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Cell Surface Modifications Induced by Calcium Ion in the Myxobacterium *Stigmatella aurantiaca*

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Calcium ion induces in the myxobacterium *Stigmatella aurantiaca* the ability to glide on solid surfaces and to become cohesive (D. F. Gilmore and D. White, J. Bacteriol. 161:113–117, 1985; B. J. Womack, D. F. Gilmore, and D. White, J. Bacteriol. 171:6093–6096, 1989). The addition of calcium ion to the growth medium resulted in the formation of extracellular fibrils, the appearance in the membrane fractions of a 30-kDa protein, and the accumulation in a low-speed centrifugal pellet of 10 polypeptides that cross-reacted with affinity-purified antibody to one of the polypeptides. One of the polypeptides, a 55-kDa protein, was present in the membrane fraction of control cells not incubated with calcium ion and was apparently translocated to the extracellular matrix during incubation in medium containing calcium ion. The 55-kDa protein was immunologically related to a 65-kDa protein located on the fibrils of another myxobacterium, *Myxococcus xanthus*.

Stigmatella aurantiaca, a myxobacterium, is a gram-negative soil bacterium that moves on solid surfaces by gliding motility and constructs multicellular fruiting bodies under conditions of nutrient deprivation (15, 19, 22). In *Stigmatella* spp., calcium ion induces gliding motility and an energy-dependent cohesion system (9, 24). The induction is prevented by inhibitors of protein synthesis. A related myxobacterium, *Myxococcus xanthus*, also requires calcium ion for gliding motility and possesses an energy-dependent cohesion system (6, 17). Cohesion in *M. xanthus* requires the involvement of components of a group motility system, called the S (social) system, and is correlated with the appearance of extracellular fibrils (1, 2, 18). The cell surface of the myxobacteria is of obvious importance to gliding motility and to cohesion but has not been well characterized. The cells can produce, and become embedded in, a chemically complex extracellular matrix consisting of polysaccharide, protein, and lipid, as well as cell surface fibrils (19). The data presented here demonstrate that the addition of calcium ion to the growth medium promotes the synthesis of extracellular fibrils in *S. aurantiaca*, causes the translocation of a 55-kDa protein from the membrane fraction to a low-speed centrifugal pellet which we suggest is the extracellular matrix, promotes the appearance in the low-speed pellet of nine additional polypeptides that cross-react with the antibody to the 55 kDa-protein, and results in the accumulation of a 30-kDa protein in the membrane fraction. Affinity-purified antibody to the 55-kDa protein cross-reacted with several *M. xanthus* proteins, including a 65-kDa protein located in the extracellular fibril fraction (3). These modifications of the *Stigmatella* cell surface and matrix may be related to the calcium-dependent acquisition of the ability to glide and to cohere in cell clumps (9, 24).

MATERIALS AND METHODS

Strains and cultivation. Bacteria were grown in 1% tryptone (Difco Laboratories, Detroit, Mich.) and 8 mM $MgSO_4$ at 30°C with shaking at 200 rpm. Calcium-induced cells were obtained by including 1 mM $CaCl_2$ in the growth medium. Under these circumstances, the cells grew clumped. Noninduced refers to cells grown in the absence of added calcium. Such cells grew as a dispersed culture. *S. aurantiaca* DW135 was previously described (9). *M. xanthus* MD207 was obtained from M. Dworkin (University of Minnesota).

Isolation of cell fractions. Cells were harvested by centrifugation and washed once with cold 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; pH 7.2) containing 1 mM phenylmethanesulfonyl fluoride (PMSF) and stored frozen at −80°C. The defrosted cells were resuspended in cold 10 mM HEPES and passed through a French pressure cell four times at 17,200 lb/in^2. Four cell fractions were obtained via differential centrifugation: pellet I (3,020 × *g*, 5 min), which contained extracellular matrix material and some unbroken cells; pellet II (6,780 × *g*, 20 min), which contained matrix; the membrane fraction (160,000 × *g*, 120 min); and the soluble supernatant from the 160,000 × *g* centrifugation. The soluble supernatant was precipitated with 2 volumes of cold 95% ethanol overnight, and the precipitate was sedimented for further analysis. Purified *Myxococcus* fibrils were obtained from M. Dworkin.

Sucrose density centrifugation. Pellets were resuspended in 25% (wt/wt) sucrose in 10 mM HEPES buffer, layered onto a step gradient (30 to 55% [wt/wt] sucrose in 10 mM HEPES), and centrifuged in a Beckman SW28 rotor at 27,000 rpm for 24 h. Fractions (0.5 ml) were collected from the bottom of the centrifuge tube. Absorbancies were measured at 280 nm, and the refractive indices were measured with a Bausch & Lomb Abbe-3L Refractometer (Bausch & Lomb, Inc., Rochester, N.Y.).

Detergent treatment. Pellets were resuspended to a final protein concentration of 5 mg/ml in 10 mM HEPES containing 1 mM EDTA (pH 7) and 2% Zwittergent 3-14 (Calbiochem, San Diego, Calif.). The suspension was gently stirred at 4°C for 1 h and then centrifuged at 100,000 × *g* for 1 h to yield a pellet enriched for the 55-kDa protein. Zwittergent 3-14-treated pellets were sometimes further extracted with lauryldimethylamine oxide (Calbiochem) or octylglucoside (Sigma) in an identical fashion. After centrifugation at 100,000 × *g* for 1 h, the supernatants were precipitated with 2 volumes of cold 95% ethanol overnight, and the precipitates were harvested by centrifugation for further analyses.

