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Permeability of the Boundary Layers of *Bdellovibrio bacteriovorus* 109J and Its Bdelloplasts to Small Hydrophilic Molecules

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Measurements of the sucrose-permeable and -impermeable volumes during *Bdellovibrio bacteriovorus* attack on *Escherichia coli* or *Pseudomonas putida* showed that the volume of the bdelloplast increased over that of the substrate cell. Although the pattern of the increase differed with the two organisms, the volumes reached maximum at about 60 min into the bdellovibrio growth cycle. By this time, the cytoplasmic membranes of the attacked cells were completely permeable to sucrose. The kinetics of increase in sucrose-permeable volumes were similar to the kinetics of attachment and penetration (Varon and Shilo, J. Bacteriol. **95**:744–753, 1968). These data show that the original cytoplasmic and periplasmic compartmentalization of the substrate cell ceases to exist with respect to small hydrophilic molecules during bdellovibrio attack. In contrast, the effective pore size of the outer membrane of the substrate cell to small oligosaccharides remains unaltered during bdelloplast formation as was shown by direct measurements of its exclusion limits. The major porin protein of *E. coli*, OmpF, was recoverable from the bdelloplast outer membrane fraction until the onset of lysis. The Braun lipoprotein was removed from the bdelloplast wall early, and OmpA was lost in the terminal part of the bdellovibrio growth cycle.

Bdellovibrio bacteriovorus has an obligatory two-stage life cycle consisting of an attack phase and a growth phase. The growth phase is initiated by an attack on a suitable gram-negative bacterium that will serve as the substrate for bdellovibrio growth. The attack is completed by the penetration of the bdellovibrio through the outer membrane and peptidoglycan layer into the periplasm of the substrate cell. The complex of a substrate cell penetrated by a bdellovibrio has been termed the bdelloplast (18). It is a unique environment in which a bdellovibrio has exclusive access to a rich source of nutrients and within which it grows with an unusually high efficiency (17).

The bdelloplast has four morphologically distinct boundary layers: the bdelloplast wall derived from the wall layers of the substrate cell; what was the cytoplasmic membrane of the substrate cell; and the wall and cytoplasmic membrane of the included bdellovibrio. The permeability characteristics of these layers must regulate the potential for exchange of materials between the growing bdellovibrio, its growth chamber, and the environment external to the bdelloplast. It can be inferred from indirect evidence that the outer membrane of the bdellovibrio, within the bdelloplast, is permeable to fatty acids, amino acids, and nucleoside monophosphates, since these exogenously supplied molecules are incorporated into the macromolecules of the growing bdellovibrio (20). It is also known that the outer membrane of the bdellovibrio permits entry of penicillin and lysozyme since either compound, without pretreatment of the cell, will cause the conversion of the free-living bdellovibrio to a peptidoglycan-free spheroplast (23). The mechanism(s) responsible for these permeability characteristics has not been investigated.

With respect to the substrate cell-derived boundary layers, it is clear from previous studies that both are altered structurally and functionally early in bdellovibrio attack (20). The cytoplasmic membrane of the substrate cell becomes permeable to lactose, and its respiratory potential is eliminated (19). Its banding characteristics on sucrose gradients change markedly, and there is a rapid release of $^{42}K^+$ from prelabeled substrate cells (6). The lipopolysaccharide of its outer membrane is partially degraded (14). Its peptidoglycan is deacetylated, and long-chain fatty acids are covalently attached to the structure (25, 26). There is also evidence that the Braun lipoprotein originally covalently linked to peptidoglycan is removed or degraded early in the bdellovibrio growth cycle (25).

The effects of these, and possibly other, alterations during bdelloplast formation and subsequent intraperiplasmic growth on the permeability of the substrate cell boundaries have not been fully characterized. In this paper, we present data that more specifically define the permeability of the cytoplasmic membrane of the substrate cell and the exclusion limit, i.e., the effective pore size, of its outer membrane for hydrophilic oligosaccharides before and after bdellovibrio attack. In addition, the effect of bdelloplast formation on the outer membrane protein profile of the substrate cell is described. Data for hydrophobic molecules are given in the following paper (2).

