JOURNAL OF BACTERIOLOGY, Feb. 1988, p. 646–652
0021-9193/88/020646-07$02.00/0

Vol. 170, No. 2

Metabolism of Periplasmic Membrane-Derived Oligosaccharides by the Predatory Bacterium *Bdellovibrio bacteriovorus* 109J

EDWARD G. RUBY* AND JOAN B. McCABE

Department of Biological Sciences, University of Southern California, Los Angeles, California 90089

Received 24 August 1987/Accepted 26 October 1987

Membrane-derived oligosaccharides (MDO), a class of osmotically active carbohydrates, are the major organic solutes present in the periplasm of *Escherichia coli* and many other gram-negative bacteria when cells are grown in a medium of low osmolarity. Analyses of growing cells of *Bdellovibrio bacteriovorus*, a gram-negative predator of other bacteria, have confirmed that they also synthesize a characteristic MDO-like class of oligosaccharides. The natural growth environment of bdellovibrios is the periplasm of other gram-negative bacteria. Because of this location, prey cell MDO constitute a potential source of organic nutrients for growing bdellovibrios. Using cells of *E. coli* whose MDO were ^{3}H labeled, we examined the extent to which *B. bacteriovorus* 109J metabolizes these prey cell components. Interestingly, there was neither significant degradation nor incorporation of prey cell MDO by bdellovibrios during the course of their intracellular growth. In fact, bdellovibrios had little capability either to degrade extracellular MDO that was made available to them or to transport glucose, the major monomeric constituent of prey cell MDO. Instead, periplasmic MDO were irreversibly lost to the extracellular environment during the period of bdellovibrio attack and penetration. Thus, although prey cell periplasmic proteins are retained, other important periplasmic components are released early in the bdellovibrio growth cycle. The loss of these MDO may aid in the destabilization of the prey cell plasma membrane, increasing the availability of cytoplasmic constituents to the periplasmic bdellovibrio.

The members of the genus *Bdellovibrio* have evolved a dimorphic developmental cycle that links their ability to grow with their entry into the periplasmic space of gram-negative bacteria (23). This location provides the bdellovibrio with easy and exclusive access to both the periplasmic and cytoplasmic contents of its prey. The intracellular bdellovibrio can grow solely upon the prey cell contents, utilizing proteins, lipids, and nucleic acids in a remarkably efficient manner (22, 23). In fact, complex prey cell molecules have been shown to be transported into, and even incorporated intact and functional as part of, the growing bdellovibrio cell (13, 16, 31).

The mechanism by which a bdellovibrio enters the prey cell is not well understood; however, electron micrographs (3) have revealed that penetration occurs through a discrete entry pore. This localized breach of the prey cell envelope is achieved by a partial enzymatic degradation that is terminated after the pore has been produced (32, 33). Following the entry of the bdellovibrio into the periplasmic space, the entry pore is believed to close by self-annealing of the prey cell outer membrane (23). The final outcome of the process is a lodging of the bdellovibrio within the prey cell periplasm, with only a negligible loss of periplasmic contents, as judged by the retention of most or all of a number of periplasmic proteins (18).

The bdellovibrio cell utilizes the cytoplasmic contents of its prey, which it obtains access to (4), degrades (5, 8, 24), transports (25, 26), and incorporates (13, 16, 31) during intracellular growth. Much less is known about the extent to which the prey cell periplasm, which can contain a significant percentage of the total organic mass of the cell, contributes to the nutrition of the bdellovibrio. Stock et al. (30) have shown that 20 to 40% of the total volume of a typical gram-negative bacterium is periplasm and that this cell compartment is iso-osmotic with the cytoplasm. Kennedy and co-workers (10, 34) have reported that when cells are grown in medium of low osmolarity the major soluble osmolytes in the periplasm are a class of glycerophosphate-substituted, short-chain carbohydrates originally described as membrane-derived oligosaccharides (MDO).

MDO are a class of compounds that occur in enteric bacteria, pseudomonads, and members of the family *Rhizobiaceae* and thus may be a general, if not a universal, constituent of gram-negative bacteria (1, 11, 15). Results of structural analyses have described linear, anionic glucans in enteric bacteria and cyclic, neutral glucans in soil bacteria (15). In both cases the concentration of oligosaccharides in the periplasm has been shown to increase to as much as 7% of the total dry weight of the cell when the bacteria are grown in a medium of low osmotic strength (10). This relationship has supported the hypothesis that MDO serve to decrease the osmotic pressure experienced by the cytoplasmic membrane (11).

In this report we consider two questions that focus on the importance of MDO in the metabolism of *Bdellovibrio bacteriovorus* 109J: first, whether bdellovibrios synthesize MDO-like compounds and, if so, whether these compounds are similar to those of either enteric or soil bacteria, and second, whether the MDO of a prey cell are metabolized or incorporated by the predatory bdellovibrio as part of its intracellular growth.

