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Acquisition of *Escherichia coli* Outer Membrane Proteins by *Bdellovibrio* sp. Strain 109D

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The ability of *Bdellovibrio* sp. to acquire the OmpF major outer membrane protein from its *Escherichia coli* prey was examined to determine if there were other outer membrane proteins which could or could not be acquired. Growth of bdellovibrios on mutant prey which were defective in the expression of outer membrane proteins revealed that *Bdellovibrio* sp. could acquire the OmpC protein in the absence of the OmpF protein. However, the OmpA, LamB, and protein 2 proteins could not be found in the *Bdellovibrio* Triton-insoluble outer membrane. The disappearance of the OmpF and OmpC proteins from the bdelloplast surface was measured, and it was determined that *Bdellovibrio* sp. exhibited a kinetic and temporal preference for the OmpF protein. Bdellovibrios could be grown on porin-deficient prey, and the progeny bdellovibrios possessed outer membranes with a protein mass deficiency.

The bdellovibrios are a remarkable group of predatory gram-negative bacteria which are capable of breeching the outer membrane and peptidoglycan of their gram-negative bacterial prey. They establish residence in the periplasmic space and acquire from the prey virtually all the required nourishment for growth and division (25, 26). Bdellovibrios grow with a high metabolic efficiency explainable in part by their ability to incorporate preformed cellular constituents from their prey (25, 26). For example, the fatty acid composition of *Bdellovibrio* phospholipids and lipopolysaccharide reflects the fatty acid composition of the prey (16, 19).

We (9) and others (14) have reported that *Bdellovibrio* sp. appears to mobilize outer membrane proteins from *Escherichia coli* prey and transfer them to the *Bdellovibrio* outer membrane. Guerrini et al. (14) concluded from specific radioactivity measurements that the protein found in the *Bdellovibrio* outer membrane could not have been synthesized by the bdellovibrios, and we (9) demonstrated with density gradient-purified bdellovibrios that the presence of the apparently prey-derived OmpF protein conferred sensitivity to colicin A. The colicin A receptor complex is known to require the OmpF protein (5, 6), and we showed that bdellovibrios without the OmpF protein were resistant to colicin A as a result of their failure to bind colicin (9).

We present in this study additional experimental evidence for the ability of *Bdellovibrio* sp. to acquire major outer membrane proteins from its prey. The flexibility and limitations of this process are examined, and experiments are shown which directly measure the loss of the OmpF and OmpC porins from the outer membranes of *Bdellovibrio*-infected prey.

MATERIALS AND METHODS

Strains, media, and growth conditions. *Bdellovibrio bacteriovorus* 109D was used throughout this study (9, 24). *E. coli* K-12 strains were: CS138 (W1485 F$^-$ *gyrA*) (8, 9, 12), previously published as PB105 (2); DD1002 (CS138 OmpF); DD1032 (DD1002 OmpC); and CS328 (22). DD1002 and DD1032 were spontaneous mutants selected with the OmpF- and OmpC-specific bacteriophages Sϕ108 and Sϕ, respectively (see below). Strain CS328 expresses only protein 2 and the OmpA protein (22).

High-viability *Bdellovibrio* stock cultures were maintained as bdelloplasts (30) frozen in 7 to 10% dimethyl sulfoxide at −70°C. Routine propagation of *Bdellovibrio* sp. employed stationary-phase prey cultures of *E. coli* CS138 in YP medium (31). Mutant prey would not grow in YP medium so it was grown in either Trypticase soy broth (TSB) (BBL Microbiology Systems, Cockeysville, Md.) or nutrient broth (Difco Laboratories, Detroit, Mich.). Mutant prey was grown to stationary phase, washed, and suspended in 5 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer at pH 7.4 before infection with *Bdellovibrio* sp. All experiments were performed with *Bdellovibrio* sp. and prey suspensions in HEPES buffer. A Klett-Summerson colorimeter was used for adjusting prey density, and bdellovibrios were quantitated with the aid of a Petroff-Hausser counting chamber. Bdellovibrios grown on mutant prey were cycled a minimum of four transfers on the mutant prey before experimental use.

NaN_3 was added to a final concentration of 5.0 mM to *Bdellovibrio* batch cultures grown for the preparation of Triton-insoluble outer membranes. This was done at the time of bdelloplast lysis to minimize peptidoglycan turnover (32) and to prevent errors in the amino acid analyses (see below).

Membrane preparation. Batch cultures with NaN_3 were harvested and washed as previously described except that 5 to 15% Ficoll gradients were used to purify the bdellovibrios (16) in place of sucrose gradients (9). The density gradient-purified bdellovibrios were broken in a French pressure cell, and the Triton-insoluble outer membranes were prepared as previously described (8, 9). The Triton-insoluble outer membranes of control prey cultures were prepared by the previously published method (10).

