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Caulobacter crescentus Cell Envelope: Effect of Growth Conditions on Murein and Outer Membrane Protein Composition

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The murein and membrane protein compositions of *Caulobacter crescentus* strains CB13B1a and CB15 have been characterized, and the influence on cell envelope constituents of culture conditions which affect morphogenesis have been studied. Amino acid and amino sugar analysis of murein sacculi revealed a simple $A1_\gamma$ murein configuration typical of gram-negative bacteria. The membranes of *C. crescentus* had low levels of 2-keto-3-deoxyoctonate relative to enteric bacteria, in addition to the absence of lipid A components (Shapiro et al., Science **173:** 884-892, 1971; Chow and Schmidt, J. Gen. Microbiol. **83:**369-373, 1974). Nevertheless, *C. crescentus* membranes could be fractionated into inner and outer membrane components by sucrose density gradient centrifugation procedures developed for *Escherichia coli.* The proteins of the outer membrane were distributed between three major (I, II, and III) and two minor (IV and V) protein classes. Class I proteins were ≥74,000 daltons and constituted the primary proteins of the outer membrane. Class I proteins were separated into approximately 50 polypeptides by two-dimensional gel electrophoresis; the protein composition of this class was affected by culture conditions in both CB13B1a and CB15. Class II (47,000 to 39,000 daltons) and III (20,000 to 11,500 daltons) proteins differed in each strain in composition and response to culture conditions.

The bacterial cell envelope constitutes the primary interface for sensing environmental change and transducing this information to the genome where appropriate cellular responses are elicited. In the gram-negative bacterium *Caulobacter crescentus,* different culture conditions such as phosphate limitation or changes in carbon source result in altered expression of surface structures and arrest of the developmental program (18, 22). In addition, morphological changes in the cell envelope and appendant structures are the principal manifestation of the *Caulobacter* cell cycle (16, 21). In studying morphogenesis in *Caulobacter,* it was thus important to characterize the effects of growth conditions on cell envelope constituents to distinguish between those changes induced by culture conditions versus those which occur during development. We report here the initial biochemical characterization of the cell envelope of two principally studied *Caulobacter* strains, *C. crescentus* CB15 and CB13Bla. These studies indicate that the outer membrane of *C. crescentus* is different from that of enteric bacteria such as *Salmonella* and *Escherichia* and that its components can reflect changes in the growth conditions of the cells.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *C. crescentus* wild-type strains CB15 and CB13Bla were used in these studies. Cells used for sacculi or membrane fractionations were grown at 30°C with shaking in either peptone-yeast extract medium (PYE) (1, 16) or in minimal salts medium supplemented with 1% Hutner modified mineral base (22) and 0.2% glucose (HMG) or 0.2% lactose (HML). Stalk elongation medium (WPYE) contained PYE supplemented with 0.2% glucose-0.05% NH_4Cl-1% Hutner modified mineral base. Solidified medium also contained 1% agar (Difco). Heterogeneous mid-exponential-phase (optical density at 660 nm = 0.5 to 0.7) cultures were used in these studies. In some experiments, cells were harvested after 6 h of growth in lactose, when the population exists mainly as predivisional intermediate cells and are referred to as lactose-blocked (HML-Bl) cultures (22).

Isolation of murein sacculi. Murein sacculi were prepared by adaptation of the procedures of Schwarz et al. (19). Late exponential-phase cells (optical density at 660 nm = 0.8 to 1.0) were collected by centrifugation at 12,000 rpm for 15 min in a Sorvall GSA rotor and stored as frozen cell pellets. Cells (0.5 to 2.0 g) were thawed and suspended in 8 to 12 ml 0.1 M $MgSO_4$ containing 50 μg of DNase I (Worthington) per ml. Acid-washed glass beads (0.17-mm diameter) were added to the cell suspension (1 g/ml) and mixed

in a Sorvall Omnimixer for 20 min at speed-control settings of 5 to 7.5. Cell disruption was assessed by phase-contrast microscopy. Empty cell walls were separated from glass beads by centrifugation in a clinical centrifuge for 5 min. Cell walls were pelleted in a Sorvall SS-34 rotor at 18,000 rpm for 40 min, and the pellet was suspended in 5 ml of water. All of the above procedures were carried out at 4°C. The cell wall suspension was added dropwise to 30 to 50 ml of boiling 4% (wt/vol) sodium dodecyl sulfate (SDS). After addition was complete, the solution was boiled with constant stirring for 5 min, and then stored overnight at room temperature.