MATERIALS AND METHODS

Bacterial strains and media. *B. bacteriovorus* 109J (20), the wild-type strain, was used for intracellular growth experiments. It was routinely maintained as previously described (27) on suspensions of *Escherichia coli* ML35. Before use in single-cell growth experiments, bdellovibrio cells were washed and suspended in HM buffer (1 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid [HEPES], ad-

* Corresponding author.

justed to pH 7.6 with NaOH, plus 1.0 mM $CaCl_2$ and 0.1 mM $MgCl_2$). A prey-independent mutant, strain 109J-KA1, was derived from strain 109J by K. Gray, University of Southern California, using a procedure described previously (29). Axenic growth of 109J-KA1 was supported on nutrient broth (NB) medium containing 0.8% nutrient broth (Difco Laboratories, Detroit, Mich.) and 0.5% yeast extract (Difco), adjusted to pH 7.6 with NaOH. *E. coli* BB26-36 (*plsB*), which requires exogenous glycerol or glycerolphosphate for growth (10), was kindly provided by E. P. Kennedy. It was maintained on NB medium supplemented with 0.5 mM glycerol.

Labeling of MDO. To produce radioactively labeled MDO, cells of strain BB26-36 were inoculated into 50 ml of CAA medium (10), consisting of 1 mM KH_2PO_4, 1.5 mM $(NH_4)_2SO_4$, 0.08 mM $MgCl_2$, 0.5 mg of $FeSO_4$ per liter, and 2 mg of thiamine per liter. The medium was supplemented with 0.5% Casamino Acids (Difco) and 1.1 mM glycerol and was adjusted to pH 7.0 with Tris-free base. To this medium was added 50 μCi of [2-^{3}H]glycerol (500 μCi/μmol), and the cells were grown to stationary phase at 30°C. The labeled glycerol became incorporated into the structure of MDO as previously described (11, 12). Strain 109J-KA1 was labeled in 50 ml of NB medium to which was added 50 μCi of [2-^{3}H]glycerol. After inoculation, the culture was incubated at 30°C until growth had ceased. Attempts to label nongrowing, attack-phase (27) cells of strain 109J were made by suspending 3×10^{11} cells in 30 ml of CAA medium containing 15 μCi of [2-^{3}H]glycerol and incubating them with agitation at 30°C for 7 h.

Fractionation of cell material. MDO from BB26-36 and labeled MDO-like material from bdellovibrios were isolated, as previously described (10), by extracting a washed cell pellet with 5% (wt/vol) trichloroacetic acid (TCA). Almost all the TCA-soluble, glycerol-labeled cell material eluted as a single peak during Sephadex (Pharmacia Fine Chemicals, Piscataway, N.J.) chromatography (see Fig. 1). The labeled constituents of such peaks were defined as either MDO (10) or, for bdellovibrios, MDO-like material. MDO-like material isolated from bdellovibrios was ether insoluble and was almost completely (84%) adsorbed to activated charcoal, from which it could be extracted with pyridine as has been previously described for *E. coli* MDO (10).

Analytical assays. Total carbohydrate was determined by means of the anthrone method (7), with glucose as the standard. After acid hydrolysis for 4 h in 1.0 N HCl at 100°C, the glucose content of MDO was determined enzymatically with glucose oxidase (Sigma Chemical Co., St. Louis, Mo.). Phosphate was assayed by the ascorbate-molybdate method (2). Cell protein was determined by using a modified Lowry assay (19) with bovine serum albumin as the standard.

Single-cycle growth experiments. To obtain a synchronized single cycle of bdellovibrio attack and growth, equal volumes of suspensions of *B. bacteriovorus* 109J (2×10^{10} to 3×10^{10} cells per ml) and *E. coli* BB26-36 (1×10^{10} cells per ml) were mixed in HM buffer and incubated with vigorous shaking at 30°C (21). As judged by microscopic observation of bdelloplast formation, attack and penetration were completed within 50 min when strain BB26-36 served as prey, and the single cycle of growth ended within 2.5 to 3 h.

Permeation of MDO into bdelloplasts. The permeability characteristics of cells of *E. coli* (strains BB26-36 and ML35), as well as bdelloplasts made from these bacteria by attacking *B. bacteriovorus* 109J, were determined by the method described by Cover et al. (4). Briefly, a washed suspension of 6×10^{10} *E. coli* cells, or bdelloplasts from a 45-min synchronized cycle of bdellovibrio attack, was pelleted in a microcentrifuge (Fisher Scientific Co., Pittsburgh, Pa.) at 13,000 × *g* for 4 min. The supernatant fluid was discarded, and the pellet was suspended in 0.5 ml of ice-cold HM buffer containing either ^3H_2O, [^{14}C]sucrose, [^{3}H]dextran (16,000 daltons [Da]), or [^{3}H]MDO purified from *E. coli* BB26-36. After equilibration for 5 min at 0°C, the suspension was recentrifuged and the supernatant fluid was completely removed. The radioactivity (counts per minute per milliliter) of the fluid (supernatant 1) was determined by scintillation counting. Enough chilled HM buffer was added to suspend the pellet to a total volume of 1 ml, and the suspension was incubated at 0°C for 10 min. After centrifugation the total supernatant fluid (supernatant 2) was counted for radioactivity. Dividing the radioactivity (counts per minute) of the total volume of supernatant 2 by the radioactivity concentration (counts per minute per microliter) of supernatant 1 gave the volume of the cell pellet occupied by the labeled compound used. By using standard compounds of known permeation, the cytoplasmic, periplasmic, and total cell volumes could be calculated. The values we obtained for the volumes of *E. coli* cells and bdelloplasts were within 5% of those determined previously (4).

Substrate transport by bdellovibrios. The ability of *B. bacteriovorus* 109J to accumulate a variety of metabolites was determined by using a transport assay method described previously (26). Washed cells were suspended in HM buffer containing radioactively labeled compounds, and the suspension was shaken at 30°C. At 30-s intervals, aliquots of the suspension were removed, the cells were collected on filters, and the cellular radioactivity was determined by scintillation counting. Rates of substrate transport were calculated from the degree of accumulation that occurred during the first 2.5 min.