Analytical procedures. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed in tris-glycine slab gels containing 8.0 M urea by the procedure of Pugsley and Schnaitman (23), with the exception that we used 12% gels with an acrylamide/bisacrylamide ratio of 52.2. Slab gels were stained with Coomassie blue by the

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procedure of Fairbanks et al. (11), and densitometry was performed with a Hoefer model 1650 scanning densitometer (Bio-Rad Laboratories, Richmond, Calif.). Quantitation from densitometer scans was accomplished by excising peaks and weighing them with an analytical balance.

Triton-insoluble outer membrane samples were hydrolyzed and subjected to amino acid analysis to determine the glycine/diaminopimelate ratios by procedures previously described (8, 12).

Acquisition kinetics. Experiments to measure the loss of porin from the cell surface of bdelloplasts were performed by establishing conditions under which the attachment rates of porin-specific bacteriophages were proportional to the amount of porin in the outer membrane of the prey. Bacteriophage Sϕ specific for the OmpC porin and bacteriophage Sϕ108 specific for the OmpF porin were used (J. A. Fralick and D. L. Diedrich, manuscript in preparation).

E. coli CS138 was employed for the prey. This strain was grown in TSB for one experiment to suppress the level of the OmpF porin (2). Experiments 2 and 3 employed prey grown in minimal medium A with glucose as a carbon source (7) to permit approximately equal expression of the OmpF and OmpC porins (2).

Prey was harvested from its medium and suspended with a lysate of *Bdellovibrio* sp. which had been cultivated several cycles on porin-deficient *E. coli* DD1032 in HEPES buffer. Sufficient *Bdellovibrio* lysate was used to yield an input multiplicity of five bdellovibrios per prey, and this was determined from counts with a Petroff-Hausser counting chamber. The infected prey were incubated for 15 min at 30°C and subsequently pelleted by a differential centrifugation at 2,000 × *g* for 5 min to leave most of the unattached bdellovibrios in the supernatant. The *Bdellovibrio*-prey complexes were resuspended in 5.0 mM HEPES buffer at pH 7.4 to a density of 100 Klett units. Incubation was continued at 30°C in a gyratory water bath shaker. Estimations made from microscopic examination indicated that at least 95% of the prey had attached bdellovibrios.

Duplicate 0.5-ml samples were removed at zero time and at 30-min intervals. These samples were diluted into two tubes containing 0.4 ml of L broth (2) and 0.1 ml of either bacteriophage Sϕ or Sϕ108 at a titer of 1×10^6 to 2×10^6 PFU/ml. Samples were removed from the bacteriophage assay tubes at 2, 5, and 10 min, and these were rapidly diluted in L broth over chloroform for plating by the overlay method with *E. coli* CS138 as the bacteriophage indicator. The L agar overlay plates were incubated at 37°C. Both the medium and the temperature arrest the development of any surviving bdellovibrios.

The bacteriophage attachment kinetics were plotted for each bacteriophage at each sample time. The attachment rate constant was calculated for each curve either from the data directly or occasionally, when necessary, from linear regression analysis of the data. The attachment rate constants were calculated according to the discussion by Adams (1), and they were plotted as a percentage of the zero time attachment rate constant.

These plots quantitate the loss of porin receptor from the surface of the bdelloplast during the intraperiplasmic phase of the *Bdellovibrio* life cycle. The experiments were terminated before the lysis of the bdelloplasts since the released bdellovibrios reexpose the acquired proteins, and these become available for bacteriophage inactivation (data not shown).

RESULTS

Influence of mutant prey on prey-derived protein. Our initial studies showed that the OmpF porin from either *E. coli* or *Salmonella typhimurium* could be found in the Triton-insoluble outer membrane preparations of density gradient-purified bdellovibrios which had been grown on these prey. We expanded this study to determine whether there were other outer membrane proteins of *E. coli* which could or could not be acquired by *Bdellovibrio* sp.

Figure 1a shows the SDS-PAGE profiles of the Triton-insoluble outer membrane proteins of an *E. coli* mutant lacking the OmpF porin and the proteins of *Bdellovibrio* sp. grown on this strain. A stained band corresponding to the OmpF protein is absent from the *Bdellovibrio* outer membrane proteins (Fig. 1a, lane 2). However, another band

