined more closely to shed light on the process of its disappearance from the spore coat during germination. A time course of germination (activated by heat) revealed SP80 to decrease gradually in level in the mutant but remain constant in normal spore coats (Fig. 2). The decrease in DL119 followed emergence of amoebae from the spore coat. Analysis of the germination medium revealed that a new mAb 83.5-reactive band appeared over a similar time course. The new mAb 83.5-reactive band had an apparent MW of ca. 65 kD. Since SP80 disappeared coordinately with the appearance of the 65 kD band and all other mAb 83.5-reactive bands were unaffected, the new band appears to be a fragment of SP80 which retained the mAb 83.5 epitope (which recognizes GA-X).

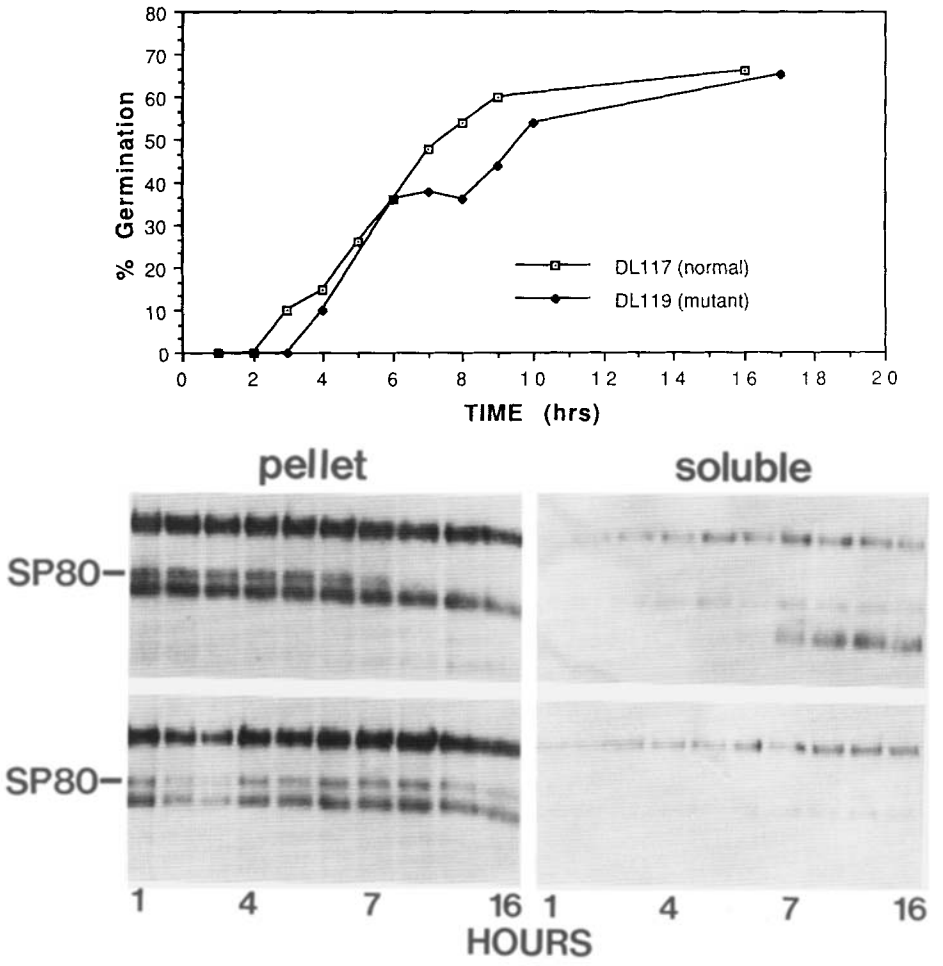

Fig. 2. Cleavage of SP80 during spore germination. Spores were germinated as described in the legend to Table I. Samples were collected hourly for measurement of germination (as described in the legend to Table I) and for analysis by Western blotting after centrifugation (at 100,000g × 1 h) to separate sedimentable (spores and spore coats) from soluble material. 3 × 10^5 spores and spore coats, or the germination fluid corresponding to this number of spores and spore coats, were loaded into each lane. The major bands above and below SP80 (which is labeled) are SP96 and SP75, respectively. Minor lane-to-lane fluctuations in levels of SP96 and SP75 reflect variable recoveries of the small amount of material sampled, for the ratio of SP96 to SP75 is consistent in the original nitrocellose blot, and fluctuations in total labeling are not reproducible, in this and two other trials. Germination is plotted in the graph. The Western blots show that cleavage of SP80 occurs only in the mutant (DL119) after germination and is accompanied by release of the major fragment (ca. 65kD) into the germination medium. Similar results were obtained for strain DL118 (not shown).