The SDS was removed from the murein sacculi preparations by five successive washings of the resuspended pellets in water (Sorvall SS-34 rotor at 18,000 rpm for 70 min). The first three washes were done at room temperature; the temperature was lowered in subsequent washes so that the last centrifugation was at 4°C. The final pellet was resuspended in 1 to 2 ml of water and treated first with 10 μg of Pronase per ml at 37°C for 30 and 45 min, respectively. The enzymatically treated sacculi suspension was washed twice, as described above; the resulting pellet was suspended in 10 ml of 1% deoxycholic acid (membrane [Millipore Corp., 0.45 μm HA] filtered) and incubated at 30°C for 30 min in a shaking water bath. The murein sacculi were then washed two or three times more with water by resuspending the pellet and centrifuging as above. The final pellet was stored frozen.

Amino acid analysis. Samples were hydrolyzed for 2, 6, and 12 h; at 5 to 6 h, hydrolysis of peptidoglycan was essentially complete with minimal destruction of amino sugar moieties. Accordingly, purified sacculi were hydrolyzed under vacuum at 100°C for 5 to 6 h in 2.0 ml of constantly boiling HCl. HCl was removed by rotoevaporation, and the dried murein hydrolysate was stored frozen prior to amino acid analysis. Analysis was carried out with a Beckman model 120 C analyzer, using the technique of Spackman et al. (23).

Preparation of cell envelope membrane fractions. Outer and cytoplasmic membranes were prepared according to the general procedure described by Koplow and Goldfine (10), except that the initial cell homogenization to remove capsular and flagellar material was eliminated. Late exponential-phase cells were collected by centrifugation at 12,000 rpm for 15 min in a Sorvall GSA rotor, and washed once in 50 mM tris(hydroxymethyl)aminomethane-hydrochloride containing 1 mM ethylenediaminetetraacetic acid at pH 7.8. This initial washing resulted in the release of approximately 10% of the cellular lipopolysaccharide (LPS) as determined by crossed immunoelectrophoresis (4). Pellets of 2 to 4 g were stored frozen.

To separate outer from cytoplasmic membranes, 15 to 25 mg of total membrane protein was layered onto a discontinuous sucrose gradient containing 5 ml of 2.02 M sucrose (enzyme grade, Schwarz/Mann), 17 ml of 1.44 M sucrose, and 12 ml of 0.77 M sucrose made in HEPES (10 mM *N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid) (Sigma Chemical Co.), pH 7.4. Gradients were centrifuged at 25,000 rpm for 16 h in a Spinco SW27 rotor. Fractions (10 drops, approximately 0.8 ml) were collected dropwise from the bottom of the gradient. Peak fractions were pooled and diluted approximately 10-fold in 10 mM HEPES, pH 7.4, and processed as described previously (10). The washed pellets were suspended in 0.3 ml of distilled water. All of the above procedures were carried out at 4°C.

Electrophoretic techniques. Fractionated membrane proteins were analyzed in one dimension by SDS-polyacrylamide slab gel electrophoresis (SDS-PAGE) and also by two-dimensional gel electrophoresis. For one-dimensional analyses, 10% acrylamide slab gels were prepared by the general procedure of Laemmli (11) and poured into a slab gel apparatus as described by Studier (24), but with the procedural modification of Hopper et al. (8). The resolving gels were 160 mm in length. Membrane fractions were suspended in SDS sample buffer, heated for 3 min in a boiling water bath, and applied to the gel after cooling to room temperature. The migratory behavior in gels of any of the protein preparations was not affected by the length of boiling in the SDS sample buffer. Slab gels were run at 17 mA.