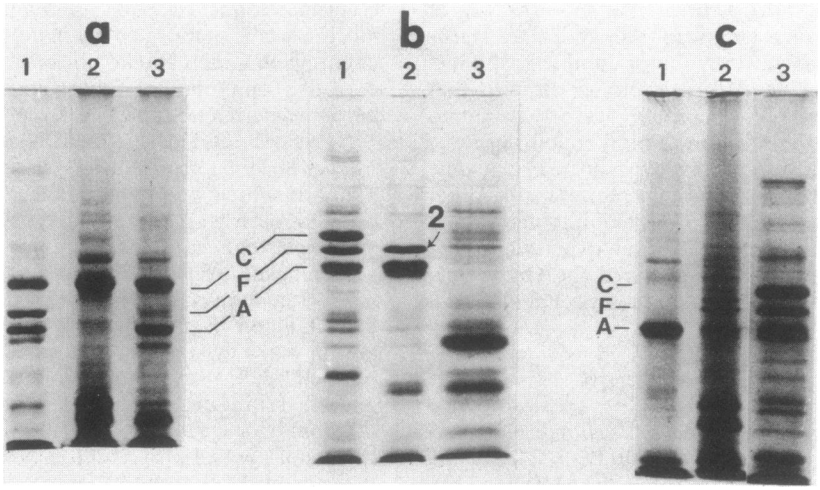

FIG. 1. SDS-PAGE of Triton-insoluble outer membrane proteins of *E. coli* and *Bdellovibrio* sp. (a) Acquisition of the OmpC protein by *Bdellovibrio* sp. Lane 1, *E. coli* CS138 showing the reference positions for the OmpC (C), OmpF (F), and OmpA (A) proteins; lane 2, *Bdellovibrio* sp. strain 109D grown on *E. coli* DD1002 lacking the OmpF protein; lane 3, *E. coli* DD1002. (b) Failure to acquire protein 2 by *Bdellovibrio* sp. lane 1, *E. coli* CS138 reference control; lane 2, *E. coli* CS328 with the reference position marked for protein 2; lane 3, *Bdellovibrio* sp. strain 109D grown on strain CS328. (c) Outer membrane proteins of bdellovibrios grown on a strain lacking both the OmpC and OmpF proteins. Lane 1, *E. coli* DD1032; lane 2, *Bdellovibrio* sp. strain 109D grown on strain DD1032; lane 3, *E. coli* CS138 reference control.

appeared which exhibited the same electrophoretic mobility as the *E. coli* OmpC porin (Fig. 1a, lane 3). The bdellovibrios appear to be capable of acquiring the OmpC porin in the absence of the OmpF porin. Our original study showed that bdellovibrios acquired only the OmpF protein when they were grown on prey which contained approximately equal levels of the OmpF and OmpC porins. The apparent selectivity exhibited in the initial experiment and the results shown in Fig. 1 led us to suspect that the acquisition of a prey-derived protein is a specific process which possesses some flexibility.

We continued to examine this apparent flexibility by growing bdellovibrios on prey which expressed only protein 2 and the OmpA protein. Outer membrane protein 2 of *E. coli* is a major protein encoded by the PA-2 prophage (29), and its expression is regulated by temperature and catabolite repression (10, 12, 29). This protein is a peptidoglycan-associated porin, and it is similar in its chemical and physical properties to the OmpF and OmpC porins (9, 10, 21, 27). It probably has greatest homology with the OmpF porin since we have been able to construct host-range mutants of an OmpF-specific bacteriophage which recognize both the OmpF porin and protein 2 or only protein 2 (Fralick and Diedrich, manuscript in preparation). This has not been possible with the OmpC-specific bacteriophage (unpublished observation).

Figure 1b shows the SDS-PAGE profiles of the Triton-insoluble outer membrane proteins of *E. coli* CS328 (Fig. 1b, lane 2) and the bdellovibrios produced by growth on CS328 prey (Fig. 1b, lane 3). The *Bdellovibrio* outer membrane does not contain a major protein with the same electrophoretic mobility as protein 2. An apparent major mass of protein appears at a lower molecular weight in the *Bdellovibrio* sample, and the origin of this band is unknown. Figure 1b, lane 3, additionally shows that the bdellovibrios are not capable of acquiring the OmpA protein, and we have observed in other experiments that bdellovibrios are incapable of acquiring the LamB protein (data not shown). Although peptidoglycan associated, the LamB protein is a specific porin for maltose and maltose oligosaccharides (4, 13, 17, 20, 33). The OmpA protein is not a porin and it is not peptidoglycan associated (10, 15).

The inability of *Bdellovibrio* sp. to acquire protein 2 establishes a limit to the apparent flexibility of the prey-derived protein acquisition process. This observation provides further evidence that the presence of prey-derived outer membrane proteins in the Triton-insoluble *Bdellovibrio* outer membrane is not likely to be a preparative artifact. The failure of *Bdellovibrio* to acquire protein 2 additionally shows that the acquisition of prey-derived proteins is not an essential process in the intraperiplasmic phase of the *Bdellovibrio* life cycle. However, it remained possible that porin-deficient *E. coli* may not be suitable prey.

Figure 1c shows the SDS-PAGE profiles of the Triton-insoluble outer membrane proteins from porin-deficient *E. coli* DD1032 (Fig. 1c, lane 1) and the bdellovibrios resulting from predation of this strain (Fig. 1c, lane 2). *Bdellovibrio* sp. propagates on porin-deficient prey and exhibits no major protein mass in the portion of the gel where any of the major outer membrane proteins of *E. coli* would be found (Fig. 1c, lane 3). However, we have observed a partial penetration defect of porin-deficient bdellovibrios grown on porin-deficient prey. This will be the subject of another communication (manuscript in preparation).