MATERIALS AND METHODS

Organisms and growth procedures. *B. bacteriovorus* 109J was the experimental organism. *Escherichia coli* strains ML35 and K-12 and *Pseudomonas putida* N15 served as substrate cells for bdellovibrio growth.

Substrate cells used for synchronous single cycle bdellovibrio cultures were grown overnight (16 h) at 30°C in Difco nutrient broth (8 g/liter) supplemented with yeast extract (5 g/liter) (pH 7.6) and harvested by centrifugation. The substrate cells were washed twice with HM buffer (10^{-3} M *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid [HEPES], 10^{-3} M $CaCl_2$, and 10^{-4} M $MgCl_2$, adjusted to pH 7.6 with NaOH) and resuspended to the desired cell density in the same buffer. To maintain bdellovibrio stock cultures and to obtain cells for experimental purposes, *E. coli* ML35 cul-

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tures, grown overnight as described above, were diluted with 1.5 volumes of HM buffer and inoculated with bdellovibrios. The cultures were reincubated overnight at 30°C by which time essentially all *E. coli* cells were lysed. The progeny bdellovibrios were harvested, washed, and suspended in HM buffer as described above for the substrate cells. Cell numbers of the various suspensions were determined from turbidity measurements by reference to standard curves based on plaque assays (27) for *B. bacteriovorus* and colony counts on nutrient agar plates of the substrate organisms.

Synchronous, single cycle cultures were initiated by mixing buffer suspensions of bdellovibrios and substrate cells to give final concentrations of about 6×10^{10} bdellovibrios and 3×10^{10} substrate cells per ml. Cultures were incubated at 30°C with shaking in a water bath. Lysis of *E. coli* bdelloplasts in such cultures was first observed at about 135 min and was essentially complete at about 180 min. The cycle was somewhat slower for *P. putida* and was not completed until about 210 min.

Determination of membrane permeability. The permeability characteristics of the outer and cytoplasmic membranes of the bdellovibrio, the substrate cell, and corresponding structures in the bdelloplast were determined by measuring the distribution of paired radioactive molecules between cell compartments and the surrounding aqueous environment, as described by Decad and Nikaido (3). A washed suspension containing either 6×10^{10} substrate cells, 16×10^{10} bdellovibrios, or the cells and bdelloplasts in 1 ml of a synchronous culture was centrifuged in a 1.5-ml microcentrifuge tube at $5,000 \times g$ for 5 min in a table top centrifuge (Fisher Scientific Co., Pittsburgh, Pa.). The supernatant fluid was discarded, and 0.5 ml of a mixture of one of three types of paired radioactive molecules (^{14}C, 0.15 μCi; ^{3}H, 0.4 μCi) was added to the sedimented cells (cell pellet): [^{14}C]sucrose and [^{3}H]dextran (16,000 daltons); [^{14}C]sucrose and [$^{3}H_2O$]; or [^{14}C]sucrose and [^{3}H]oligosaccharide. The cell pellet was mixed with the solution of labeled compounds by vortexing for 30 s and the suspension was allowed to equilibrate for 5 min at either room temperature or 4°C. The suspension was then recentrifuged for 5 min at $5,000 \times g$, and the radioactivity (counts per minute per microliter) of the supernatant fluid (supernatant I) was determined by scintillation counting. The cell pellet was resuspended to 1 ml of total volume in HM buffer and incubated for 10 min at either room temperature or 4°C. The suspension was recentrifuged as described above, and the radioactivity of the total supernatant fluid (supernatant II) was determined.