Chemical reagents. Reagent grade chemicals were used throughout. Pharmacia Fine Chemicals was the source of Sephadex gel filtration material and the DEAE-Sephacel cellulose ion-exchange column. Labeled *meso*-[1,7-^{14}C] diaminopimelic acid, L-[U-^{14}C]alanine, and UDP-[U-^{14}C]glucose were purchased from ICN Radiochemicals, Irvine, Calif. D-[U-^{14}C]glucose, [U-^{14}C]sucrose, [*methoxy*-^{3}H]dextran, ^3H_2O, and D-[G-^{3}H]galactose were obtained from New England Nuclear Corp., Boston, Mass., and L-[G-^{3}H] glutamine, D-[U-^{14}C]alanine, [2-^{3}H]glycerol, and D-[1-^{14}C] glucosamine were obtained from Amersham Corp., Arlington Heights, Ill.

RESULTS

Synthesis of an MDO-like compound(s). To determine whether bdellovibrios produce MDO-like cell constituents, *B. bacteriovorus* 109J-KA1 was cultivated axenically in an NB medium containing [^{3}H]-glycerol. After 17 h (about a 25-fold increase in cell mass), 82% of the cell-associated label was found in the ether-soluble lipid fraction of the TCA-insoluble material. An additional 1% to 2% of the ^{3}H-labeled material, accounting for about 0.1% of the total dry weight of the cells, appeared in the TCA-soluble cell fraction. This nonlipid material could be adsorbed to activated charcoal and eluted with pyridine in a manner characteristic of MDO (10). When the bdellovibrio MDO-like material was chromatographed on Sephadex G-25, it appeared in the eluate as a single peak with an apparent molecular size that was greater than that of MDO isolated from *E. coli* (Fig. 1A). An apparent size of about 4,000 Da was estimated by chromatography on Sephadex G-50, as

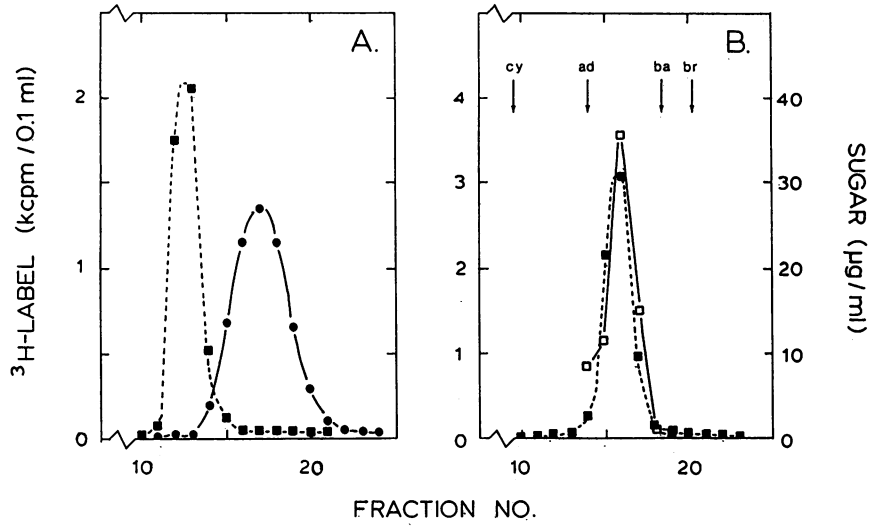

FIG. 1. Sephadex column chromatography of labeled MDO fractions. (A) TCA-soluble extracts of [2-^{3}H]glycerol-labeled cells of *E. coli* BB26-36 (●) or *B. bacteriovorus* 109J-KA1 (■) were applied to a Sephadex G-25 column (13 cm by 0.9 cm [diameter] which had been equilibrated with 0.15 M ammonium acetate in 7% (vol/vol) aqueous *n*-propanol (12). The column was eluted with the same buffer at the rate of 2.3 ml/h, fractions (0.5 ml) were collected, and the amount of label in each was quantified by liquid scintillation counting. (B) Labeled TCA-soluble extracts of *B. bacteriovorus* 109J-KA1 cells were chromatographed on a Sephadex G-50 column under conditions similar to those described above. The radioactivity (■) and total anthrone-reactive sugar content (□) of each fraction were determined. The arrows indicate the point of elution of the following molecular mass standards: cy, cytochrome *c* (13,000 Da); ad, adrenocorticotropic hormone (4,600 Da); ba, bacitracin (1,450 Da); and br, bromphenol blue (692 Da).

compared with protein standards (Fig. 1B). That the bdellovibrio MDO-like material contained sugar residues was indicated by the anthrone-positive reaction of the label-containing fractions. No MDO-like material was produced by nongrowing *B. bacteriovorus* 109J cells during a 7-h incubation in CAA medium containing labeled glycerol (data not shown).