Outer membrane protein mass measurements. The propagation of *Bdellovibrio* sp. on porin-deficient prey should result in a *Bdellovibrio* outer membrane with a demonstrable protein mass deficiency. Quantitation from densitometer tracings of the Triton-insoluble outer membrane proteins of bdellovibrios derived from prey cultures containing the OmpF protein (strain CS138) result in the OmpF protein accounting for 35.2% of the relative protein mass. The relationship between this relative value and the actual protein mass of the *Bdellovibrio* outer membrane was determined by quantitating the glycine/diaminopimelate ratio by amino acid analysis of Triton-insoluble outer membrane protein hydrolysates. We have used this approach previously (8, 12). The peptidoglycan of gram-negative bacteria probably exists as a monomolecular layer, so the quantitation of diaminopimelate becomes a measurement of the cell surface area. Diaminopimelate has been shown to be a component of *Bdellovibrio* peptidoglycan (32). Glycine, which is absent from peptidoglycan, is an abundant amino acid in proteins. Triton-insoluble outer membranes represent quantitative recovery of the protein-peptidoglycan complex with a depletion of the outer membrane phospholipids and lipopolysaccharides. Thus, the glycine/diaminopimelate ratio of Triton-insoluble membranes becomes an index of the absolute protein density (protein per unit area) of the cell surface.

We determined the value of the glycine/diaminopimelate ratio for the Triton-insoluble outer membranes of bdellovibrios grown on OmpF-containing prey (strain CS138) and compared it with values obtained from bdellovibrios grown on porin-deficient *E. coli* DD1032 and on *E. coli* CS328, which expresses a porin (protein 2) unsuitable for acquisition by *Bdellovibrio* sp. The glycine/diaminopimelate ratio of strain DD1032-derived bdellovibrios was 35.5% (standard deviation, ±0.3%) lower than that of the OmpF-containing bdellovibrios. Bdellovibrios derived from *E. coli* CS328 yielded a glycine/diaminopimelate ratio which was 41.4% (standard deviation, ±0.2%) lower than the value obtained for the OmpF-containing bdellovibrios.

These data show that bdellovibrios grown on porin-deficient prey possess outer membranes which are protein deficient. The relative contribution of the prey-derived OmpF protein to the protein mass of the outer membrane accurately reflects its absolute mass contribution to the outer membrane. It is likely that *Bdellovibrio* sp. strain 109D does not compensate for this deficiency. Initially, we suspected that the low-molecular-weight protein masses observed in Fig. 1b, lane 3, and Fig. 1c, lane 2, could at least partially offset the loss incurred by porin deficiency. However, the contribution of these proteins probably is illusionary and results from the amplification of minor proteins caused by loading large samples on the gels. Unfortunately, a reliable and bona fide *Bdellovibrio* protein is not apparent for use as an internal reference.

Conservation of prey-derived protein. The bdellovibrios used to start the preparative cultures for the experiments presented in Fig. 1 had been cycled for several transfers on the specific mutant prey examined in the experiment. This was done on the assumption that prey-derived protein from previous prey was conserved and required dilution by growth to reach undetectable levels (14). Alternatively, it was possible that the prey-derived protein was lost during the penetration phase of a new round of the life cycle. We tested this possibility by infecting porin-deficient prey with bdellovibrios which contained the OmpF protein (from *E. coli* CS138 grown in YP medium) under conditions under which there only were sufficient prey for a single cycle of growth. The Triton-insoluble outer membranes were pre-

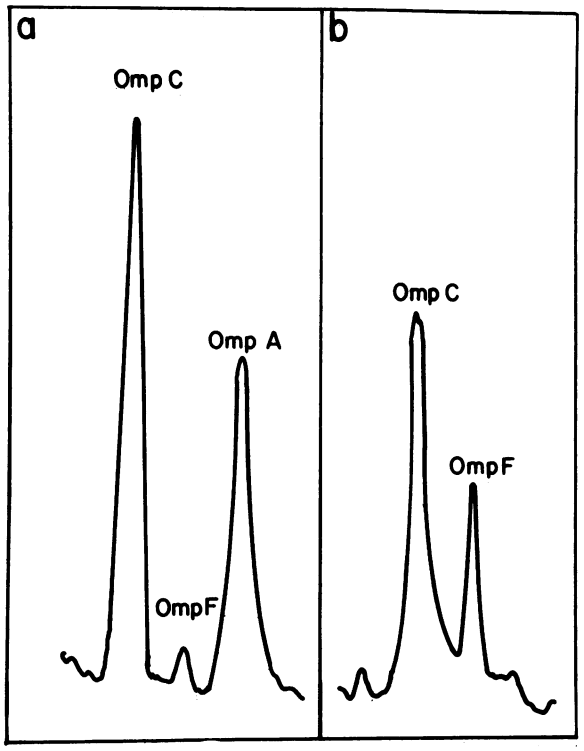

FIG. 2. Densitometer tracing of SDS-polyacrylamide slab gel stained with Coomassie blue and containing the Triton-insoluble outer membrane proteins of (a) *E. coli* CS138 grown in TSB medium to suppress the level of the OmpF protein and (b) *Bdellovibrio* sp. strain 109D grown on the TSB-cultivated *E. coli* CS138 shown in (a). Only the portion of the gel containing the major proteins is shown.

pared from the OmpF-containing input bdellovibrios and those derived from the single growth cycle on porin-deficient prey. The relative contribution of the OmpF protein to the total protein mass of the *Bdellovibrio* outer membrane was quantitated from densitometer tracings of the SDS-PAGE gels of the two preparations.