Two-dimensional gel electrophoresis was carried out by the technique of O'Farrell (14) as adapted for membrane proteins by Ames and Nikaido (2). Isoelectric focusing was performed at 400 V for 16 h followed by 500 V for 2 h. The second dimension utilized 10% SDS-polyacrylamide slab gels, 2.5 mm in thickness and 100 mm in length; gels were run at 400 mA. Gels were stained for 1 h in the solution described (14).

Quantitative determinations. Protein content was determined by the method of Lowry et al. (12), using bovine serum albumin (Sigma) as standard. 2-Keto-3-deoxyoctonate (KDO) content was assayed (15), using 0.8 to 1.0 mg of membrane protein per assay.

Enzymatic assays. Succinic dehydrogenase (SDH) activity was assayed colorimetrically by following the reduction of 2,6-dichlorophenol indophenol in a phenazine methosulfate-coupled system (6). A molar extinction coefficient [ϵ_M (cm^{-1} mol^{-1} × 10^{-3})] of 21 at 600 nm, pH 7.5 (3), was used to calculate specific activity.

RESULTS

Murein composition of CB13Bla and CB15. The amino acid and amino sugar composition of CB13Bla and CB15 are shown in Table 1. Alanine, glutamic acid, and diaminopimelic acid (DAP) are present in molar ratios of 2:1:1, respectively. Muramic acid and glucosamine are present in approximately 1:1 ratios with respect to each other and 1:2 with respect to alanine. These data are consistent with a simple, direct cross-linkage-type murein structure commonly found in a widespread variety of gram-negative bacteria and designated $A1_\gamma$ in the nomenclature of Schleifer and Kandler (17). The molar ratios of some other amino acids detected in our preparations are also listed in Table 1; slightly elevated levels of glycine are consistently found. The molar ratios of the remaining amino acids were below those deter-

TABLE 1. *Amino acid and amino sugar composition of C. crescentus murein*

Amino acid or amino sugar	Molar ratio[a] for strain:	
	CB15	CB13Bla
Ala	2.00	2.00
Glu	1.04	1.04
DAP	1.01	1.03
Muramic acid	0.98	0.92
Glucosamine	0.84	0.78
Gly	0.21	0.22
Asp	0.14	0.12
Ser	0.11	0.09
Thr	0.04	0.05
Leu	0.14	0.09

[a] Molar ratio is expressed relative to alanine at 2.0. Values are derived from the average of three determinations.

mined for threonine and are not included in Table 1.

A basic feature of gram-negative murein composition is its stability and independence of composition relative to growth stages and environmental factors (17). Since the manipulation of culture conditions is used in *Caulobacter* studies for achieving cell synchrony (20), arrest of growth and morphogenesis (22), and induction of stalk elongation (18), the murein composition was analyzed in sacculi prepared from cultures grown under each of these special conditions (Table 2). In all cases, murein sacculi retained the morphological characteristics of *Caulobacter* stalked and swarmer cells. The murein composition remains constant whether cells are grown in WPYE or morphogenesis is blocked at the elongated stalked cell stage (HML-Bl) (Table 2). Interestingly, both CB13Bla and CB15 cells grown in HMG have significantly increased molar ratios of glutamic acid in hydrolyzed sacculi preparations. This observation may be related to an accumulation of polyglutamic acid under these particular growth conditions rather than changes in murein structure (9); this question is under further study. The presence of high molar ratios of glutamic acid, however, appears to be related to growth in minimal glucose medium HMG, and not to the presence of glucose (WPYE) or to minimal nutrient conditions (i.e., HML) per se.

Fractionation of cell envelopes. Total cell membrane fractions, prepared as described above from CB13Bla and CB15 cells grown under a variety of nutrient conditions, were separated into cytoplasmic and outer membrane fractions on discontinuous sucrose gradients. Membrane fractionations of CB13Bla or CB15 cells produced two bands, similar to those described for *Escherichia coli*. Results of a typical membrane fractionation of CB15 cells grown in WPYE, PYE, HMG, and HML are shown in Fig. 1. A dense, white band formed in the lower portion of each gradient corresponds with outer membrane; a reddish-colored band in the less-dense region of the gradient corresponds with the cytoplasmic membrane. The relative yield of the inner and outer membrane varied with the preparation, but peaks were routinely well separated, and the average density of the membrane fractions was not significantly altered by growth conditions.