Inclusion of the protease inhibitors 1 mM PMSF, 0.17 TIU/ml aprotinin, and 10 μg/ml leupeptin in the germination medium did not inhibit cleavage of mutant SP80. Inclusion of 1 mM NEM and 40 mM EDTA in addition to these three inhibitors did block cleavage, but since germination was also blocked under these conditions, it is possible that cleavage of SP80 did not occur because it is dependent on germination.

Thus no conclusions could be drawn about the possibility of involvement of a specific class of protease.

Properties of the Mutant Spore Coat Prior to Germination

The results indicate that the mutant spore coat is altered during germination leading to the selective cleavage and release of SP80. This change does not occur early enough to explain the urea sensitivity of the mutant spore. The following experiments were directed toward determining whether there is an earlier change in the mutant spore coat, prior to germination.

First, mutant and normal spores were incubated for 3 h at room temperature in several solutions which disturb molecular contacts to compare the elutability of spore coat proteins. Spores were removed by centrifugation and the supernatants analyzed by Western blotting followed by immunoprobing with mAbs 83.5 and 16.1 (Fig. 3). Urea/2-mercaptoethanol was the most efficient extractant, releasing approximately one-half (by comparing with the spore pellet on Western blots) of the total SDS/2-mercaptoethanol extractable spore coat proteins detected by these two antibodies (SP96, SP85, SP80, and SP75). Urea/2-mercaptoethanol was similarly efficient for both DL117 and DL118 spores, with the exception of high MW material. Since high MW

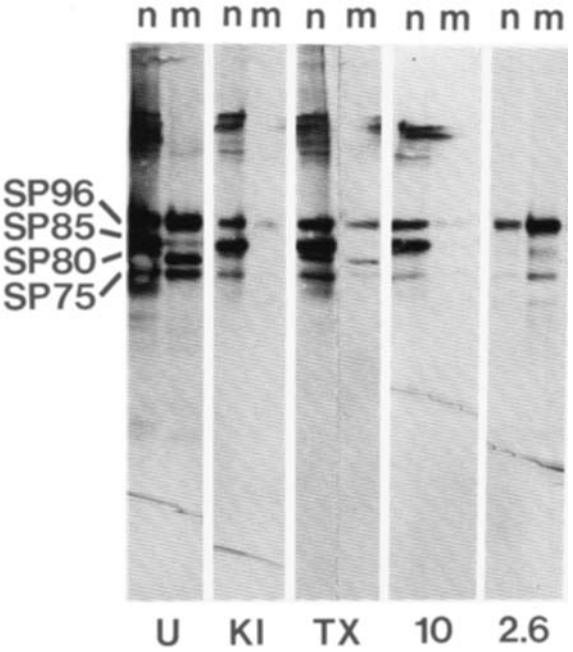

Fig. 3. Extraction of intact spores. Intact 1-day-old spores were resuspended into the following solutions: **U**) 6 M urea, 1% 2-mercaptoethanol, 20 mM Tris-HCl, pH 8.0; **KI**) 1 M KI, 20 mM Tris-HCl, pH 8.0; **TX**) 1% Triton X-100, 1% 2-mercaptoethanol, 20 mM Tris-HCl, pH 8.0; **10**) 20 mM EDTA, 20 mM cyclohexylaminopropane sulfonic acid, pH 10.0; **2.6**) 0.1 M glycine-HCl, pH 2.6, and incubated for 3 h. Spores were removed by centrifugation for 5 min at 10,000*g* and equal volumes of supernatants were electrophoresed and Western blotted. Blots were probed with a mixture of mAbs 83.5 and 16.1. Note that SP85, labeled by mAb 16.1, is not detected in the mutant. The positions of individual spore coat proteins are denoted in the margin. n = the normal strain DL117; m = the mutant strain DL118. Similar results were obtained for both strains DL118 and DL119 (not shown). The urea-extracted DL117 spore sample is distorted by a bubble.

material has been previously shown to consist of aggregates of the lower MW spore coat proteins [10], its absence in gels of mutant spore extracts indicates that mutant proteins have less of a tendency to aggregate. Three of the extractants acted more efficiently on DL117 compared to DL118 spores; these included 1 M KI, 1% Triton X-100/2-mercaptoethanol, and EDTA/cyclohexylaminopropane sulfonic acid (pH 10.0). There did not seem to be a differential effect on individual proteins. In contrast, spore coat proteins were more efficiently extracted from DL118 spores by 0.1 M glycine-HCl (pH 2.6). Similar results were found for both 1- and 7-day-old spores (not shown). The results show that, even in spores which have not been germinated, there are differences in the mutant spore coat as reflected by the distinctive kinds of chemical forces which retain glycoproteins in the matrix.