The volume in the cell pellet occupied by a particular test compound was calculated from the following formula:

$$\text{volume occupied (microliters)} = \frac{\text{counts per minute (total supernatant II)}}{\text{counts per minute/microliter (supernatant I)}}$$

For the organisms used, the outer membranes were impermeable to 16-kilodalton dextran and the cytoplasmic membranes were impermeable to sucrose, whereas both membranes were freely permeable to tritiated water. Thus, the sucrose minus dextran pellet volume was the sucrose-permeable cell volume and, by definition, the volume of the periplasmic space. Similarly, the sucrose-impermeable cell volume, i.e., the cytoplasmic compartment, was the difference between the tritiated water and the [^{14}C]sucrose pellet volumes. The sum of the sucrose-permeable and -impermeable volumes equaled the total cell volume. The oligosaccharide-permeable volumes were determined by subtracting the sucrose minus oligosaccharide volume from their sucrose minus dextran volume. The percent penetration of a test oligosaccharide was defined as 100 times the ratio of the oligosaccharide-permeable volume to the sucrose-permeable volume.

Isolation of outer membrane proteins and polyacrylamide gel electrophoresis. Outer membranes were isolated from *E. coli* and its corresponding bdelloplasts and from newly released intraperiplasmically grown bdellovibrios by the method of Diedrich et al. (5) based on Triton X-100 insolubility of the outer membrane. Electrophoresis of the associated membrane proteins was done as described by Laemmli (10) with a 1-mm slab gel with a linear gradient of 7.5 to 15% acrylamide and 1.6% bisacrylamide in the separating gel. Gels were stained with Coomassie blue R250 (7).

Radioactive chemicals. [$U^{14}C$]sucrose, [^{3}H]raffinose, and [methoxy-^{3}H]dextran were purchased from New England Nuclear Corp, Boston, Mass.; [^{3}H]stachyose and [^{3}H]verbascose were provided by H. Nikaido, University of California, Berkeley. Radioactive impurities were removed by gel filtration (13).

Liquid scintillation counting. All samples were counted in PCS solubilizer scintillation fluid (Amersham Corp., Arlington Heights, Ill.) with an LS200 scintillation spectrometer (Beckman Instruments, Inc., Irvine, Calif.).

RESULTS

Sucrose-permeable and -impermeable volumes of cells and bdelloplasts. Table 1 shows the results of a typical experiment in which the volumes occupied by three radioactive probes were measured in cells sedimented from a starting culture and from a 60-min synchronous single cycle bdellovibrio culture. The dextran cell pellet volume was essentially the same in the two samples. This result showed that the substrate cell outer wall remained impermeable to dextran after bdellovibrio attack and that the packing of the cells in the sediments had not changed significantly despite the change in shape of the attacked cells from rods to spheres during bdelloplast formation. Thus, the observed increases in the [^{14}C]sucrose and $^{3}H_2O$ pellet volumes and the decrease in sucrose-impermeable volume resulted from changes in cellular volumes occupied by these molecular species.

Summary data reporting the sucrose-permeable, -impermeable, and total cellular volumes of the bdellovibrio, two substrate cells, and the corresponding 60-min bdelloplasts are presented in Table 2. Since the input ratio of all bdellovibrio cultures was 2:1 (bdellovibrios/substrate cells), the actual experimental data for the 60-min cultures were corrected by subtracting the appropriate volumes for the free bdellovibrios, one per bdelloplast, to obtain bdelloplast volumes. It was assumed that by 60 min all substrate cells had been attacked and penetrated by one bdellovibrio (20) and that the volumes and permeability characteristics of the free bdellovibrios had not changed.

Considering first the starting cells per se, the total volume of the bdellovibrio was about 1/7 that of the *E. coli* and about 1/10 that of the pseudomonad. These results correlate closely with previous data showing that the dry weight and protein contents of the bdellovibrios are also about 1/7 that of *E. coli* (17). In contrast, the sucrose-permeable volume of the bdellovibrios, i.e., their periplasmic space, comprised a significantly larger percent of the total cell volume of the

bdellovibrio than in either *E. coli* or *Pseudomonas putida*. The data for the latter two organisms are also in accord with previously published information (3, 11, 22).