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The 55-kDa protein was insoluble in 2% Zwittergent 3-14, 5% lauryldimethylamine oxide, 0.1% octylglucoside, and 0.2% sodium dodecyl sulfate (SDS). Coomassie blue staining revealed that the Zwittergent 3-14-treated pellet II was enriched for the 55-kDa protein as well as nine additional polypeptides. Seven of the nine bands (46, 44, 41.5, 40, 36, 27, and 25 kDa) stained with affinity-purified anti-55-kDa protein antibody. Whole cells were extracted for 10 min at room temperature with 0.2% SDS in 10 mM HEPES buffer containing 1 mM PMSF and 1 mM EDTA. After extraction, the suspension was centrifuged at 12,100 × *g* for 10 min and the supernatant was centrifuged at 100,000 × *g* for 1 h. Both the 12,100 × *g* and the 100,000 × *g* pellets were enriched for the 55-kDa protein.

Isolation of LPS. Lipopolysaccharide (LPS) was extracted by the phenol-water method (23). After exhaustive dialysis, the water extracts were lyophilized and resuspended in distilled water for further analysis.

Protein gel electrophoresis. SDS-polyacrylamide gels (10 to 15% gradient) containing 0.07% SDS were made according to the method of Laemmli (11). Samples were diluted 1:3 in 4× treatment buffer (0.25 M Tris-Cl [pH 6.8], 8% SDS, 40% glycerol, 20% 2-mercaptoethanol) and heated at 100°C for 1 min. Two-dimensional gel electrophoresis was carried out according to the method of O'Farrell (13). Proteins were stained with Coomassie brilliant blue (7).

LPS gel electrophoresis. Prior to gel electrophoresis, LPS was treated with 1 mg of protease K (Sigma) per ml for 90 min at 57°C in 62.5 mM Tris buffer (pH 7.6). Samples were electrophoresed in SDS–15% polyacrylamide gels and stained with silver nitrate according to the method of Tsai and Frasch (21).

Immunogold labeling. Cells were washed once with 10 mM HEPES buffer and resuspended in buffer to 2×10^{10} cells per ml. Five microliters of cell suspension was spotted on a cold tryptone plate (1% Bacto Tryptone, 8 mM $MgSO_4$, 1.5% BBL agar [BBL Microbiology Systems, Becton Dickinson and Co., Cockeysville, Md.]), and the spot was allowed to dry in at 4°C. The spot was overlaid with 5 μl of affinity-purified anti-55-kDa protein antibody and incubated for 2 h at 4°C, 5 μl of 1:10 goat anti-mouse immunoglobulin G antibody conjugated with 30-nm colloidal gold (Janssen Scientific, Olen, Belgium) was added, and incubation was continued for an additional hour at 4°C. The cells were scraped off the plate with 2% glutaraldehyde, incubated in Eppendorf tubes for 1 h at room temperature, and washed once with room temperature distilled water. The fixed cells were resuspended in 10 μl of distilled water; 5 μl was placed on Formvar-carbon-coated grids and air dried.

Negative staining. Five microliters of cell suspension (10^9 cells per ml) was placed on Formvar-carbon-coated grids and allowed to settle for 5 min. The grids were washed once by being dipped in distilled water, and 1 drop of 2% phosphotungstic acid (pH 7.2) containing 30 μg of bovine serum albumin (BSA) per ml was placed on each grid for 30 s. The stained grids were washed once in distilled water, air dried, and observed with a Philips 300 transmission electron microscope.

Radioactive labeling. Cells were grown for 2 days in 1% tryptone medium containing 5 μCi of [^{35}S]methionine (1,095 Ci/mmol; ICN Biomedicals Inc., Costa Mesa, Calif.) per ml. Labeled cells were washed once with 10 mM HEPES buffer and then treated with 2% Zwittergent 3-14 containing 1 mM PMSF and 1 mM EDTA for 10 min at room temperature. The suspension was then centrifuged at 12,100 × *g* for 10 min, and the supernatant was centrifuged at 100,000 × *g* for 1 h. The supernatant from the high-speed centrifugation was precipitated with 2 volumes of cold 95% ethanol overnight, and the precipitate was recovered by centrifugation.

Radioactivity measurements. The 55-kDa protein in all of the samples was separated via two-dimensional gel electrophoresis. The gels were dried, placed on X-Omat film (Eastman Kodak Co., Rochester, N.Y.), and exposed for 10 days at room temperature. The developed film was layered over the gel, and the spot corresponding to the 55-kDa protein was excised from the dried gel. Each spot was rehydrated in 0.5 ml of water for 30 min. The protein was eluted from the rehydrated gel by incubation in 0.5 ml of SOLVABLE (DuPont Products) for 30 min at 50°C. The scintillation cocktail was FORMULA-989 (NEN Research Products). Total radioactivity was determined by adding the counts per minute in all of the insoluble fractions separated after detergent treatment, including the ethanol precipitate of the supernatant.