The quality of inner membrane contamination in outer membrane preparations was assessed by measurement of SDH activity in the outer membrane fractions. Table 3 documents typical SDH activities obtained from preparations of this type; approximately 80% of the total SDH activity was recovered in the two peak membrane fractions. In general, separations of the CB15 membranes resulted in slightly less cytoplasmic contamination of the outer membrane fraction than in similar CB13Bla preparations. Rebanding of isolated outer membrane preparations on sucrose density gradients did not substantially change the quality of separation as assessed by SDH activity.

When inner membrane samples were assayed for outer membrane contamination by measurement of KDO, using the technique of Osborn et al. (15), it was determined that the overall KDO levels in *Caulobacter* were approximately 10 to 15 μg of KDO per mg of total membrane protein; the reaction product of KDO in *Caulobacter*

TABLE 2. *Effect of growth media on murein composition*

Amino acid or amino sugar	Molar ratio of strain:				
	CB15				CB13Bla
	WPYE	HML	HML-Bl	HMG[a]	HMG[a]
Ala	2.00	2.00	2.00	2.00	2.00
Glu	0.96	1.08	1.38	4.11	2.98
DAP	0.90	1.44	1.18	0.92	1.03
Muramic acid	0.72	0.88	0.93	0.78	0.94
Glucosamine	0.53	0.52	0.57	0.71	0.78
Gly	0.21	0.24	0.45	0.51	0.07
Asp	0.10	0.17	0.22	0.33	0.05
Ser	0.09	0.10	0.26	0.35	0.03
Thr	0.08	0.06	0.12	0.17	0.03
Leu	0.18	0.14	0.30	0.34	0.07

[a] Values reported are the average of two determinations.

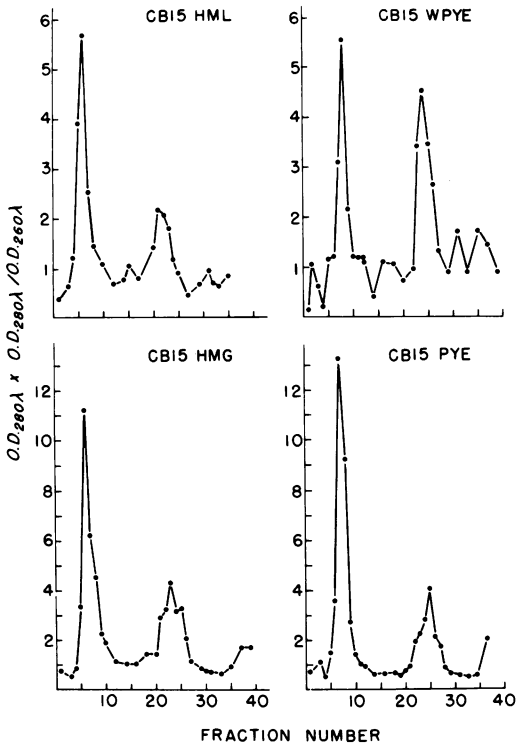

FIG. 1. *Protein profiles of total membranes fractionated by sucrose density gradient centrifugation as described in Materials and Methods. CB15 preparations were obtained from cells grown under a variety of culture conditions as described in the text. The direction of sedimentation is from right to left. Data is plotted according to the convention of Koplow and Goldfine (10).*

membranes had an identical absorption spectrum as authentic KDO in the assay. The KDO levels detected in *Caulobacter* are thus approximately two-thirds lower than those reported for rough mutants of *Salmonella* and approximately 10-fold less than wild-type *S. typhimurium* (15). The KDO assay was therefore impractical for assessing contamination of cytoplasmic membrane by outer membrane due to the large sample volumes needed for its detection. Assessment of the KDO levels was further complicated by the release of approximately 10% of the *Caulobacter* LPS after treatment with 50 mM tris(hydroxymethyl)aminomethane-hydrochloride and 1 mM ethylenediaminetetraacetate, pH 8.0, in initial stages of the membrane fractionation procedure. Immunological assay, however, did not reveal significant levels of LPS in the cytoplasmic membrane fractions (data not shown).