With respect to the 60-min bdelloplast, it is evident (Table 2) that their sucrose-permeable space was greatly increased over that of the starting substrate cells. Considering the magnitude of the increase, it is quite evident that the cytoplasmic membrane of the attacked substrate cells had become permeable to sucrose before the 60-min sampling time. One would have expected, with that membrane permeable, that the sucrose-impermeable volume of the bdelloplast should have equaled the sucrose-impermeable space of the intraperiplasmic bdellovibrios, i.e., 0.7 $\mu l/10^{10}$ cells. The observed larger impermeable volumes, 2.3 and 3.3 $\mu l/10^{10}$ cells for the *E. coli* and *P. putida* bdelloplasts, could have been due to the presence of some unattacked substrate cells in the culture and to an increase in size of the intraperiplasmic bdellovibrios and hence in their sucrose-impermeable volume. In this connection, it was recently found that bdellovibrios prematurely released from 60-min bdelloplasts appeared considerably thicker than the free-swimming forms (21). Finally, the data (Table 2) show that the total volume of the 60-min bdelloplasts was significantly greater than that of the substrate cells from which they were derived; 23 and 9% for *E. coli* and *P. putida*, respectively.

Kinetics of increase in permeability of the cytoplasmic membrane of the substrate cell to sucrose. To determine how rapidly bdellovibrio attack rendered the cytoplasmic membrane of the substrate cell permeable to sucrose, samples of single cycle synchronous bdellovibrio cultures growing on *E. coli* or *P. putida* were assayed at 10-min intervals for sucrose-permeable and -impermeable volumes. With both substrate cells, the sucrose-permeable volume increased concomitantly with a decrease in sucrose-impermeable volume (Fig. 1). The increase was detectable in both cultures within the first 10 min of the cycle and continued until about 60 min. After 60 min, sucrose-permeable or -impermeable volumes did not change appreciably until early lysis began at about 135 min.

Total cellular volume showed little change in either culture over the first 30 min into the cycle (Fig. 1). Since by this time most substrate cells had been penetrated by a bdellovibrio, the bdelloplasts formed had a larger volume than the starting substrate cells. Otherwise, the total cellular volume of the culture would have declined in proportion to the number of intraperiplasmic bdellovibrios. Between 30 and 60 min, the bdelloplasts formed from *E. coli* (Fig. 1A) increased in total cellular volume, which then remained almost constant until the onset of lysis. In contrast, the bdelloplasts formed from *P. putida* (Fig. 1B) showed, if anything, a small decrease in total volume after 30 min.

TABLE 1. Changes in sucrose-permeable and -impermeable cell pellet volumes during growth of *B. bacteriovorus* 109J on *E. coli* ML35[a]

Culture sample (min)	Cell pellet volumes (μl/ml of culture)			
	I		II	
	[^{14}C]sucrose	[^{3}H]dextran	$^{3}H_2O$	[^{14}C]sucrose
5	24	19	38	20
60	42	21	55	47

[a] Duplicate 1-ml samples of a synchronous bdellovibrio culture initially containing 6×10^{10} bdellovibrios per ml and 3×10^{10} *E. coli* cells per ml were removed, centrifuged, washed, and recentrifuged. The distribution of the radioactive probes in the cell pellets was then determined as described in the text.

TABLE 2. Changes in cellular volumes during growth of *B. bacteriovorus* 109J on *E. coli* ML35 and *P. putida*[a]

Sample	Cellular volumes ($\mu l/10^{10}$ cells or bdelloplasts)			
	Sucrose permeable (a)	Sucrose impermeable (b)	Total ($a + b$)	$\frac{100a}{(a + b)}$
B. bacteriovorus	0.3	0.7	1.0	30
E. coli	1.4	5.9	7.3	19
Bdelloplasts (60 min)	6.7	2.3	9.0	74
P. putida	0.8	9.0	9.8	9
Bdelloplasts (60 min)	7.4	3.3	10.7	69

[a] Experimental data were normalized to $\mu l/10^{10}$ cells or bdelloplasts (see text). Results are averages of six experiments with *E. coli* and three with *P. putida*.