We found that the bdellovibrios derived from porin-deficient prey had a relative OmpF protein content of 8.1%. This was approximately one-third of the 24.5% OmpF protein content of the bdellovibrios before infection of the porin-deficient prey. This value is consistent with the dilution value predicted for the observed burst size of three to four progeny bdellovibrios per bdelloplast. If any of the *Bdellovibrio* prey-derived protein was lost on subsequent predation, the loss could not have been extensive.

Preference for the OmpF protein. Our previous study showed that the growth of *Bdellovibrio* sp. on prey which contained equal levels of the OmpF and OmpC porins resulted in bdellovibrios which contained only the OmpF porin (9). Although bdellovibrios are capable of acquiring the OmpC porin (Fig. 1a, lane 2), the preference for the OmpF porin is illustrated further in Fig. 2. Figure 2a shows a densitometer tracing of an SDS gel containing the major outer membrane proteins of *E. coli* CS138 grown in TSB medium. The level of the OmpF protein is suppressed (2), and results in an OmpC/OmpF mass ratio of 18.4. Bdellovibrios derived from growth on the culture shown in Fig. 2a possessed a Triton-insoluble outer membrane with the protein composition shown in Fig. 2b. The OmpC/OmpF ratio in the bdellovibrios is 2.3. This is an eightfold relative increase of the OmpF protein.

This preference exhibited by *Bdellovibrio* sp. for the OmpF protein was examined again by a substantially different approach. Bacteriophages specific for the OmpF and OmpC proteins were used to titrate the loss of these proteins from the surface of *Bdellovibrio*-infected *E. coli* (bdelloplasts). Samples of the synchronously infected prey were assayed periodically throughout the infection cycle for their ability to bind and inactivate the protein-specific bacteriophages. The experiment was performed under conditions in which the attachment rate constants (K) of the bacteriophages were proportional to the amount of receptor present on the bdelloplast surface, and K was calculated from attachment kinetic plots consisting of three or four early data points before their deviation from first-order kinetics. A less-refined precedent for this approach has been published (29).

Figure 3 shows the change in the attachment rate constants of Sϕ108 (specific for the OmpF protein) and Sϕ (specific for the OmpC protein) on bdelloplasts of *E. coli*. The *E. coli* cells had been grown in TSB to have an outer membrane protein composition identical to that shown in Fig. 2a. The data show that the OmpF protein disappeared from the bdelloplast surface earlier in the infection cycle than the OmpC protein even though the OmpF protein was present initially at a substantially lower concentration. Additionally, this experiment showed that the disappearance of protein from the bdelloplast surface occurred throughout

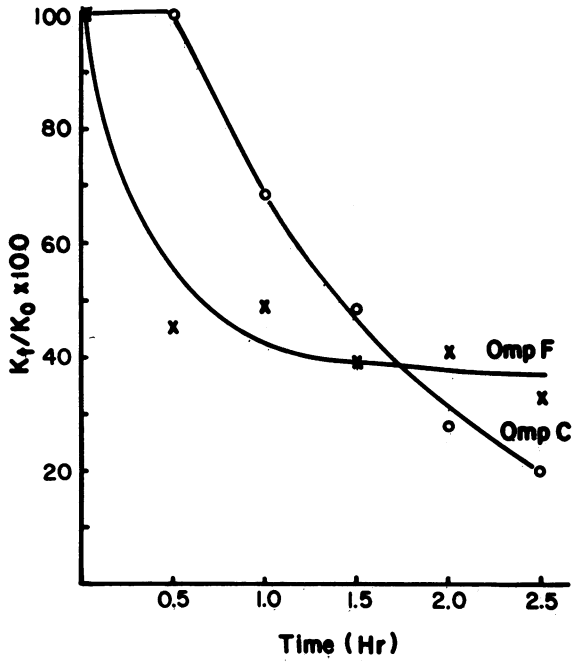

FIG. 3. Changes in bdelloplast surface concentrations of the OmpF and OmpC proteins measured by the attachment rate constants (K) of the OmpF-specific bacteriophage Sϕ108 and the OmpC-specific bacteriophage Sϕ. The *E. coli* CS138 prey had been grown in TSB medium to have an outer membrane protein composition identical to that shown in Fig. 2a. The bdellovibrios used to infect the *E. coli* cells had been cycled several times on porin-deficient *E. coli* DD1032 to eliminate any residual prey-derived protein in their outer membranes which would interfere with the bacteriophage binding assay. Each time point represents a K value plotted as a percentage of the zero-time K value. Each K value was determined from a 10-min attachment kinetics plot containing four data points. Symbols: ×, change in the attachment rate constant K for the OmpF-specific bacteriophage Sϕ108; ○, change in the attachment rate constant of the OmpC-specific bacteriophage Sϕ.

most of the infection cycle. Bdelloplast lysis began ca. 30 min after the last sample (2.5 h). Samples beyond 2.5 h were not examined since the lysis of the bdelloplasts reexposes functional bacteriophage receptor. Zero time on the experiment illustrated in Fig. 3 was ca. 20 min postinfection by the bdellovibrios since time was consumed in the *Bdellovibrio* pulse, differential centrifugation, and bdelloplast density adjustment steps of the experimental protocol.