Western immunoblotting and antibody staining. Proteins were electrophoretically transferred from SDS-polyacrylamide gels to nitrocellulose under a constant voltage of 30 V for 24 h at 4°C, using a Bio-Rad Trans-Blot Cell (Bio-Rad Laboratories, Richmond, Calif.). Molecular weight markers were also transferred and visualized with amido black (16). After transfer, the membranes were stored frozen in 0.15 M phosphate-buffered saline (PBS; pH 7.2) until further use. For antibody staining, nitrocellulose strips were blocked with PBS containing 0.5% Tween 20 (Sigma) for 1 h at room temperature and then incubated overnight with antibody diluted 1:1,000 in PBS–0.5% Tween 20 at 4°C. After incubation with antibody, the membranes were washed three times with PBS–0.5% Tween 20, 5 min per wash, and then incubated at room temperature for 2 h with goat anti-mouse immunoglobulin G antibody-conjugated alkaline phosphatase (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) diluted 1:4,000 in PBS–0.5% Tween 20. After 2 h, the membranes were washed twice with PBS–0.5% Tween 20, each time for 5 min, and once with 150 mM barbital (pH 9.6; Sigma) for 5 min at room temperature. The membranes were stained with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (Sigma) according to the manufacturer's instructions (4).

Antibody preparation. The 55-kDa protein was purified by two-dimensional gel electrophoresis and transblotted to nitrocellulose. The nitrocellulose spot containing approximately 1 μg of the 55-kDa protein was implanted subcutaneously in the necks of 2-week-old BALB/c Wat female mice. After 30 days, the mice were boosted with subcutaneous injection in the leg with homogenized polyacrylamide containing about 2 μg of the 55-kDa protein purified by one-dimensional SDS-gel electrophoresis. The homogenization fluid contained 2 ml of Freund's adjuvant (Cappel Laboratories, West Chester, Pa.). To obtain large quantities of antibody, the mice were given ascites tumors. Two weeks after being boosted, the mice were injected with 0.5 ml of Pristane (Sigma), and 4 days later they were injected with ascites myeloma cells. The ascites fluid was collected 10 days after injection of the myeloma cells. Antibody titer to the 55-kDa protein was determined by using serial dilutions of the serum or ascites fluid to stain Western blots. A monoclonal antibody (MAb) to *Myxococcus* fibrils (MAb 2105) was obtained from M. Dworkin.

Affinity purification of antibody. Affinity-purified antibody was prepared as previously described, with minor modifications (14). The nitrocellulose strip containing the 55-kDa protein was blocked in PBS containing 10% BSA at room

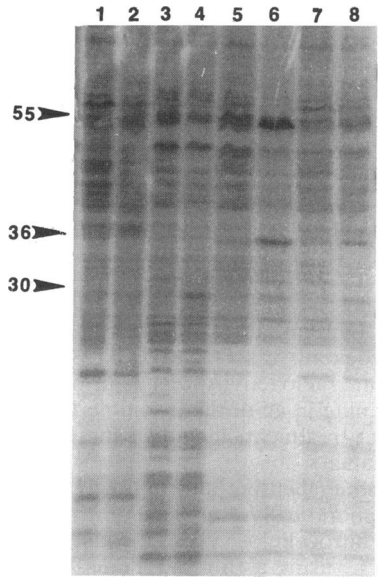

FIG. 1. SDS-polyacrylamide gel (10 to 15% gradient) of *S. aurantiaca* proteins stained with Coomassie blue. Each well contained 40 μg of protein. Lanes: 1, noninduced, supernatant; 2, calcium induced, supernatant; 3, noninduced, membrane fraction; 4, calcium induced, membrane fraction; 5, noninduced, pellet II; 6, calcium induced, pellet II; 7, noninduced, pellet I; 8, calcium induced, pellet I. Sizes are indicated in kilodaltons.

temperature for 2 h with gentle shaking. After 2 h, the blocking solution was replaced with 1:10 antiserum or ascites fluid in PBS containing 1.5% BSA and 0.1% Tween 20 and incubated overnight at 4°C. The strip was then washed two times with PBS containing 0.1% Tween 20 at room temperature for 30 min each time, and the antibody was eluted with elution buffer (0.2 M glycine, 0.2 M NaCl, 100 μg of BSA per ml [pH 2.8]). The eluted antibody was immediately neutralized with NaOH to pH 8.8 and dialyzed against 0.5 mM HEPES buffer overnight at 4°C. The antibody solutions were lyophilized and stored at 4°C. Just before use, the dry antibody was dissolved in 0.5% BSA.

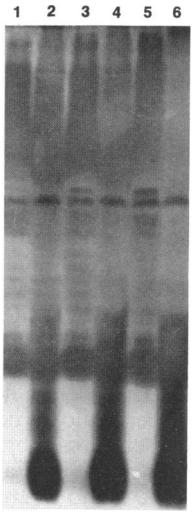

FIG. 2. SDS-acrylamide gel analysis of LPS. A silver stain of an SDS–15% polyacrylamide gel of LPS is shown. LPS was extracted from pellet II and the membrane from calcium-induced cells. Lanes: 1, 3, and 5, 5, 10, and 15 μg, respectively, from pellet II; 2, 4, and 6, 5, 10, and 15 μg, respectively, from the membrane fraction.