Chemical characterization of bdellovibrio MDO-like material revealed that about 30% of the total sugar residues of the molecule were glucose and that there was a significant quantity of phosphate residues attached (Fig. 2). As has been observed during chromatography of *E. coli* MDO (34), the individual chemical components did not peak in identical fractions, suggesting that the material was slightly heterogeneous in nature. The fractions that constituted the major peak obtained by Sephadex G-50 chromatography (Fig. 1B) were combined and desalted on a small Sephadex G-15 column. The labeled MDO-like material in this preparation was partially bound by DEAE-Sephadex (pH 7.5 to 8.0), but was not bound by CM-Sephadex (pH 6.5 to 7.6), suggesting that at least some of it was anionic. Attempts to resolve this material by column chromatography on DEAE-Sephacel have been unsuccessful.

Subjecting the glycerol-labeled MDO-like material isolated from bdellovibrios to alkaline hydrolysis or to treatment with alkaline phosphatase (12) did not change the point at which either the label or the phosphate eluted from a Sephadex G-50 column (data not shown), suggesting that neither of these moieties is attached to the rest of the molecule by the labile ester linkages characteristic of *E. coli* MDO.

Bacteria grown in low-ionic-strength media typically have an increased concentration of MDO in their periplasm (11, 15). As the osmolarity is increased, the concentration of MDO decreases, reaching a minimum value at an ionic strength of about 0.2 M. Because of the requirement of a rich nutrient medium (NB) for the growth of the axenic bdellovibrios, the minimum ionic strength at which they could be grown was 0.14 M. Addition of 0.1 M NaCl to this medium did not decrease the concentration of MDO-like material produced (data not shown), suggesting that the ionic strength of NB may have already limited the extent of MDO synthesis. This may account for the relatively low concentrations (0.1% of the cell dry weight) of bdellovibrio MDO-like material obtained during growth in NB.

Utilization of prey cell MDO. To determine whether the prey cell MDO are utilized by intracellularly growing bdellovibrios, we combined a suspension of ^{3}H-labeled *E. coli* with *B. bacteriovorus* 109J to initiate a synchronous single

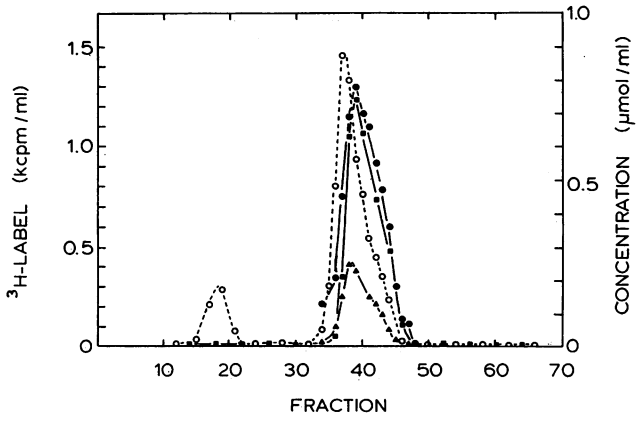

FIG. 2. Chromatography of MDO-like material from *B. bacteriovorus* 109J-KA1. TCA-soluble material from cells grown in the presence of [2-^{3}H]glycerol was chromatographed on a column of Sephadex G-50 (23 cm by 1.5 cm [diameter]) and eluted as described in the legend to Fig. 1B. Labeled material (○) was detected by liquid scintillation counting. The concentrations of total anthrone-reactive sugar (●), glucose (▲), and phosphorus (■) in each fraction were determined as described in Materials and Methods.

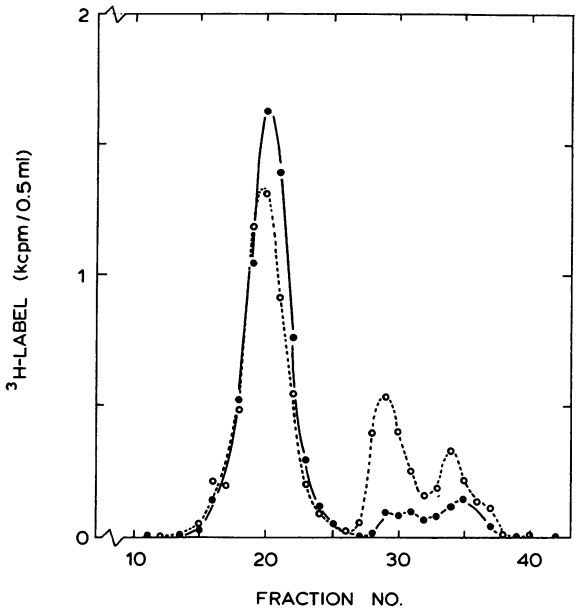

FIG. 3. Persistence of *E. coli* MDO after attack by bdellovibrios. Samples (1 ml) of a cell suspension of ^{3}H-labeled *E. coli* BB26-36 and unlabeled *B. bacteriovorus* 109J in HM buffer were removed at 0 min (●) and 200 min (○) after initiation of a single cycle of bdellovibrio attack. The entire sample was acidified with TCA (final concentration, 5% [wt/vol]) and left on ice for 30 min. TCA-insoluble material was removed by centrifugation, and the supernatant fluid was applied to a Sephadex G-25 column and eluted as described in the legend to Fig. 1A.

cycle of bdellovibrio attack and intracellular growth. Samples of the combined suspension were removed both immediately after mixing and at the end of the bdellovibrio growth cycle, following lysis of the prey cells. The total TCA-soluble material was recovered from each portion of cell suspension and was applied to a Sephadex G-25 column. Comparision of the amount of ^{3}H-labeled, TCA-soluble material present in fractions numbered 15 to 25 (Fig. 3) before and after bdellovibrio attack and growth indicated that more than 87% of the *E. coli* MDO apparently remained unaltered. In addition, there was an increase in ^{3}H-labeled small material. Treatment of *E. coli* cells with EDTA in Tris buffer (16) released labeled material that eluted in about the same position (data not shown), suggesting that the glycerol label in these peaks was associated with lipopolysaccharide released from the prey cell wall. This finding is consistent with the bdellovibrio-dependent solubilization of prey cell lipopolysaccharide described previously (32).