Outer membrane proteins. The proteins of the fractionated outer membrane were analyzed by one- and two-dimensional gel electrophoresis as described in Materials and Methods. The one-dimensional (SDS-PAGE) protein profiles of CB13Bla and CB15 outer membranes prepared from cells grown under various culture conditions are shown in Fig. 2A and B, respectively. The outer membrane proteins may be divided into five classes: the major proteins fall into three classes, those greater than or equal to 74,000 daltons are designated class I, those in the range of 47,000 to 39,000 daltons, class II, and those in the range of 20,000 to 11,500 daltons, class III. Minor protein components are also resolved at 63,000 to 54,000 daltons (class IV) and 28,000 to 13,000 daltons (class V).

The primary contribution to the outer membrane proteins is derived from class I. Within this class the 74,000- and 81,000-dalton protein bands are represented in both strains under all culture conditions tested. The remaining proteins form a heterogeneous group of protein bands whose relative proportion and complexity varies with culture conditions. Growth in HML, for example, results in the appearance of a prominent protein band of approximately 110,000 daltons in both CB13Bla and CB15.

Proteins of class II display different responses to growth conditions in each of the strains. In CB15 there are three proteins, 47,000, 45,000, and 39,000 daltons, which are found in cells grown in any of these media. In CB13Bla, however, growth in lactose results in the appearance of a protein at 47,000 daltons and an increased recovery of protein component at 39,000 daltons which appears to consist of at least two protein bands under these conditions; the presence of glucose in the growth media is correlated with the recovery of the 45,000-dalton protein and an apparent loss of the 47,000-dalton protein.

The 11,500-dalton class III protein is found in both CB13Bla and CB15 strains in all growth media tested. The 20,000-dalton protein species is recovered primarily from CB13Bla, where it is a major membrane protein. A protein of this molecular weight is also present, but in very low levels, in CB15 outer membrane preparations.

TABLE 3. *SDH activity in fractionated membranes of CB15*

Growth conditions	SDH[a] (μmol/min per mg of protein)		
	Membrane		
	Total	Outer	Inner
WPYE	101.5	19.0	150.3
PYE	24.1	13.3	52.1
HMG	16.7	3.2	47.6
HML	87.0	50.1	154.5

[a] Values represent specific determinations in samples obtained from membrane fractionations shown in Fig. 1.