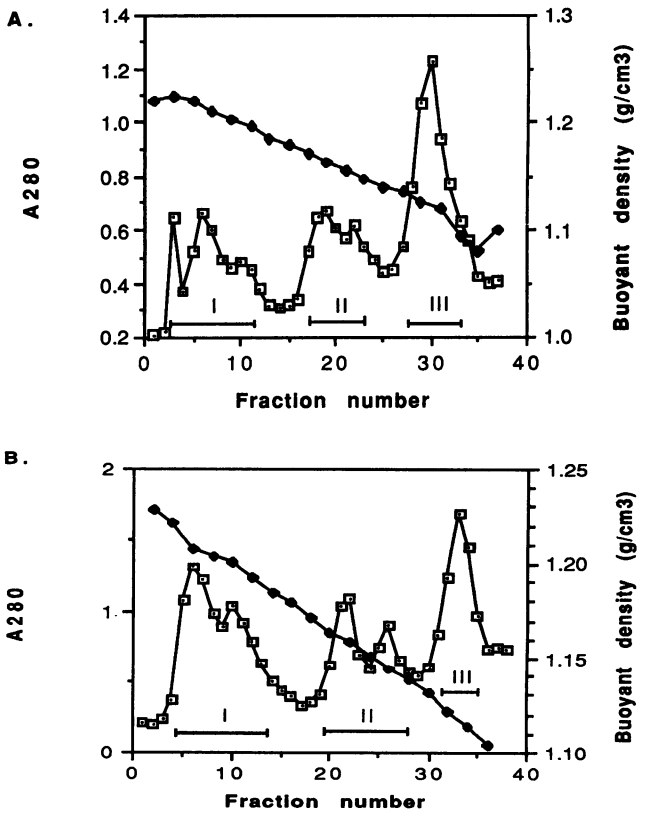

FIG. 3. Sucrose density gradient analysis of the matrix fraction (pellet II). (A) Calcium induced; (B) noninduced. Approximately 1 mg of protein was layered on top of each gradient.

Chemical assays. Total carbohydrate was measured by the phenol-sulfuric acid assay, using glucose as the standard (10). Protein was determined by the assay of Lowry et al. (12), with BSA as the standard.

RESULTS

Accumulation of the 55-kDa protein in pellet II of calcium-induced cells. Calcium incubation resulted in the accumulation of a 55-kDa polypeptide in pellet II (Fig. 1, lane 6). Occasionally, pellet I was also enriched in the 55-kDa protein. There was also a 30-kDa protein that was consistently found in the membranes of calcium-induced cells (lane 4) but not in the membrane fraction from noninduced cells (lane 3). A 35-kDa protein was occasionally observed in pellet II of calcium-induced cells (lane 6) that was present in much smaller amounts in noninduced cells (lane 5). Transmission electron microscopy of negatively stained material indicated that pellets I and II consisted primarily of amorphous material and some membrane vesicles (6a).

LPS. Gel electrophoresis of the aqueous phase of the phenol extract of pellet II revealed a series of bands (Fig. 2, lanes 1, 3, and 5) that migrated identically to LPS (lanes 2, 4, and 6) but lacked low-molecular-weight material. Similar low-molecular-weight material in *M. xanthus* was identified

TABLE 1. Buoyant densities of pooled peaks from sucrose density gradient of pellet II

Sample	Buoyant density (g/cm^3)		
	Peak 1	Peak 2	Peak 3
Calcium induced	1.214	1.162	1.122
	1.197	1.150	
Noninduced	1.208	1.158	1.118
	1.201	1.144	

as incomplete LPS, i.e., lipid A and core without the oligosaccharide portion (8).

Sucrose density gradient analysis. Pellet II consisted of three major peaks of material separable by their buoyant densities (Fig. 3). Peaks 1 and 2 were orange, and each consisted of two overlapping peaks. The material in peak III was colorless and had an absorption maximum at 260 nm, indicating nucleic acid. The buoyant densities are listed in Table 1. Analysis of the proteins by gel electrophoresis revealed that most of the 55-kDa protein in calcium-induced cells was in peak 2 (Fig. 4, lane 3).

Purification of the 55-kDa protein by gel electrophoresis and preparation of antibody. Partial purification of the 55-kDa protein was obtained by differential solubilization in detergent. The Zwittergent 3-14-treated pellet II was subjected to two-dimensional gel electrophoresis. The 55-kDa protein had a pI of approximately 6.8 and was well separated from other proteins (Fig. 5). The 55-kDa protein purified via two-dimensional gel electrophoresis was electrophoretically transferred to nitrocellulose and implanted into mice to raise polyclonal antibodies used in the Western blot analyses and the cell localization experiments.

Western blot analysis of cell fractions and cellular location of the 55-kDa protein. Pellet II from calcium-induced cells contained nine polypeptides, not present in noninduced cells, that cross-reacted with anti-55-kDa protein antibody (Fig. 6). Affinity-purified antibody stained the same bands as did the nonpurified antibody, suggesting that the 10 polypeptides shared a common epitope. The 55-kDa protein was found in approximately equal amounts in both calcium-induced and noninduced cells, but the cellular location was different. Figure 7 (lane 1 [whole cells, calcium induced] and lane 2 [whole cells, noninduced]) indicates that the total amounts of the 55-kDa protein were approximately the same

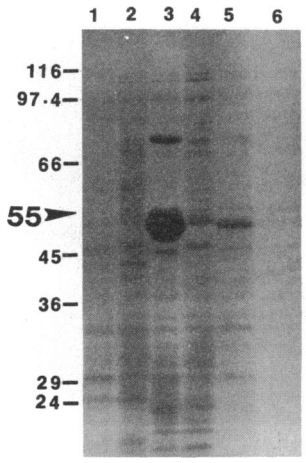

FIG. 4. Coomassie blue-stained SDS-polyacrylamide gel (10 to 15% gradient) of fractions from the sucrose gradient. Each well contained 40 μg of protein. Lanes: 1, peak 1, calcium induced; 2, peak 1, noninduced; 3, peak 2, calcium induced; 4, peak 2, noninduced; 5, peak 3, calcium induced; 6, peak 3, noninduced. Sizes are indicated in kilodaltons.