To see whether the apparently unchanged prey cell MDO were accumulated inside the bdellovibrio during its intracellular life cycle, we determined the location of prey cell MDO as a function of bdellovibrio attack and growth. Most of the TCA-soluble, ^{3}H-labeled MDO, initially found only within the prey cell, was released from the cellular fraction between 20 and 60 min after prey cells and bdellovibrios were combined (Fig. 4). The release of MDO was concurrent with the attack process and did not occur without the addition of bdellovibrios. Thereafter, essentially all of the MDO remained extracellular; that is, it was not associated with either the bdelloplasts or the subsequently produced progeny bdellovibrios.

This leakage of prey cell MDO might result from the physical damage sustained during the attack and entry of the bdellovibrios. However, because the intrabdelloplast volume was small relative to the total volume in which they were suspended, the appearance of essentially all of the prey cell MDO outside the bdelloplast could have resulted from a biochemical modification of the bdelloplast outer membrane that left it freely permeable to charged, polar molecules of a few thousand daltons in mass. If this were the case, then the interior of the bdelloplast should be equally accessible to entry by external MDO. To test this hypothesis, we determined the extent to which bdelloplasts were permeable to a high concentration of extracellularly added, labeled *E. coli* MDO (Table 1). Because the volume occupied by added MDO after equilibration was more similar to that of the large (16,000-Da), impermeable dextran molecules than to that of the outer-membrane-permeable sucrose molecules, it can be concluded that extracellular MDO was generally unable to penetrate the outer membranes of bdelloplasts. Similar data were obtained with bdelloplasts of strain BB26-36 in the absence or presence of an inhibitor of metabolic energy generation or when bdelloplasts of the larger ML35 strain were tested. Although clearly not as great as the sucrose-occupied volume, the volume occupied by the labeled MDO was uniformly and significantly greater than that of the dextran control (Table 1). This effect could result from minor chemical differences between dextran and MDO, and because it was also expressed by the unattacked BB26-36 cells, it is not a specific function of the process of bdelloplast formation.

The total amount of ^{3}H-labeled, TCA-soluble material released into the cell-free suspension was about 125% of the initial amount of TCA-soluble material in the prey cell. Most of this excess material appeared between 10 and 40 min after initiation of attack (Fig. 4), at the same time as the release of the additional low-molecular-weight material seen in Fig. 3. Less than 10% of the total initial TCA-soluble label remained

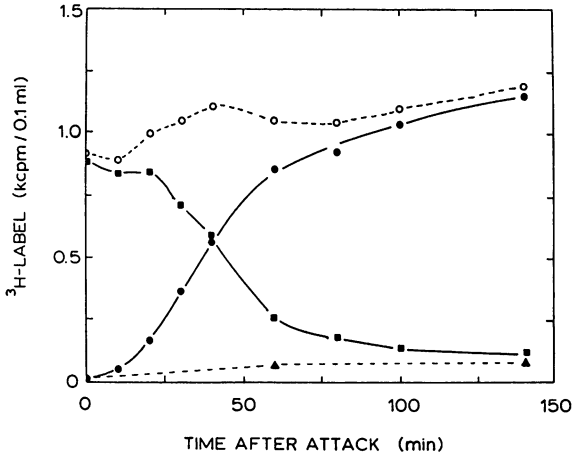

FIG. 4. Location of *E. coli* MDO during attack and growth of bdellovibrios. A single-cycle growth experiment was conducted as described in Materials and Methods. At different times after bdellovibrios and MDO-labeled prey cells had been combined, 1 ml of culture was removed and the cells were separated from the suspending fluid by centrifugation at 13,000 × *g* for 5 min. The TCA-soluble material in both the pellet fraction (■) and the cell-free supernatant (●) was extracted, and the amount of radioactivity present in each was quantified by liquid scintillation counting. The total (cellular plus cell-free) TCA-soluble label in the culture was also determined (○). Negligible amounts of TCA-soluble material (▲) were released into the suspending fluid when MDO-labeled prey cells were incubated in the absence of bdellovibrios.

TABLE 1. Permeability of bdelloplasts and cells to MDO

Cell suspension	Vol[a] (μl/10^{10} bdelloplasts or cells) penetrated by:			
	Water	Sucrose	Dextran	MDO
ML35 bdelloplasts	12.3	9.9	4.4	5.5
BB26-36 bdelloplasts	11.3 ± 0.7	8.8 ± 0.5	3.7 ± 0.1	4.7 ± 0.1
BB26-36 bdelloplasts (plus 10 mM azide)	ND[b]	ND	4.1	4.6
BB26-36 cells	ND	ND	3.2 ± 0.0	4.2 ± 0.1

[a] Values listed with standard deviation range are means of triplicate determinations; other values are single determinations.
[b] ND, Not determined.

in the cellular fraction at the end of bdellovibrio growth and lysis from the bdelloplast. Thus, there was no evidence for a significant degree of scavenging of prey cell MDO during the growth of bdellovibrios.