Effect of 0.3 M NaCl on the sucrose-permeable volume of the bdelloplast. It is known (3) that the sucrose-permeable volume of *E. coli* is increased in buffer containing 0.3 M NaCl due to plasmolysis of the protoplast (see zero time values in Table 3). If, as concluded from the experiments above, the cytoplasmic membrane of the substrate cell is rendered permeable to sucrose by bdellovibrio attack, then NaCl should also be permeable and have no effect on the volume sucrose occupies after bdelloplast formation beyond small increases due to plasmolysis of the intraperiplasmic and free-swimming bdellovibrios. As predicted, the addition of NaCl to the samples had a decreasing effect with time on the sucrose-permeable volume. In the particular experiment shown, it had no effect after 45 min when the ratio of sucrose-permeable volumes in the presence and absence of NaCl (x/y, Table 3) reached unity. The reciprocal of the rate of decrease of that ratio roughly paralleled the rate of increase in sucrose-permeable volume illustrated in Fig. 1A.

Effect of bdelloplast formation on the effective pore size of the outer membrane. The degree of penetration of a series of [^{3}H]oligosaccharides of increasing molecular weight relative to [^{14}C]sucrose was measured in substrate cells and 60-min bdellovibrio cultures. Since the sucrose-permeable volume of the free bdellovibrios was small relative to the bdelloplasts in the 60-min culture (less than 5%, see Table 2), the data from this experiment (Table 4) were not corrected for the excess bdellovibrios present. The degree of penetration of this series of oligosaccharides did not change significantly from that of the substrate cells after the bdelloplasts were formed. The observed partial penetration of stachyose and complete penetration of raffinose into the *E. coli* periplasm was in agreement with previously published data (3). *P. putida*, like other pseudomonads (8), had a larger effective pore size and allowed complete penetration of stachyose. The data show that the permeability of the substrate cell outer membrane to hydrophilic sugars remained unaltered after bdellovibrio attack. In addition, the results show that the outer membrane of *B. bacteriovorus* 109J had a larger effective pore size than that of *E. coli* and was freely permeable to stachyose. Limited supplies prevented the testing of verbascose, but the bdellovibrio outer membrane proved impermeable to 4,000-dalton polyethyleneglycol. That is, the pellet volume of this compound was, on the average, equal to that of the excluded 16,000-dalton dextran.