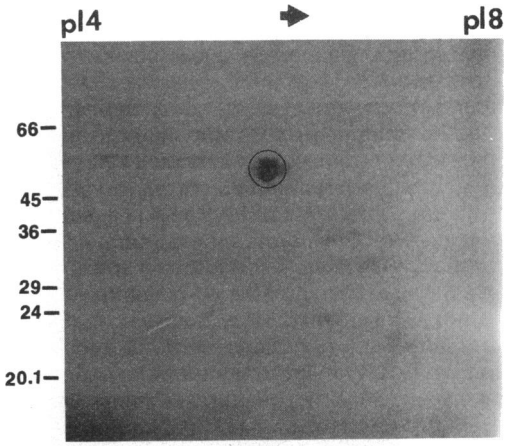

FIG. 5. Coomassie blue-stained two-dimensional gel of 2% Zwittergent 3-14-treated pellet II from calcium-induced cells. Each tube gel was loaded with 100 μg of protein. The 55-kDa protein is circled. Sizes are indicated in kilodaltons.

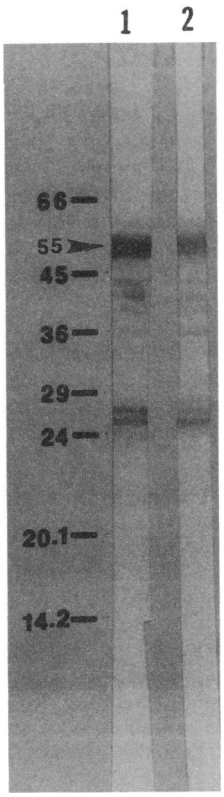

FIG. 6. Western blot analysis of pellet II from calcium-induced cells stained with antibody to the 55-kDa protein. Protein (30 μg per well) was separated on a 10 to 15% gel and electrophoretically transferred to nitrocellulose. Lanes: 1, non-affinity-purified antibody; 2, affinity-purified antibody. Sizes are indicated in kilodaltons.

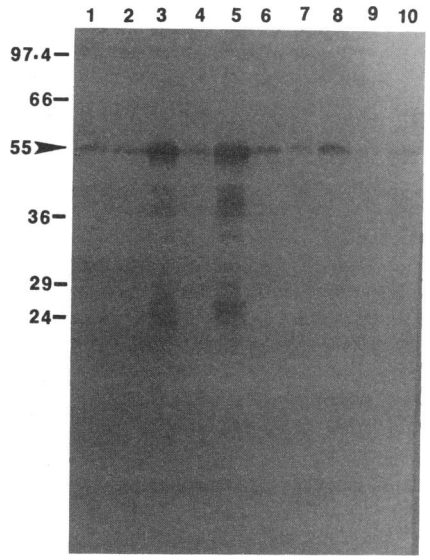

FIG. 7. Western blot analysis of cell fractions stained with antibody to the 55-kDa protein. Protein (30 μg per well) was separated on a 10 to 15% polyacrylamide gel and electrophoretically transferred to nitrocellulose. Lanes: 1, total cell protein, calcium induced; 2, total cell protein, noninduced; 3, pellet I, calcium induced; 4, pellet I, noninduced; 5, pellet II, calcium induced; 6, pellet II, noninduced; 7, membrane fraction, calcium induced; 8, membrane fraction, noninduced; 9, supernatant, calcium induced; 10, supernatant, noninduced. Sizes are indicated in kilodaltons.

in the two cell types. However, the membranes of noninduced cells (lane 8) contained more 55-kDa protein than did the membranes of induced cells (lane 7), whereas pellets I (lane 3) and II (lane 5) of induced cells contained much more 55-kDa protein than did the corresponding pellets from noninduced cells (lanes 4 and 6). These data suggest that calcium induced the translocation of the 55-kDa protein from the membranes to pellets I and II. Immunogold labeling with affinity-purified antibody revealed gold particles entirely in the extracellular matrix (Fig. 8A). It should be emphasized that the affinity-purified antibody stained several protein bands on Western blots, the 55-kDa protein being the major one (Fig. 6, lane 2). We cannot be certain of the identities of the proteins stained in Fig. 8A. The label was generally concentrated between closely apposed cells. Translocation, rather than de novo synthesis, was further substantiated by radioactive labeling experiments.

Radioactive labeling. Whole cells were solubilized with 2% Zwittergent 3-14, and the fractions were separated by differential centrifugation. Antibody labeling of Western blots of one-dimensional gels indicated that the 55-kDa protein was located primarily in the insoluble fraction after centrifugation at 100,000 × *g* for 1 h (Fig. 9, lanes 3 and 4). Only trace amounts were found in the ethanol precipitates of the detergent-solubilized fractions (lanes 5 and 6) and in the 12,100 × *g* fractions (lanes 1 and 2). Cells were labeled with [^{35}S]methionine and extracted with detergent, and both the insoluble fractions and the ethanol precipitates from the soluble fractions were analyzed via two-dimensional gel electrophoresis. Autoradiographs demonstrated that the protein was well separated from other radioactive spots (Fig. 10). The amounts of 55-kDa protein were compared with the levels of radioactivity in total protein and with the amounts of a reference 60-kDa protein, also purified by two-dimen-