The spore coats of non-germinated spores were also compared by probing with a lectin which by EM immunolabeling has been shown to bind to a component of the inner layer of the spore coat, the gal/galNAc polysaccharide [9]. We reasoned that if the spore coat of the intact spore was, as suggested by the extraction data, altered in the mutant prior to germination, this might be reflected by a difference in ability of FITC-RCA-I to gain access to the inner layer of the coat and bind to the gal/galNAc polysaccharide. FITC-RCA-I failed to bind to intact spores of either DL117 or DL119 (Fig. 4). If spores were first extracted with hot urea/2-mercaptoethanol, DL119 spores became fluorescent after 1 h of lectin labeling. Under these conditions, DL117 spores remained unlabeled. No difference in urea-extracted protein between the two strains could be observed by Western blotting (Fig. 3, and data not shown). This observation reinforces the conclusion that the *mod*B-mutant spore coat is altered, and suggests that the alteration leads to a more open architecture after urea extraction.

DISCUSSION

In considering the specificity of the spore defects in the *mod*B mutant, we will compare them with the defects found in the spores of HL250, which carries a mutation leading to the absence of fucosylation [10].

Both mutations have a phenotype during spore germination. Young (1- or 2-day-old) *mod*B mutant spores are only inefficiently induced to germinate by urea though they are induced normally by heat. In contrast, young HL250 spores are equally activatable by either urea or heat. Older (7-day-old) HL250 spores cannot, however, be induced by any activation protocol tested [10].

Both mutations alter the glycosylation of spore coat proteins. However, the glycoproteins affected appear to be different. By epitope analysis, SP80 and SP85 are affected in *mod*B mutants. Though SP85 could not be monitored, SP80 was cleaved and the larger fragment released (the order of events is unknown) from the spore coat after emergence of amoebae. By epitope and MW-shift analysis, SP70, SP75, SP80, and SP96 are affected in HL250 [10]. Both SP70 and SP96 were cleaved after emergence, though the fragment of SP96 which retained its marker epitope was not released from the spore coat following its cleavage. SP75 could not be monitored. SP80 was not cleaved, in contrast to its fate in the *mod*B mutant. Thus different but overlapping sets of glycoproteins were observed to be altered in the two mutants, and this correlated in part with which proteins were cleaved after germination. Cleavage of SP80, whose glycosylation is affected in both mutants, was, however, specific for the deficiency in the *mod*B-dependent epitope.