The preference of the OmpF protein illustrated kinetically in Fig. 3 was examined further by performing a similar experiment in which the OmpF and OmpC porins were present at approximately equal amounts in the prey. We predicted that sufficient OmpF protein should be present to satisfy the bdellovibrios and that the OmpC protein concentration on the bdelloplast surface should remain constant. This predicted result would account for our published observation that bdellovibrios grown on prey with equal OmpF and OmpC porins contained only the OmpF protein (9). The results of this experiment are shown in Fig. 4.

The rapid decline in the OmpF-specific bacteriophage binding capacity of the bdelloplast was similar to that shown in Fig. 3. However, there also was a significant decline in the OmpC-specific bacteriophage binding capacity of the bdelloplasts. This observation initially appeared to be in contrast to our prediction and previous data. However, this experiment and the one illustrated in Fig. 3 employed bdellovibrios which were porin deficient to prevent the input bdellovibrios from binding and inactivating bacteriophage. Our previous experiments (9) employed bdellovibrios which already contained OmpF protein before attacking their prey. It seemed likely that prey with equal levels of the OmpF and OmpC porins would provide sufficient OmpF porin if the input bdellovibrios already contained their conserved OmpF protein from previous prey. However, the protein requirements of protein-deficient bdellovibrios may exceed the amount of the OmpF protein present in this prey.

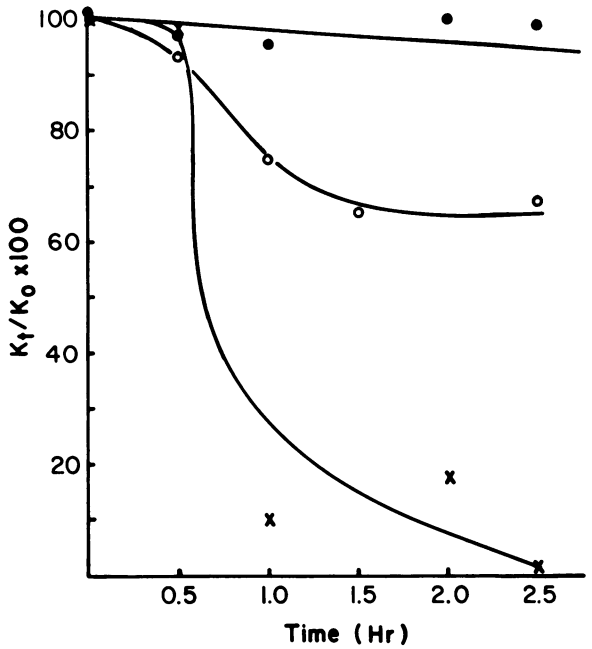

FIG. 4. Changes in the bdelloplast surface concentration of the OmpF and OmpC proteins measured identically to the measurements in Fig. 3 by the attachment rate constants of protein-specific bacteriophages. The *E. coli* CS138 prey had been grown in a minimal medium with glucose for a carbon source so that it would possess an outer membrane with equal levels of the OmpF and OmpC porins (2). Bdellovibrios were porin deficient similar to those employed in the experiment shown in Fig. 3. Symbols: ×, change in the attachment rate constant K for the OmpF-specific bacteriophage Sϕ108; ○, change in the attachment rate constant K for the OmpC-specific bacteriophage Sϕ; ●, change in the attachment rate constant K for the OmpC-specific bacteriophage Sϕ when the input bdellovibrios were not protein deficient because of their prior growth on *E. coli* CS138.

This hypothesis was tested by repeating the experiment shown in Fig. 4 with the exception that the bdellovibrios were propagated on prey containing the OmpF protein. Only the OmpC protein was followed since the input bdellovibrios contained the OmpF protein and would interfere with the assay. The results of this experiment are plotted in the topmost curve in Fig. 4 (filled circles) and show that the bdelloplast surface concentration of the OmpC protein appeared not to be altered when the input bdellovibrios were not protein deficient.