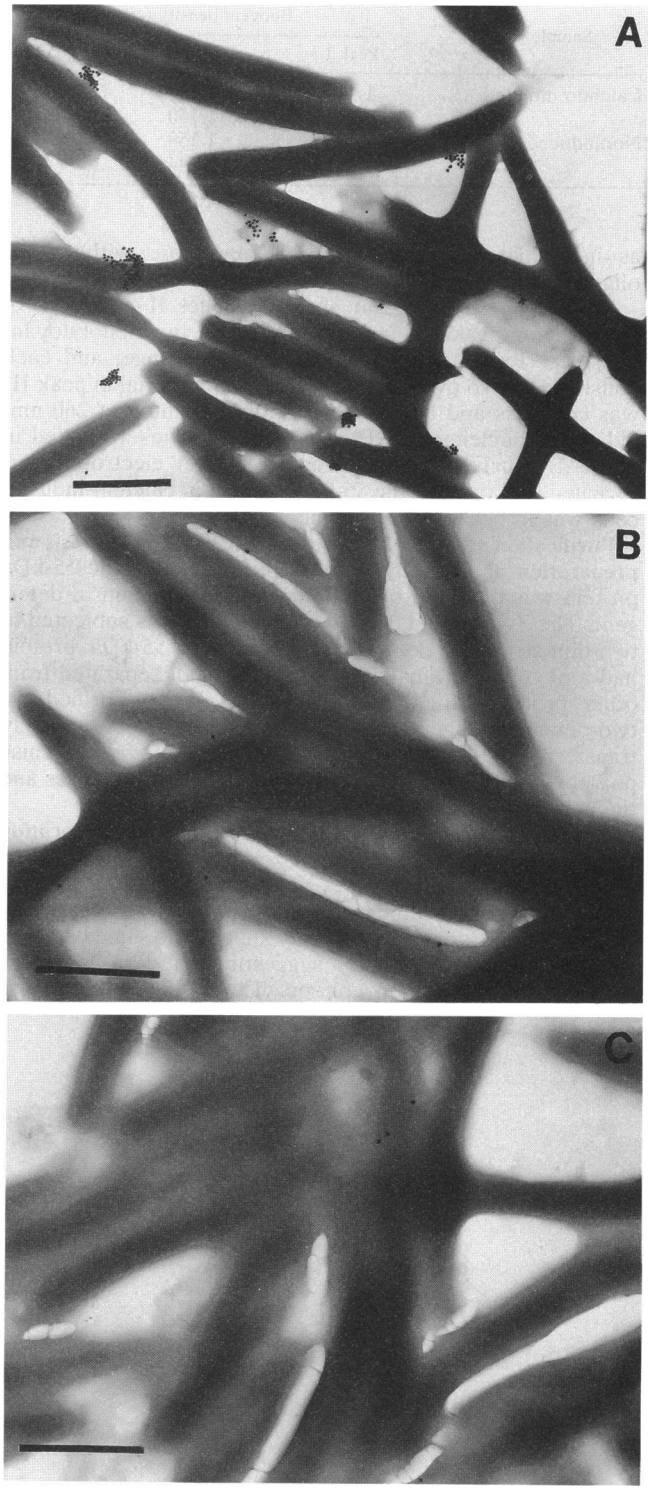

FIG. 8. Immunogold labeling of whole cells with affinity-purified anti-55-kDa protein antibody as the primary antibody. (A) Calcium-induced cells; (B) noninduced cells; (C) calcium-induced cells, no primary antibody. Bars represent 1 μm.

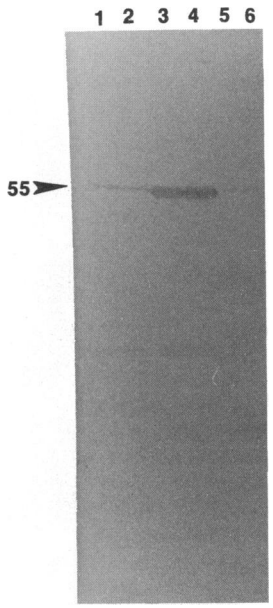

FIG. 9. Western blot analysis of the 55-kDa protein after Zwittergent 3-14 treatment of whole cells. Cells were solubilized with 2% Zwittergent 3-14, and the fractions were separated by differential centrifugation. Each well contained 40 μg of protein. The primary antibody was an anti-55-kDa protein antibody. Lanes: 1, 12,100 × *g* sediment from calcium-induced cells; 2, 12,100 × *g* sediment from noninduced cells; 3, 100,000 × *g* sediment from calcium-induced cells; 4, 100,000 × *g* sediment from noninduced cells; 5, ethanol precipitate of high-speed supernatant from calcium-induced cells; 6, ethanol precipitate of high-speed supernatant from noninduced cells.