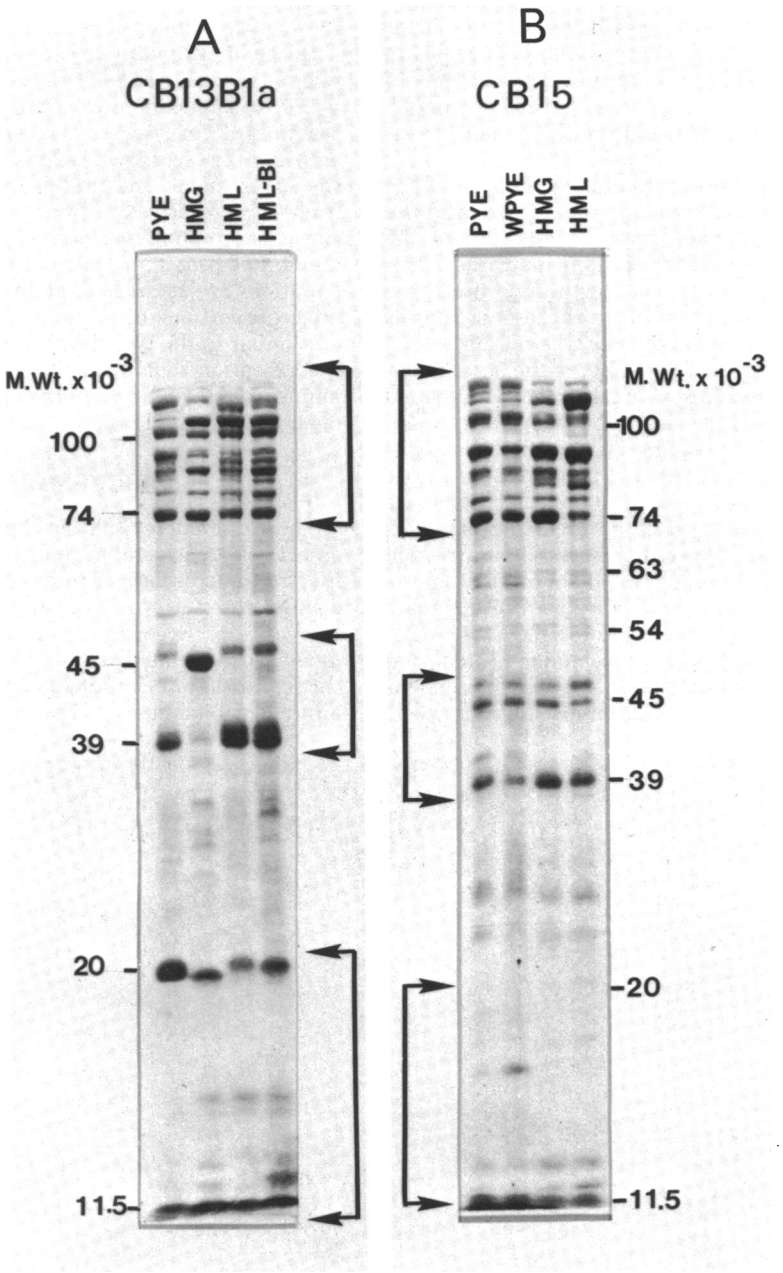

FIG. 2. *One-dimensional SDS-PAGE of outer membrane proteins. Outer membranes were obtained from peak fractions of sucrose density gradients; proteins of these outer membrane fractions were displayed by SDS-PAGE of samples (20 μg/slot) as described in Materials and Methods. (A) Outer membrane proteins of CB13B1a obtained from cells grown in PYE, HMG, HML, and HML-Bl. (B) Outer membrane proteins of CB15 obtained from cells grown in PYE, WPYE, HMG, and HML. Roman numerals I, II, and III refer to the major protein classes described in text.*

The minor outer membrane protein components have been divided into two general classes on the basis of molecular weight. Class IV proteins in the 63,000- and 54,000-dalton range are found in both strains. The remaining minor protein constituents are found in class V. Proteins

which fall into this class have been found at 28,000 to 25,000, 17,000 to 16,000, and 14,000 to 13,000 daltons in both strains. The proteins of classes IV and V have not been studied in detail; in general their occurrence in the membranes is not correlated with culture conditions.

Class I proteins are the major proteins of the *C. crescentus* outer membrane and are also the most heterogeneous class. In addition to the complexities of class I protein patterns introduced by variations in growth conditions, the protein bands are difficult to resolve in this molecular-weight range. For this reason, an assessment of the heterogeneity of the outer membrane proteins was made by two-dimensional electrophoresis, as described in Materials and Methods. The proteins of the CB13Bla outer membranes analyzed by these methods are shown in Fig. 3. The proteins of class I are resolved into at least 50 different protein spots by this method. The major 74,000-dalton protein band, which is found in all outer membrane preparations from both strains, appears upon two-dimensional electrophoresis (SDS-PAGE) to be composed of a single major protein and two to three minor protein species; the remaining bands on SDS-PAGE exhibit more complexity upon two-dimensional electrophoresis.

Inner membrane proteins. The cytoplasmic membrane fractions appear to be enriched by 70 to 85% as determined by immunological assay of LPS in cytoplasmic membrane fractions and, in some cases, by KDO assay. Nevertheless, the inner membrane protein profiles obtained from *C. crescentus* by either one- or two-dimensional electrophoresis closely resemble the outer membrane protein pattern. The major class distributions are retained, and, in general, the relative proportions of proteins within each class are similar in the two membrane fractions, with the exception that there are a large number of minor proteins which contributed to the inner membrane protein profile.

DISCUSSION

The *Caulobacter* cell envelope has been the object of considerable morphological study. Electron microscopy of thin-sectioned cells has demonstrated a typical gram-negative cell envelope structure consisting of a unit cytoplasmic membrane (6.0-nm diameter) and a multilayered cortical structure containing a murein layer (4.0 nm) and a bilaminar outer cell membrane (16).