Effect of bdelloplast formation on the outer membrane

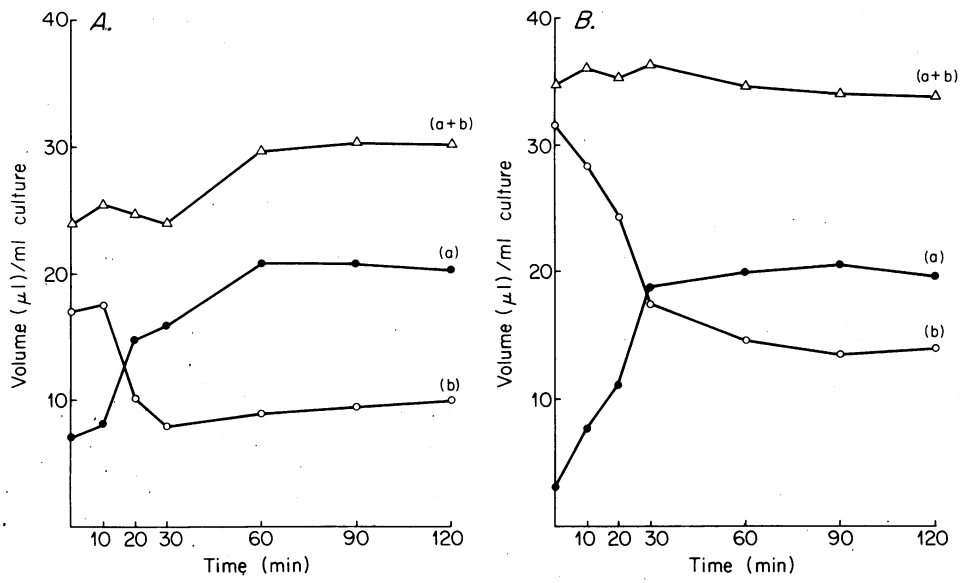

FIG. 1. Kinetics of change in (a) sucrose-permeable, (b) sucrose-impermeable, and (a + b) total cell and bdelloplast volumes of cultures of bdellovibrios grown synchronously on *E. coli* (A) and on *P. putida* (B). Initial cultures contained 6×10^{10} bdellovibrios and 3×10^{10} substrate cells per ml. Cells in 1-ml samples were taken at indicated times and assayed immediately.

protein profile of the substrate cell. The data (Table 4) suggested that the porin proteins in the *E. coli* outer membrane that regulate the permeation of small hydrophilic molecules (12) remained unaltered in the bdelloplast wall. To test this inference, outer membrane proteins were prepared from samples of equal volumes taken at intervals from a synchronous bdellovibrio culture and applied to gels for electrophoresis. The outer membrane protein profile of *E. coli* ML35 showed the presence of intensely stained bands of the porin proteins, OmpF and OmpA, as well as less intense bands of the Braun lipoprotein and other outer membrane components (Fig. 2, lanes a and j). The OmpF band was resolved into two components, OmpF and OmpC, by an increased time of electrophoresis (data not shown). The corresponding profile of the input bdellovibrio (Fig. 2, lane b) showed an intense band at about the same position as OmpF, but no bands at equivalent positions of OmpA or the Braun lipoprotein. As predicted, the outer membrane protein profiles of the synchronous culture showed little change in intensity of the band at the OmpF position over the first 120 min of the growth cycle (Fig. 2, lanes c to g). The intensity of that band then decreased in parallel to lysis of the bdelloplasts (Fig. 2, lanes h and i) which, in the experiment illustrated, started somewhat before 180 min. It has recently been reported (4) that *B. bacteriovorus* 109D and *B. stolpii*, when grown on *E. coli* strains having the OmpF porin, incorporate that protein into their outer membranes. Whether the protein of similar Rf found in *B. bacteriovorus* 109J grown on *E. coli* ML35 (Fig. 2, lanes b and i) is indeed OmpF will be considered elsewhere. For the purposes of this paper, it is important to note that the bulk of the outer membrane protein of the bdellovibrio culture migrating to the OmpF position is in the bdelloplast wall since its quantity decreases markedly with the lysis of the bdelloplast.

In contrast, bdellovibrio growth influenced the recoverability of OmpA. A decrease in intensity of this protein band was evident by 60 min, and it had completely disappeared by 120 min. These data indicate that OmpA was either degraded or released from the outer membrane. As OmpA was lost, at least one new lower-molecular-weight (ca. 22,000) polypeptide band appeared by 60 min, possibly a proteolytic product of OmpA (Fig. 2, lane e). In conformity with earlier data (25), the Braun lipoprotein was completely lost by 30 min into the bdellovibrio growth cycle.

DISCUSSION

The few measurements made on free-swimming attack phase bdellovibrios suggest that their outer and cytoplasmic membranes are typical of other gram-negative bacteria (3) with respect to permeability of small hydrophilic molecules. That is, its outer membrane has an exclusion limit of somewhere between 700 and 4,000 daltons for this class of molecules, whereas its cytoplasmic membrane is impermeable to sucrose. Its periplasmic volume measured some 30% of total cell volume, a value in the middle of the range reported by Stock et al. (22) for other gram-negative bacteria, but considerably larger than that found for stationary phase *E. coli* and *Salmonella typhimurium* by Stock (cited by Nikaido [15]). That the bdellovibrio outer membrane is permeable to lysozyme (23) (ca. 14,000 daltons) and not to

TABLE 3. Effect of 0.3 M NaCl on the sucrose-permeable cellular volumes of a synchronous culture of *B. bacteriovorus* growing on *E. coli*[a]

Time (min)	Sucrose-permeable cell pellet volume (μl/ml of culture)		
	+ NaCl (*x*)	− NaCl (*y*)	*x/y*
0	4.0	1.3	3.1
15	5.3	3.0	1.8
30	7.1	5.3	1.3
45	6.0	6.1	0.98
60	6.7	6.2	1.1
90	6.4	6.0	1.1
135	5.3	5.4	0.98

[a] Data normalized to initial cell population of 2×10^{10} bdellovibrios and 1×10^{10} *E. coli* per ml of culture.