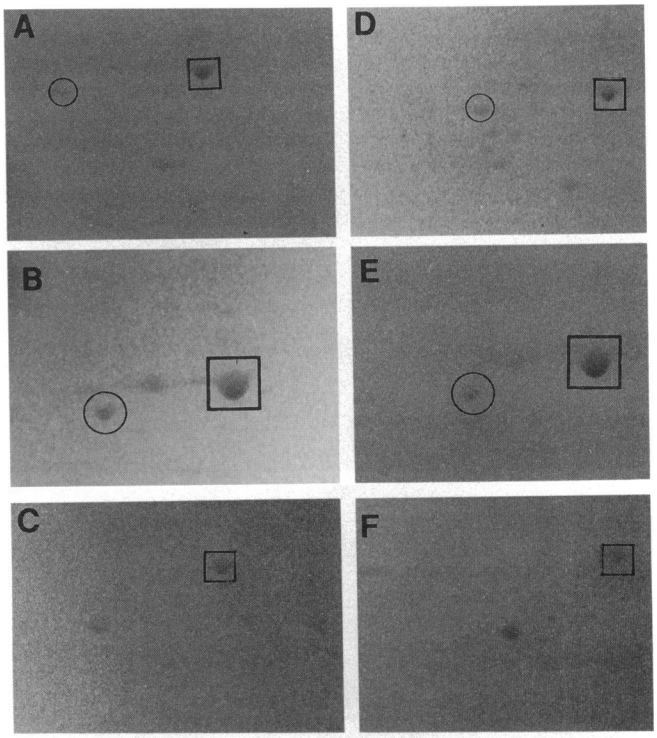

FIG. 10. Autoradiographs of differential centrifugation fractions from radioactive cells solubilized with 2% Zwittergent 3-14. One hundred micrograms of protein was separated by two-dimensional gel electrophoresis. (A) Calcium induced, 12,100 × *g* pellet; (B) calcium induced, 100,000 × *g* pellet; (C) calcium induced, ethanol precipitate of supernatant; (D) noninduced, 12,100 × *g* pellet; (E) noninduced, 100,000 × *g* pellet; (F) noninduced, ethanol precipitate of supernatant. Circles, 55-kDa protein; squares, 60-kDa reference protein.

sional gel electrophoresis, whose levels were not increased by calcium ion, as judged by Coomassie blue staining of one-dimensional gels. The results of one such experiment are shown in Table 2. Calcium did not increase the total amounts of 55-kDa protein. Since the 55-kDa protein was located primarily in the membrane of noninduced cells (Fig. 7, lane 8), it appears that the membrane served as the chief source of the protein.

Negative stain of whole cells. Negative staining of whole cells revealed that the matrix of calcium-induced cells consisted of many cell-associated fibrils in comparison with noninduced cells (Fig. 11). Scanning electron micrographs of critical-point-dried cells also demonstrated that calcium-induced cells produced many more fibrils than did noninduced cells (data not presented). Since the procedures for immunogold labeling did not reveal the fibrils, we were not able to determine whether the 55-kDa protein was located on the fibrils.

Cross-reactivity to *M. xanthus* proteins. Cell fractions were prepared via French press and differential centrifugation from *M. xanthus* in a procedure identical to that used for the preparation of fractions from *S. aurantiaca*. The proteins were separated by one-dimensional gel electrophoresis, transferred to nitrocellulose, and stained with affinity-purified antibody prepared against the *Stigmatella* 55-kDa protein. Pellets I and II of *M. xanthus* contained a 65-kDa polypeptide that cross-reacted with the anti-55-kDa protein antibody (Fig. 12, lanes 1 to 4). There was no indication that the amounts of the 65-kDa polypeptide increased as a result of calcium ion incubation or that the protein moved from the membranes to the matrix. The 65-kDa protein is a fibril protein in *M. xanthus* (3). The affinity-purified anti-55-kDa protein antibody also stained several polypeptides extracted from the membranes of *M. xanthus* (lanes 5 and 6). An MAb to the *Myxococcus* 65-kDa protein (MAb 2105) stained the 55-kDa protein weakly (data not shown).

DISCUSSION

Electron micrographs of negatively stained cells revealed both attached fibrils and amorphous material surrounding *Stigmatella* cells. The density of fibrils was greatly enhanced upon incubation of cells with calcium ion. Calcium ion also induces gliding motility and the ability to cohere in *Stigmatella* cells (9, 24). The induction is prevented by inhibitors of protein synthesis, which suggests that the cells respond to calcium by synthesizing proteins that either function in

TABLE 2. Quantitation of the 55-kDa protein

Sample	Radioactivity (cpm derived from 8×10^9 cells)		
	Total[a]	55-kDa protein	60-kDa protein
Calcium induced	1.19×10^7	1.07×10^4	8.9×10^5
Noninduced	1.10×10^7	1.26×10^4	8.4×10^5

[a] Counts, after detergent treatment, in the sedimented pellets and the ethanol-insoluble supernatant fraction.

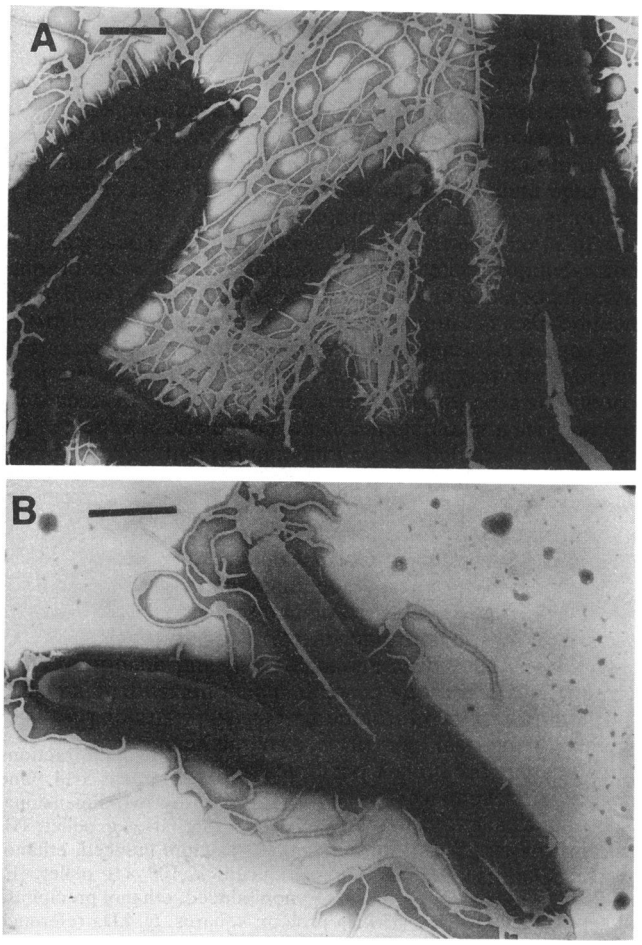

FIG. 11. Negative stain of whole cells. (A) Calcium induced; (B) noninduced. Bars represent 1 μm.