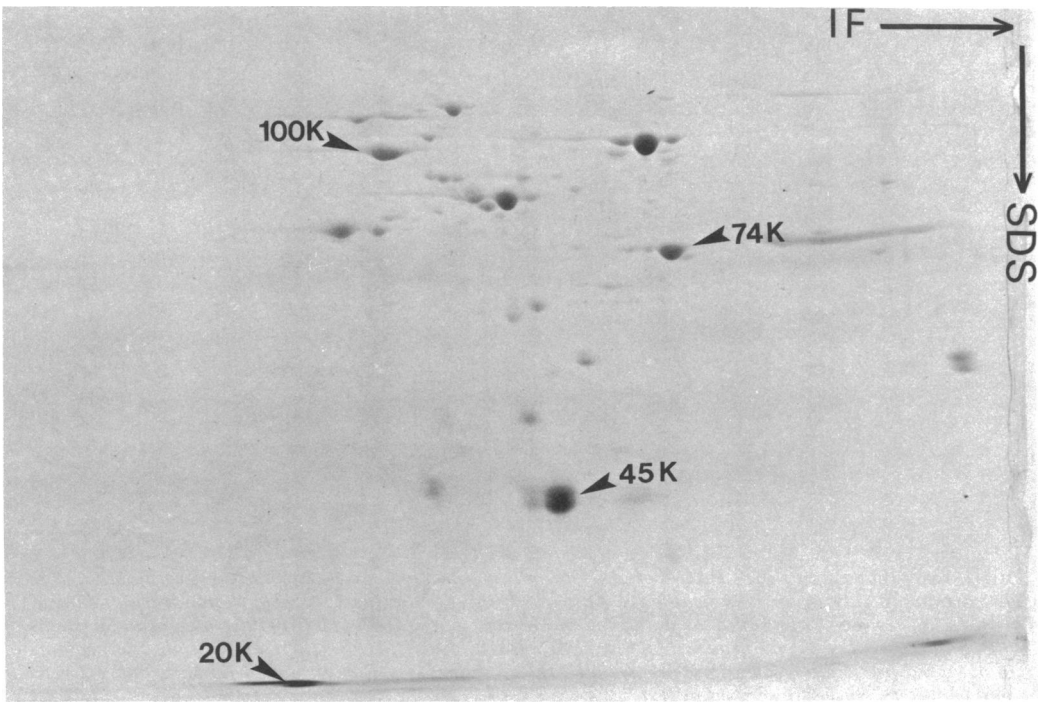

FIG. 3. *Two-dimensional gel electrophoresis of CB13B1a outer membrane proteins. Outer membrane fractions were prepared from CB13B1a cells grown in HMG, and the proteins were resolved by two-dimensional gel electrophoresis as described in Materials and Methods. IF, Isoelectric-focusing dimension; SDS, SDS-PAGE dimension. Some major membrane proteins are indicated by arrows and molecular weight.*

In this report, a study of the biochemical components of this structure has been initiated.

Murein sacculi which retained characteristic cell structure after purification were hydrolyzed, and the amino sugar and amino acid composition of *C. crescentus* murein was analyzed. The composition obtained is consistent with the simple $A1_\gamma$ murein structure which consists of alanine, glutamic acid, and DAP in a ratio of 2:1:1, respectively; this murein form is commonly found among gram-negative bacteria. Despite the fact that culture conditions can affect stalk length (WPYE) and block cell development (HML) (18, 22), no differences in the murein composition were detected under these conditions. Goodwin and Shedlarski (7) have reported the complete absence of DAP in swarmer cell populations and a 50% reduction of DAP (relative to stalked cells) in predivisonal cell populations in a related strain, CB2. Cell populations were prepared by these investigators by manipulations of culture conditions, which include all those tested in this study. It is likely that their results are attributable to the activity of murein hydrolase in this strain (P. Gill, Jr., and J. G. Shedlarski, Jr., Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, K153, p. 271). The murein isolated from either CB13Bla or CB15 cells grown in HMG contained significantly higher molar ratios of glutamic acid. Since the structure of the murein sacculus is preserved during purification, intracellular polyglutamic acid homopolymers could be copurifying with the sacculi and thus not be structural components of the sacculus itself. The accumulation of very large poly-D-glutamic acid homopolymers under minimal growth conditions has been reported in gram-positive organisms, especially *Bacillus* species (9). The existence of such a polymer in gram-negative bacteria has not been documented, and this observation may be of interest in that regard.