Because the released prey cell MDO was no longer within the confines of the bdelloplast, it was not directly available for digestion or utilization by the growing bdellovibrio. Its location outside the bdelloplast may account for the stability of the MDO during the intracellular growth of the bdellovibrio. To determine whether the growing bdellovibrio cell would use MDO if they were made accessible, the prey-independent strain 109J-KA1 was grown in NB medium to which purified, ^{3}H-labeled *E. coli* MDO were added. After bdellovibrio growth was completed, over 95% of the added MDO were still intact and extracellular (data not shown). Thus, bdellovibrios do not appear to have the capacity to either degrade or incorporate exogenously provided *E. coli* MDO.

Substrate accumulation by bdellovibrios. To determine whether bdellovibrios utilize prey cell MDO sugars if they are hydrolyzed into their component glucose residues, we determined the ability of bdellovibrios to transport monomeric sugars. The data in Table 2 summarize some substrate transport properties of *B. bacteriovorus* 109J. Over a wide range of substrate concentrations there was no detectable transport of any of the sugars or sugar derivatives tested. In contrast, there was considerable transport capacity for L-alanine and L-glutamine (Table 2) and other amino acids (data not shown), as well as nucleoside phosphates and glycerol. Interestingly, the cell-wall-specific amino acids D-alanine and diaminopimelic acid were, like the sugars, not accumulated to any detectable extent. Thus, bdellovibrio substrate transport capacities appear to be adapted to utilize only certain classes of prey cell components.

TABLE 2. Substrate transport by *B. bacteriovorus* 109J

Substrate	Concn range (μM)	V_{max}[a] (pmol/min per mg)	K_t[a] (μM)
Amino acids			
L-Alanine	5–250	500	6.2
L-Glutamine	1–150	505	17.8
D-Alanine	10–150	<9	ND[b]
meso-DAP	10–120	<4	ND
Carbohydrates			
Glucose	15–125	<8	ND
Galactose	10–120	<3	ND
Glucosamine	25–125	<8	ND
UDP-glucose	100	<6	ND
Glycerol	1–100	1,250	11.0
Nucleic acids			
UMP[c]	3–300	1,500	104
ATP[d]	10–100	1,000	51

[a] Kinetic constants were calculated from Lineweaver-Burk transformations of substrate transport rates determined as described in Materials and Methods.
[b] ND, Not determinable; substrate transport rates were below measurable levels.
[c] Reported previously (26).
[d] Reported previously (25).

DISCUSSION

Since their first isolation from *E. coli* by Van Golde et al. (34), MDO have been detected in a wide range of gram-negative bacteria (15, 28). MDO-like compounds, isolated from *B. bacteriovorus* 109J, have an average apparent molecular mass (4,000 Da) that is larger than that described for *E. coli* MDO (2,500 Da) and near to that estimated for the periplasmic cyclic glucans of the soil bacterium *Agrobacterium tumefaciens* (15). It is not clear what, if any, significance can be ascribed to the larger mass of the bdellovibrio MDO-like material. However, Cover et al. (4) have reported that for bdellovibrios, the maximum outer membrane permeability to nonpolar sugars is between 600 and 4,000 Da in molecular mass. This is a greater degree of permeability than the 600-Da maximum of the *E. coli* outer membrane (4, 17). Thus, to retain MDO within their periplasm, bdellovibrios may require a larger molecule. Nongrowing, attack-phase bdellovibrios have been reported to rapidly turn over cellular protein and RNA, although no net synthesis occurs (9). However, in this developmental stage, bdellovibrios do not produce MDO-like compounds, the synthesis of which may be restricted to growing cells.

Both the behavior during isolation and the chemical composition of the bdellovibrio MDO-like components were similar to, but not identical with, the characteristics described for the *E. coli* MDO (12). Both classes of compounds contain glycerol derivatives, glucose, and phosphate. However, although the material from *E. coli* (34) and *A. tumefaciens* (15) contains glucose as the sole carbohydrate, only about 30% of anthrone-positive MDO-like material from bdellovibrio can be accounted for by the glucose-oxidase reaction. In addition, the abundance of phosphate in the bdellovibrio material is about 3 times that of *E. coli* MDO, making it equimolar with the carbohydrate equivalences (Fig. 2). Finally, unlike *E. coli* MDO, much of the MDO-like material is not retained during ion-exchange chromatography on either DEAE-Sephacel or DEAE-Sepharose, a characteristic reminiscent of MDO from other soil bacteria (15). However, unlike the uncharged MDO fraction of *A. tumefaciens*, the bdellovibrio MDO-like material does contain phosphate. There is also no evidence that the linkage between the glycerol label and the rest of the MDO molecule is sensitive to alkaline hydrolysis or that the phosphate is sensitive to alkaline phosphatase release, as it is in *E. coli* MDO. The significance of these differences in composition

cannot be ascertained until further chemical analysis of the structure of the bdellovibrio MDO-like material is performed.

Examination of the location of prey cell MDO during growth of bdellovibrios reveals that the MDO are lost to the surrounding extracellular fluid during the period of bdellovibrio attack and entry. The kinetics of appearance of MDO in the extracellular fluid are consistent with their leakage early in the intracellular growth cycle, and not during lysis of the bdelloplasts at the end of the bdellovibrio growth cycle (Fig. 4). In addition, the inability of extracellular prey cell MDO to enter 45-min-old bdelloplasts indicated that the bdelloplast envelope does not simply become freely permeable to MDO, but instead either transiently or permanently loses its ability to retain MDO.