4,000-dalton polyethylene glycol is not unexpected since it is unlikely that this highly basic protein permeates through a normal porin channel.

With respect to the attacked substrate cell and the bdelloplast, the data presented permit three major conclusions. First, the cytoplasmic membrane of the substrate cell becomes freely permeable to small hydrophilic molecules very early in the bdellovibrio growth cycle. In effect, the original cytoplasmic and periplasmic compartmentalization of the substrate cell ceases to exist with regard to small molecules as the bdelloplast is formed. Whether the compartmentalization also disappears for macromolecules, e.g., proteins, will be considered elsewhere. Second, the total volume of the bdelloplast is larger than that of the starting substrate cell, and for the *E. coli* bdelloplast, the increase occurs in two distinct phases. Third, the permeability characteristics of the outer membrane of the substrate cell to small hydrophilic molecules are not altered by bdelloplast formation. That is, the effective pore size of the outer membrane of the bdelloplast is a property conserved from the original substrate cell and not determined by the bdellovibrio.

The physical or chemical events responsible for the increase in permeability of the cytoplasmic membrane of the substrate cell are virtually unknown. However, the increase in permeability to sucrose shows similar kinetics to other bdellovibrio-induced damage such as the unmasking of β-galactosidase activity and the decrease in respiratory potential of the substrate cell (19). All of these events parallel the bdellovibrio attachment curve reported by Varon and Shilo (27). In fact, increases in sucrose volume or decreases in the NaCl effect on that volume, like attachment (27), were measurable within 10 min into the growth cycle. The kinetics of $^{42}K^+$ release from prelabeled substrate cells are even more rapid, and the process is essentially complete by about 15 min (6). From the kinetic data it can be concluded that initiation of this alteration in the cytoplasmic membrane does not require complete penetration of the bdellovibrio into the substrate cell, since penetration lags some 10 min behind attachment (27). Whether attachment per se triggers this damage or whether some additional early event in bdellovibrio attack is responsible cannot be surmised from the data.

TABLE 4. Permeability of normal cells and 60-min bdelloplasts to oligosaccharides of increasing molecular weight[a]

Organism	Form	Degree of penetration[b]			
		Sucrose (342)[c]	Raffinose (504)	Stachyose (666)	Verbascose (828)
E. coli ML35	Cell	100	90	36	NT[d]
	Bdelloplast	100	94	28	NT
E. coli K-12	Cell	100	82	36	0
	Bdelloplast	100	77	50	12
P. putida	Cell	100	83	100	NT
	Bdelloplast	100	87	78	NT
B. bacteriovorus 109J	Cell	100	100	100	NT

[a] The penetration into cellular space was determined in cell pellets containing 6×10^{10} cells or 60-min cultures of 6×10^{10} bdellovibrios growing synchronously on 3×10^{10} substrate cells. Data are the average of three experiments.

[b] $\text{Degree of penetration} = \frac{\text{oligosaccharide cellular volume}}{\text{sucrose cellular volume}} \times 100.$

[c] Numbers in parentheses equal molecular weights.

[d] NT, Not tested.