The total membrane of *C. crescentus* may be fractionated into inner and outer membranes by techniques used for *E. coli* despite the fact that the *Caulobacter* cell envelope is quite different from that of *E. coli* in its lack of lipid A (5, 21) and very low levels of KDO in the outer membrane; this suggests an unusual alteration of LPS structure not typically found in gram-negative organisms. A highly antigenic carbohydrate-containing component has been isolated from *Caulobacter* by aqueous-phenol extraction (21; C. Lagenaur and N. Agabian, unpublished); this component has been partially purified and characterized as LPS (21); however, its mode of attachment or insertion in the outer membrane, in the absence of the inner core LPS components, is unknown. In this context, it should be mentioned that several SDS-PAGE bands in the class I region of both inner and outer membrane proteins are stained with periodic acid-Schiff reagent, suggesting some of the membrane proteins may be modified by carbohydrate.

The outer membrane proteins of *Caulobacter* fall into three major classes on the basis of molecular weight. The proteins of class I are $\geq$74,000 daltons; within each strain, it appears that this class may be further divided into two subclasses, those from 74,000 to 95,000 daltons and those $\geq$100,000 daltons. Although the relative number and proportion of these proteins varies between strains and with growth conditions, the staining patterns suggest that there may be a coordinate modulation of these protein components. These peptides may form structural arrays within the membrane, the constituents of which are modified by growth conditions.

Because of the complexity of the class I proteins, it is difficult to determine the extent of de novo protein synthesis versus changes in the relative amounts of existing proteins upon alteration of culture conditions. It has been reported (13) that the relative proportion of membrane proteins in *E. coli* K-12 changes upon alteration of growth conditions. The de novo synthesis and/or induction of specific proteins in *C. crescentus* is suggested by the SDS-PAGE protein profiles. This is especially notable when comparing class I and II proteins in CB13Bla cells grown in HMG and HML. Identification of such proteins could form the basis for studying the mechanism of protein insertion in the outer cell membrane in this system. The identification and quantitation by one- and two-dimensional electrophoresis of proteins synthesized under variable culture conditions is in progress.

The response to altered culture conditions in protein species of class II and III appears for the most part, to be different in these strains. In CB13Bla there is a striking alteration in the class II proteins in each of the culture conditions. In CB15, on the other hand, this protein class is relatively unaffected by growth conditions. The relative recovery of the 47,000- and 39,000-dalton proteins in CB15 varies, however, from one preparation to another. In pulse-labeling studies, to be reported elsewhere, it appears that the presence of the 39,000-dalton protein in CB15 strains is inversely related to the levels of 47,000-dalton protein, suggesting that it may be a cleavage product; the relative amounts of the 45,000-dalton class II protein in CB15 remains unaltered. We have not examined the possibility that the 45,000- and 39,000-dalton species in CB13Bla are related by a similar relationship. The class III proteins in CB15 and CB13Bla do

not, in general, fluctuate under these conditions; however, small but reproducible changes in the migration of the CB13Bla 20,000-dalton protein does occur and may be indicative of covalent modification.

Further resolution of the outer membrane proteins by two-dimensional electrophoresis emphasizes the complexity of the polypeptides found in this preparation. The class I proteins which appear to contain 8 to 15 polypeptides in one dimension, are resolved into 50 or more proteins upon analysis in two-dimensional gel systems.

Characterization of the membrane proteins from *Caulobacter* stalked and swarmer cells and the isolated stalk reveals changes in the class I proteins between cells of different morphology (N. Agabian, M. Evinger, C. Lagenaur, and G. Parker, submitted). In view of these findings and those discussed in this report, it becomes imperative that comparisons of membrane components during morphogenesis be made between cell preparations obtained under identical growth conditions.

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