These data have several important implications concerning the permeability of the prey cell-bdelloplast outer envelope. First, the ready loss of prey cell MDO is further evidence that this class of cell constituents is indeed periplasmically located and is freely diffusible within that compartment (28). Secondly, assays of prey-specific periplasmic enzymes before and after the entry process have suggested that there is little if any loss from the periplasm of proteins at least as small as 80,000 Da during penetration of the outer membrane (18). In addition, Cover et al. (4) showed that after bdellovibrio entry, the bdelloplast outer membrane remained impermeable to dextran and other relatively large nonpolar compounds until terminal lysis by the progeny bdellovibrios. Thus, the entry process does not result in a permanent, unsealed hole. However, the loss of prey cell MDO suggests that the process of bdelloplast formation is accompanied by the unidirectional loss of polar periplasmic constituents at least as large as 2,500 Da. It is not yet possible to tell whether the prey cell MDO (but not all periplasmic components) are lost during the actual entry of the bdellovibrio or whether the outer membrane of the entered prey cell is somehow permanently modified to lose, but not allow entry of, the polar, hydrophilic MDO molecule.

Although prey cell MDO do not appear to be used by growing bdellovibrios, the data presented here are of use in our understanding of bdellovibrio nutrition. There are strong substrate transport capacities for amino acids and nucleoside monophosphates, the breakdown products of prey cell proteins and nucleic acids (Table 2). Not surprisingly, bdellovibrios have been reported to excrete extracellular proteases and nucleases, which would produce those monomers (5, 6, 24). In contrast, bdellovibrios seem to lack two biochemical adaptations that are common among heterotrophic bacteria that utilize extracellular sugars as a source of nutrients or energy: there are no reports of generalized glycanases that could produce transportable monomers, and there is no apparent capability to accumulate those sugars.

The role that has been suggested for MDO in normal, unattacked *E. coli* cells is as an osmotic stabilizing agent that decreases the osmotic stress across the plasma membrane and transfers it to the outer membrane (11). If this is the case, it is interesting to speculate what effect the loss of the prey cell MDO might have on the bdelloplast. Conceivably, the loss of MDO from cells growing in a dilute environment would greatly decrease the ionic strength of the periplasmic compartment relative to the cytoplasm. This could lead to increased stress on the cytoplasmic membrane and, perhaps, a facilitated leakage of prey cell constituents into what had been the prey cell periplasm, where they could easily be degraded by bdellovibrio extracellular enzymes.

ACKNOWLEDGMENTS

We thank S. W. Ruby for her role in initiating the ideas leading to this work and E. P. Kennedy for his support and suggestions. K. Miller, M. McFall-Ngai, and S. C. Rittenberg provided helpful and illuminating discussions throughout the study.

This research was supported by the National Science Foundation (grant DMB85-15558).

LITERATURE CITED

1. **Amemura, A., and J. Cabrera-Crespo.** 1986. Extracellular oligosaccharides and low-M_r polysaccharides containing (1-2)-β-D-glucosidic linkages from strains of *Xanthomonas*, *Escherichia coli* and *Klebsiella pneumoniae*. J. Gen. Microbiol. **132**:2443–2452.
2. **Ames, B. N., and D. T. Dubin.** 1960. The role of polyamines in the neutralization of bacteriophage deoxyribonucleic acid. J. Biol. Chem. **235**:769–775.
3. **Burnham, J. C., T. Hashimoto, and S. F. Conti.** 1968. Electron microscopic observations on the penetration of *Bdellovibrio bacteriovorus* into gram-negative bacterial hosts. J. Bacteriol. **96**:1366–1381.
4. **Cover, W. H., R. J. Martinez, and S. C. Rittenberg.** 1984. Permeability of the boundary layers of *Bdellovibrio bacteriovorus* 109J and its bdelloplasts to small hydrophobic molecules. J. Bacteriol. **157**:385–390.
5. **Engelking, H. M., and R. J. Seidler.** 1974. The involvement of extracellular enzymes in the metabolism of *Bdellovibrio* . Arch. Microbiol. **95**:293–304.
6. **Gloor, L., B. Klubek, and R. J. Seidler.** 1974. Molecular heterogeneity of the bdellovibrios: metallo and serine proteases unique to each species. Arch. Microbiol. **95**:45–56.
7. **Hanson, R. S., and J. A. Phillips.** 1981. Chemical composition, p. 328–364. *In* P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg, and G. B. Phillips (ed.), Manual of methods for general bacteriology. American Society for Microbiology, Washington, D.C.
8. **Hespell, R. B., and D. A. Odelson.** 1978. Metabolism of RNA-ribose by *Bdellovibrio bacteriovorus* during intraperiplasmic growth on *Escherichia coli*. J. Bacteriol. **136**:936–946.
9. **Hespell, R. B., M. F. Thomashow, and S. C. Rittenberg.** 1974. Changes in the cell composition and viability of *Bdellovibrio bacteriovorus* during starvation. Arch. Microbiol. **97**:313–327.
10. **Kennedy, E. P.** 1982. Osmotic regulation and the biosynthesis of membrane-derived oligosaccharides in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **79**:1092–1095.
11. **Kennedy, E. P.** 1984. Periplasmic membrane-derived oligosaccharides and osmoregulation in *Escherichia coli*, p. 33–43. *In* E. Haber (ed.), The cell membrane. Plenum Publishing Corp., New York.
12. **Kennedy, E. P., M. K. Rumley, H. Schulman, and L. M. G. Van Golde.** 1976. Identification of a sn-glycero-1-phosphate and phosphoethanolamine residues linked to the membrane-derived oligosaccharides of *Escherichia coli*. J. Biol. Chem. **251**:4208–4213.
13. **Kuenen, J. G., and S. C. Rittenberg.** 1975. Incorporation of long-chain fatty acids of the substrate organism by *Bdellovibrio bacteriovorus* during intraperiplasmic growth. J. Bacteriol. **121**:1145–1157.
14. **Leive, L.** 1968. Studies on the permeability change produced in coliform bacteria by ethylenediaminetetraacetate. J. Biol. Chem. **243**:2372–2380.
15. **Miller, K. J., E. P. Kennedy, and V. N. Reingold.** 1986. Osmotic adaptation by Gram-negative bacteria: possible role for periplasmic oligosaccharides. Science **231**:48–51.
16. **Nelson, D. R., and S. C. Rittenberg.** 1981. Incorporation of substrate cell lipid A components into the lipopolysaccharide of intraperiplasmically grown *Bdellovibrio bacteriovorus*. J. Bacteriol. **147**:860–868.
17. **Nikaido, H., and M. Vaara.** 1985. Molecular basis of bacterial outer membrane permeability. Microbiol. Rev. **49**:1–32.
18. **Odelson, D. A., M. A. Patterson, and R. B. Hespell.** 1982. Periplasmic enzymes in *Bdellovibrio bacteriovorus* and *Bdellovibrio stolpii*. J. Bacteriol. **151**:756–763.