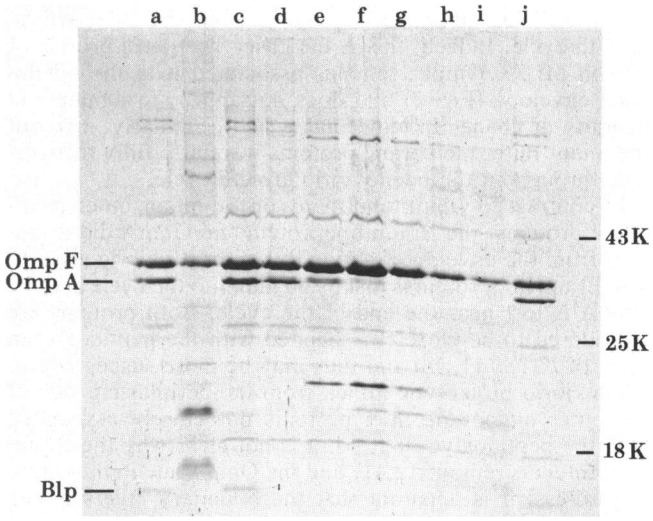

FIG. 2. Kinetic analysis of substrate cell outer membrane protein profiles during intraperiplasmic growth of *B. bacteriovorus* on *E. coli*. Samples of equal volumes were removed at 0 min (lane c), 30 min (lane d), 60 min (lane e), 90 min (lane f), 120 min (lane g), 180 min (lane h), and 240 min (after complete lysis) (lane i) from a single cycle synchronous culture of *B. bacteriovorus* 109J (6×10^{10}/ml) growing on *E. coli* ML35 (3×10^{10}/ml), the outer membrane proteins were isolated, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed as described in the text. Outer membrane preparations of the starting *E. coli* ML35 (lanes a and j), and *B. bacteriovorus* (lane b) were also processed. OmpF and OmpA, outer membrane proteins F and A respectively; Blp, Braun lipoprotein.

Penetration by the bdellovibrio into the substrate cell is largely complete by 30 min into a synchronous culture and is accompanied by an increase in cell volume and a change in shape from a rod to a sphere. This change in shape is at least partially, and possibly completely, responsible for the initial increase in cell volume. However, the bdelloplasts formed from *E. coli* continue to increase in volume for an additional 30 min. Since the total increase in bdelloplast volume occurs in two distinct steps, two separate sets of events may be involved. That is, further changes in bdelloplast envelope structure after bdellovibrio penetration may be required before the shift of the osmotic barrier from the cytoplasmic membrane to the bdelloplast wall (26) can cause a further increase in volume. It is known that during the bdellovibrio attack process, diaminopimelic acid residues continue to be released after solubilization of the peptidoglycan amino sugars ceases (24). The attendant decrease in cross-linking of the peptidoglycan strands may then permit the observed expansion of the bdelloplast. The increase in bdelloplast volume as well as the unaltered effective pore size of its boundary layer show that the penetration pore is sealed upon bdellovibrio entry.

The effective pore size of the outer membrane of the substrate cell is not affected by bdelloplast formation. This result is consistent with previous observations showing that certain small molecules are capable of entering and exiting the bdelloplast (9, 18, 19). From these data, it was predicted that the porin proteins of the substrate cell remain structural-

ly as well as functionally intact during the bdellovibrio growth cycle. Indeed, this is the case. The porin protein of *E. coli* ML35, OmpF, remains associated with the bdelloplast envelope (Fig. 2) and does not appear to diminish in quantity or change in molecular weight. Similarly, many of the minor outer membrane proteins were also fully recoverable throughout the bdellovibrio growth cycle.

In contrast to OmpF and many of the minor outer membrane proteins, the Braun lipoprotein and OmpA did disappear from the envelope fraction during bdellovibrio growth. The Braun lipoprotein is lost by 30 min into the cycle and the OmpA is lost near the end of the cycle. Both proteins are considered to be closely associated with the peptidoglycan layer of *E. coli* (1, 16) and thus may be more susceptible to bdellovibrio proteolytic attack from the periplasmic side of the outer membrane than proteins not closely associated with the peptidoglycan. If so, it is not clear why the Braun lipoprotein is removed early and the OmpA late in the cycle. Regardless, it is apparent that the boundary layers of the substrate cell that form the periplasmic and cytoplasmic compartments undergo major changes as a result of bdelloplast formation.

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LITERATURE CITED

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