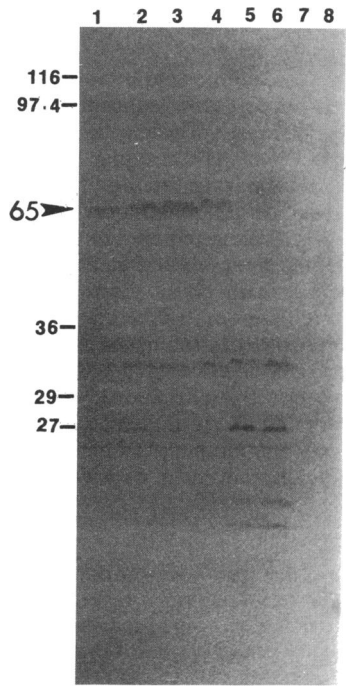

FIG. 12. Western blot analysis of cell fractions from *M. xanthus* MD207 stained with a *Stigmatella* anti-55-kDa protein antibody. French press extracts were fractionated via differential centrifugation. Protein (30 μg per well) was separated by SDS-gel electrophoresis (10 to 15% gradient), electrophoretically transferred to nitrocellulose, and stained with an affinity-purified *Stigmatella* anti-55-kDa protein antibody. Lanes: 1, calcium grown, pellet I; 2, no calcium, pellet I; 3, calcium grown, pellet II; 4, no calcium, pellet II; 5, calcium grown, membrane fraction; 6, no calcium, membrane fraction; 7, calcium grown, ethanol precipitate of supernatant; 8, no calcium, ethanol precipitate of supernatant. Sizes are indicated in kilodaltons.

preparing the cells for gliding motility and cohesion or play a direct role in gliding motility and cohesion.

We searched for new proteins in calcium-induced cells. Accordingly, broken cell extracts were fractionated by differential centrifugation into a low-speed pellet, a membrane fraction, and a soluble fraction. A 30-kDa protein was consistently found in membrane fractions from calcium-induced cells but not from noninduced cells. In addition, Coomassie blue staining of protein gels revealed a 55-kDa protein greatly enriched in the low-speed pellet. Affinity-purified antibody staining and radioactivity measurements indicated that calcium did not increase the total amounts of the 55-kDa protein but rather resulted in the translocation of the 55-kDa protein from the membrane fraction to the low-speed pellet. Nine smaller polypeptides were also stained by affinity-purified anti-55-kDa protein antibody in protein gels from low-speed pellets derived from calcium-induced cells, suggesting a common epitope.

Immunogold labeling of whole cells with the affinity-purified antibody to the 55-kDa protein showed labeling in the extracellular matrix fraction, which indicates that the antibody bound to an epitope in the matrix common to the 55-kDa protein and the nine cross-reacting proteins. We suggest that the low-speed pellet contains the extracellular matrix fraction and that the 55-kDa protein is located there. However, the evidence for this is circumstantial, since the antibody was not specific for the 55-kDa protein.

The putative matrix fraction, pellet II, consisted of a heterogeneous mixture of components, as revealed by multiple peaks in sucrose density gradients, the presence of material that had the solubility properties and electrophoretic mobility of LPS, and the presence of nucleic acid. Perhaps the putative LPS represented contamination with fragments of outer membrane. However, the stained gels suggested that the total LPS contained a fraction lacking oligosaccharide (i.e., consisting of simply lipid A and core) and that the material in pellet II did not have LPS lacking oligosaccharide. Perhaps the oligosaccharide portions of the LPS extend into the matrix, resulting in some LPS becoming bound to the isolated matrix material. The data presented do not rule out the possibility that the LPS is a component of the matrix or fibrils. The 55-kDa protein was found primarily in only one of the peaks separable by sucrose gradient centrifugation, suggesting a method for isolating the protein and possible associated structure in bulk amounts for further analysis.

An intriguing result was the finding that the 55-kDa protein was immunologically related to a 65-kDa protein located on the fibrils of another myxobacterium, *M. xanthus*. Affinity-purified anti-55-kDa protein stained the *M. xanthus* 65-kDa protein, and an MAb to the *M. xanthus* 65-kDa protein stained the *S. aurantiaca* 55-kDa protein (although weakly). The antibodies stained several other polypeptides as well,

suggesting common epitopes. Without further study, one cannot know whether the common epitopes reflect similar modifications of unrelated proteins (e.g., addition of saccharides) or similarities in the primary structure of the proteins. Data from this laboratory and others suggest that these proteins play a role in gliding motility and cohesion (1, 2, 9, 15, 24).

Although most of the literature on the physiological effects of calcium has been concerned with eucaryotic organisms, there is growing evidence that calcium has important effects on procaryotes. For example, there are at least two other examples of calcium in the growth medium influencing the chemical composition and structure of bacterial surfaces. In yersiniae, calcium represses the synthesis of temperature-induced outer membrane proteins (5). In *Rhizobium leguminosarum*, the synthesis of a 32-kDa protein subunit of flagella and an 18-kDa protein present in crude flagellin preparations, as well as a cell surface adhesin, are stimulated by calcium ion (20).

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