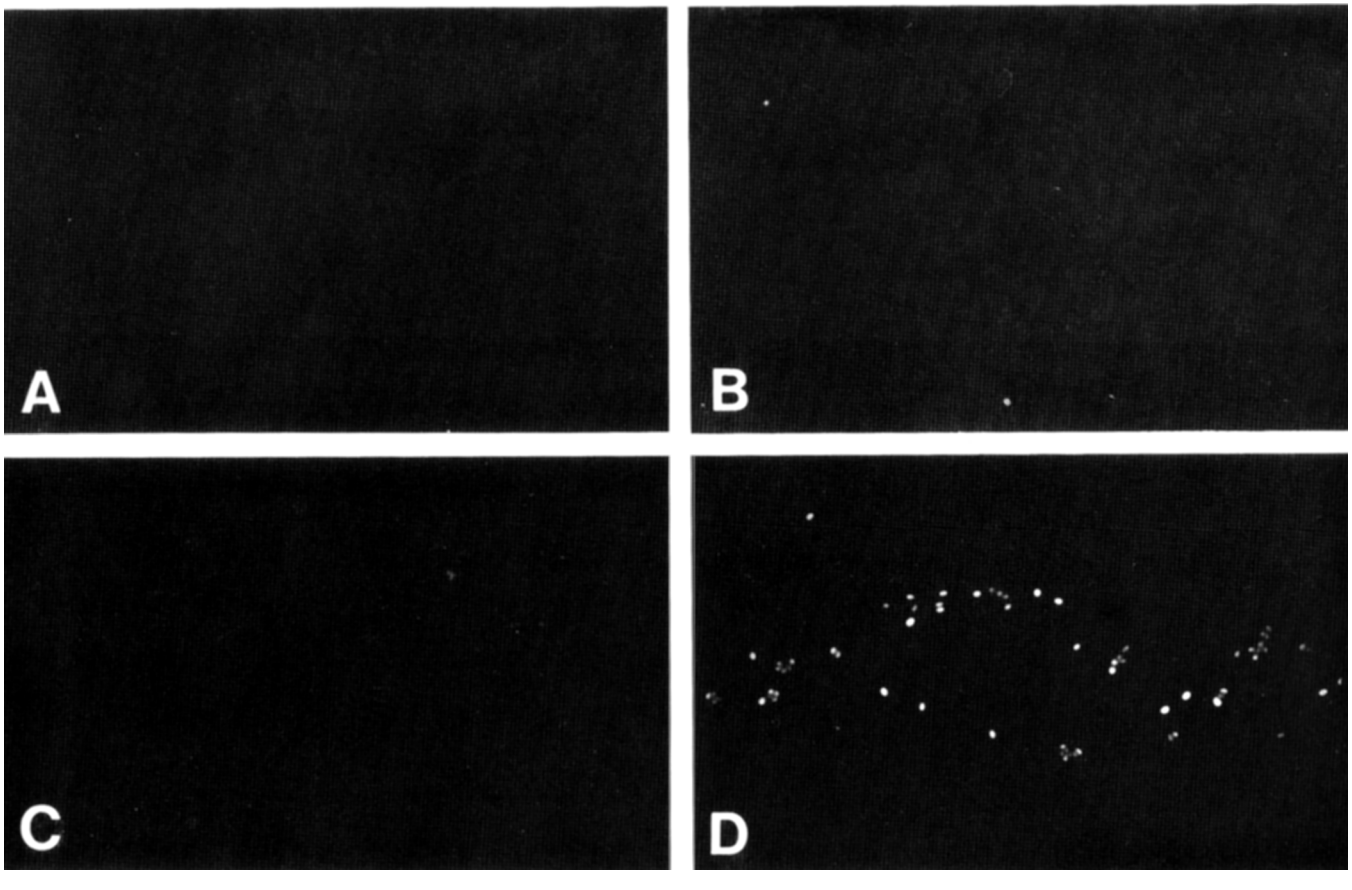

Fig. 4. Labeling of mutant spores with FITC-RCA-I. One-day-old mutant (DL119) and normal (DL117) spores were untreated or extracted with urea/2-mercaptoethanol for 3 min at 100°C and labeled with FITC-RCA-I for 1 h **A:** 40 μg/ml FITC-RCA-I + 0.25 M lactose applied to urea-treated DL119 spores. **B:** 4 μg/ml FITC-RCA-I applied to non-urea-extracted DL119 spores. **C:** 4 μg/ml FITC-RCA-I applied to urea-extracted DL117 spores. **D:** 4 μg/ml FITC-RCA-I applied to urea-extracted DL119 spores. Each fluorescent image observed in D corresponds to an individual spore. Similar results were found for strains DL118 and DL119.

Effects of both mutations could be observed in the non-germinated spore coat, as determined by the lectin labeling experiment. Both mutants were more readily labeled with the lectin after urea extraction, and this did not seem to be the result of a change in the amount of the target for lectin binding, the gal/galNAc polysaccharide. This implies a more open architecture for the mutant spore coat matrix, caused perhaps by the absence of carbohydrate structures critical to intermolecular associations. Since both mutants affect epitopes which map to the outer layer of the spore coat, which consists of a proteinaceaous layer applied to deeper cellulose-, gal/galNAc-, and protein-containing layers [9], the lesion is expressed in a position consistent with an increased accessibility of the lectin to the inner layer where the gal/galNAc polysaccharide resides. For the *mod*B mutants the idea of altered molecular associations is supported by finding that chemical conditions which perturb protein interactions, including low and high pH, divalent cation chelation, and the presence of non-ionic detergents or chaotropic agents, differentially extracted certain proteins from the two spore surfaces. Whether these differences found in the mutant spore are also responsible for the cleavage of specific proteins later during germination remains to be tested.

Numerous studies of the organization of cellulose-containing cell walls and cell coats in other organisms have reflected on the possibility that protein-linked oligosaccharides and polysaccharides serve a noncovalent bridging or cross-linking role for retaining the various elements of these matrices together [20]. Alternatively, protein-linked carbohydrate may instead play a space filling role or a role for the benefit of the carrier molecule, rather than be involved in intermolecular interaction. The relatively small apparent MW shifts observed for proteins in modB mutants seems incompatible with a major space-filling role. We find it interesting that genetic modification of protein-linked carbohydrate in the spore coat of *Dictyostelium* appears to alter the architecture of the wall in specific ways in conjunction with changes in how certain glycoproteins chemically associate with the coat, which would be expected if the carbohydrate plays a direct role in intermolecular recognition. Since assembly of the spore coat appears to begin from soluble protein precursors within vesicles inside of prespore cells [21,9], these mutants may afford new a new opportunity to study the role of protein-linked carbohydrate in the structure of an extracellular matrix.

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