DISCUSSION

We have presented several independent lines of evidence that the acquisition of the OmpF outer membrane protein of *E. coli* by *Bdellovibrio* sp. is an active and specific process. The inability to acquire protein 2 minimizes the possibility that the prey-derived protein is a preparative artifact. Additionally, it has provided us with the experimental approach to continue these studies by attempting to define domains in the primary sequence necessary for the translocation of an integral membrane protein. This movement of a bacterial outer membrane protein to another membrane as a postsecretory phenomenon to our knowledge has only one precedent. This is the transfer of protein I from *Neisseria gonnorrhoeae* to erythrocyte or artificial lipid membranes, and it is inserted into the second membrane in an inverted orientation (3, 18). Our system differs in that the membrane protein probably is interiorized and probably exists in the correct orientation in the *Bdellovibrio* outer membrane. Otherwise, bacteriophage and colicin binding would be expected to be altered.

Our experiments in which we employed bacteriophage attachment rates to measure simultaneously the surface concentration of two proteins revealed that the loss of the protein from the bdelloplast surface started early and continued throughout most of the intraperiplasmic growth of the bdellovibrios. Although it is likely that we were measuring the interiorization of the proteins, we do not know if the acquisition of the proteins by the intraperiplasmic bdellovibrios followed the same kinetics. It is possible that the interiorized proteins reside at an intermediate location and are acquired at a time closer to bdelloplast lysis. An experimental approach exists which permits the premature release of intraperiplasmic bdellovibrios (28), and the application of this technique should be useful in measuring actual acquisition kinetics.

Additionally, we do not know if there is a quantitative acquisition of the translocated protein by the bdellovibrios. Approximations could be deduced from the kinetic data, but we consider these data to be semiquantitative. Intrinsic differences between the bacteriophages are a consideration in the interpretation of the kinetic data. Bacteriophage Sϕ attaches to *E. coli* containing equal levels of OmpF and OmpC proteins at 54% the velocity of bacteriophage Sϕ108. However, there is over a 14-fold range in K for Sϕ when tested against *E. coli* which possessed high and low levels of the OmpC protein. This range in K for bacteriophage Sϕ108

was only 4.4-fold. Thus, bacteriophage Sϕ is substantially more sensitive to small changes in the receptor concentration, and K_t/K_0 values for Sϕ will appear unrealistically large relative to the values obtained for bacteriophage Sϕ108. Approximately three OmpF protein receptors would be required to disappear from the bdelloplast surface to register the same K_t/K_0 as the loss of one OmpC protein receptor.

The inability of *Bdellovibrio* sp. to acquire protein 2 may be the result of this protein never appearing in the *Bdellovibrio* habitat as a major protein. The bacteriophage which encodes this protein was isolated from a clinical strain of *E. coli* (29), and this bacteriophage would be expected to be prevalent in sewage. However, the expression of protein 2 is temperature regulated (12); and the protein is undetectable in the outer membranes of lysogens grown at 25°C. This temperature regulation may also account for several research groups failing to isolate from natural sources a bacteriophage which recognized protein 2 as its receptor. The absence of protein 2 from the *Bdellovibrio* habitat would prevent the acquisition mechanism from evolving the flexibility to manage more diverse proteins.