19. **Peterson, G. L.** 1979. Review of the Folin phenol protein quantification method of Lowry, Rosebrough, Farr and Randall. Anal. Biochem. **100**:201–220.
20. **Rittenberg, S. C.** 1972. Nonidentity of *Bdellovibrio bacteriovorus* strains 109D and 109J. J. Bacteriol. **109**:432–433.
21. **Rittenberg, S. C.** 1982. Bdellovibrios—intraperiplasmic growth, p. 379–391. *In* R. G. Burns and J. H. Slater (ed.), Experimental microbial ecology. Blackwell Scientific Publications, Ltd., Oxford.
22. **Rittenberg, S. C., and R. B. Hespell.** 1975. Energy efficiency of intraperiplasmic growth of *Bdellovibrio bacteriovorus*. J. Bacteriol. **121**:1158–1165.
23. **Rittenberg, S. C., and M. F. Thomashow.** 1979. Intraperiplasmic growth—life in a cozy environment, p. 80–85. *In* D. Schlessinger (ed.), Microbiology—1979. American Society for Microbiology, Washington, D.C.
24. **Rosson, R. A., and S. C. Rittenberg.** 1979. Regulated breakdown of *Escherichia coli* deoxyribonucleic acid during intraperiplasmic growth of *Bdellovibrio bacteriovorus* 109J. J. Bacteriol. **140**:620–633.
25. **Ruby, E. G., and J. B. McCabe.** 1986. An ATP transport system in the intracellular bacterium *Bdellovibrio bacteriovorus* 109J. J. Bacteriol. **167**:1066–1070.
26. **Ruby, E. G., J. B. McCabe, and J. I. Barke.** 1985. Uptake of intact nucleoside monophosphates by *Bdellovibrio bacteriovorus* 109J. J. Bacteriol. **163**:1087–1094.
27. **Ruby, E. G., and S. C. Rittenberg.** 1983. Differentiation after premature release of intraperiplasmically growing *Bdellovibrio bacteriovorus*. J. Bacteriol. **154**:32–40.
28. **Schulman, H., and E. P. Kennedy.** 1979. Localization of membrane-derived oligosaccharides in the outer envelope of *Escherichia coli* and their occurrence in other gram-negative bacteria. J. Bacteriol. **137**:686–688.
29. **Seidler, R. J., and M. P. Starr.** 1969. Isolation and characterization of host-independent bdellovibrios. J. Bacteriol. **100**:769–785.
30. **Stock, J. B., B. Rauch, and S. Roseman.** 1977. Periplasmic space in *Salmonella typhimurium* and *Escherichia coli*. J. Biol. Chem. **252**:7850–7861.
31. **Talley, B. G., R. L. McDade, Jr., and D. L. Diedrich.** 1987. Verification of the protein in the outer membrane of *Bdellovibrio bacteriovorus* as the OmpF protein of its *Escherichia coli* prey. J. Bacteriol. **169**:694–698.
32. **Thomashow, M. F., and S. C. Rittenberg.** 1978. Intraperiplasmic growth of *Bdellovibrio bacteriovorus* 109J: solubilization of *Escherichia coli* peptidoglycan. J. Bacteriol. **135**:998–1007.
33. **Thomashow, M. F., and S. C. Rittenberg.** 1978. Intraperiplasmic growth of *Bdellovibrio bacteriovorus* 109J: N-deacetylation of *Escherichia coli* peptidoglycan amino sugars. J. Bacteriol. **135**:1008–1014.
34. **Van Golde, L. M. G., H. Schulman, and E. P. Kennedy.** 1973. Metabolism of membrane phospholipid and its relation to a novel class of oligosaccharides in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **70**:1368–1372.