LITERATURE CITED

1. **Adams, M. H.** 1959. Bacteriophages. Interscience Publishers, Inc., New York.
2. **Bassford, P. J., Jr., D. L. Diedrich, C. A. Schnaitman, and P. Reeves.** 1977. Outer membrane proteins of *Escherichia coli*. VI. Protein alteration in bacteriophage-resistant mutants. J. Bacteriol. **131**:608–622.
3. **Blake, M. S., and E. C. Gotschlich.** 1983. Gonococcal membrane proteins: speculation on their role in pathogenesis. Prog. Allergy **33**:298–313.
4. **Braun, V., and H. J. Krieger-Brauer.** 1977. Interrelationship of the phage λ receptor protein and maltose transport in mutants of *Escherichia coli* K12. Biochim. Biophys. Acta **469**:89–98.
5. **Cavard, D., and C. Lazdunski.** 1981. Involvement of BtuB and OmpF proteins in binding and uptake of colicin A. FEMS Microbiol. Lett. **12**:311–316.
6. **Chai, T.-J., V. Wu, and J. Foulds.** 1982. Colicin A receptor: role of two *Escherichia coli* outer membrane proteins (OmpF protein and *btuB* gene product) and lipopolysaccharide. J. Bacteriol. **151**:983–988.
7. **Davis, B. D., and E. S. Mingioli.** 1950. Mutants of *Escherichia coli* requiring methionine or vitamin B_{12}. J. Bacteriol. **60**:17–28.
8. **Diedrich, D. L., and J. A. Fralick.** 1982. Relationship between the OmpC and LamB proteins of *Escherichia coli* and its influence on the protein mass of the outer membrane. J. Bacteriol. **149**:156–160.
9. **Diedrich, D. L., C. A. Portnoy, and S. F. Conti.** 1983. *Bdellovibrio* possesses a prey-derived OmpF protein in its outer membrane. Curr. Microbiol. **8**:51–56.
10. **Diedrich, D. L., A. O. Summers, and C. A. Schnaitman.** 1977. Outer membrane proteins of *Escherichia coli*. V. Evidence that protein 1 and bacteriophage-directed protein 2 are different polypeptides. J. Bacteriol. **131**:598–607.
11. **Fairbanks, G., T. L. Steck, and D. F. H. Wallach.** 1971. Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. Biochemistry **10**:2606–2617.
12. **Fralick, J. A., and D. L. Diedrich.** 1982. Studies on the expression of outer membrane protein 2 in *Escherichia coli*. Mol. Gen. Genet. **188**:139–142.
13. **Gabay, J., and K. Yasunaka.** 1980. Interaction of the LamB protein with the peptidoglycan layer in *Escherichia coli* K-12. Eur. J. Biochem. **104**:13–18.
14. **Guerrini, F., V. Romano, A. Bazzocchi, and M. Valenzi.** 1980. Parasitic modification of membrane proteins of *E. coli* and other gram-negative hosts to infection by *Bdellovibrio bacteriovorus*. Atti Assoc. Genet. Ital. **26**:175–177. (In Italian, English abstract.)
15. **Hindennach, I., and U. Henning.** 1975. The major proteins of the *Escherichia coli* outer cell envelope membrane. Eur. J. Biochem. **59**:207–213.
16. **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.
17. **Lucky, M., and H. Nikaido.** 1980. Diffusion of solutes through channels produced by phage lambda receptor protein of *Escherichia coli*: inhibition by higher oligosaccharides of maltose series. Biochem. Biophys. Res. Commun. **93**:166–171.
18. **Lynch, E. C., M. S. Blake, E. C. Gottschlich, and A. Mauro.** 1984. Studies of porins spontaneously transferred from whole cells and reconstituted from purified porteins of *Neisseria gonorrhoeae* and *Neisseria meningitidis*. Biophys. J. **45**:104–107.
19. **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.
20. **Palva, E. T., and P. Westermann.** 1979. Arrangement of the maltose-inducible major outer membrane proteins, the bacteriophage λ receptor in *Escherichia coli* and the 44K protein in *Salmonella typhimurium*. FEBS Lett. **99**:77–80.
21. **Pugsley, A. P., and C. A. Schnaitman.** 1978. Outer membrane proteins of *Escherichia coli*. VII. Evidence that bacteriophage-directed protein 2 functions as a pore. J. Bacteriol. **133**:1181–1189.
22. **Pugsley, A. P., and C. A. Schnaitman.** 1978. Identification of three genes controlling production of new outer membrane pore proteins in *Escherichia coli* K-12. J. Bacteriol. **135**:1118–1129.
23. **Pugsley, A. P., and C. A. Schnaitman.** 1979. Factors affecting the electrophoretic mobility of the major outer membrane proteins of *Escherichia coli* in polyacrylamide gels. Biochim. Biophys. Acta **581**:163–178.
24. **Rittenberg, S. C.** 1972. Nonidentity of *Bdellovibrio bacteriovorus* strains 109D and 109J. J. Bacteriol. **109**:432–433.
25. **Rittenberg, S. C.** 1982. Bdellovibrios—intraperiplasmic growth, p. 379–390. *In* R. G. Burns and J. H. Slater (ed.), Experimental microbial ecology. Blackwell Scientific Publications, Oxford.
26. **Rittenberg, S. C., and M. F. Thomashow.** 1979. Intraperiplasmic growth—life in a cozy environment, p. 80–86. *In* D. Schlessinger (ed.), Microbiology—1979. American Society for Microbiology, Washington, D.C.
27. **Rosenbusch, J. P.** 1974. Characterization of the major envelope protein from *Escherichia coli*: regular arrangement on the peptidoglycan and unusual dodecyl sulfate binding. J. Biol. Chem. **249**:8019–8029.
28. **Ruby, E. G., and S. C. Rittenberg.** 1983. Differentiation after premature release of intraperiplasmically growing *Bdellovibrio bacteriovorus*. J. Bacteriol. **154**:32–40.
29. **Schnaitman, C., D. Smith, and M. Forn de Salsas.** 1975. Temperate bacteriophage which causes the production of a new outer membrane protein by *Escherichia coli*. J. Virol. **15**:1121–1130.
30. **Starr, M. P., and J. C.-C. Huang.** 1972. Physiology of the bdellovibrios. Adv. Microb. Physiol. **8**:215–261.
31. **Stolp, H., and M. P. Starr.** 1963. *Bdellovibrio bacteriovorus* gen. et sp. n., a predatory, ectoparasitic, and bacteriolytic microorganism. Antonie van Leeuwenhoek J. Microbiol. Serol. **29**:217–248.
32. **Thomashow, M. F., and S. C. Rittenberg.** 1978. Penicillin-induced formation of osmotically stable spheroplasts in nongrowing *Bedllovibrio bacteriovorus*. J. Bacteriol. **133**:1484–1491.
33. **Yamada, H., T. Nogami, and S. Mizushima.** 1981. Arrangement of bacteriophage lambda receptor protein (LamB) in the cell surface of *Escherichia coli*: a reconstitution study. J. Bacteriol. **147**:660–669.