

The omp2 gene locus of *Brucella abortus* encodes two homologous outer membrane proteins with properties characteristic of bacterial porins.

H Marquis and T A Ficht
Infect. Immun. 1993, 61(9):3785.

Updated information and services can be found at:
<http://iai.asm.org/content/61/9/3785>

CONTENT ALERTS

These include:

Receive: RSS Feeds, eTOCs, free email alerts (when new articles cite this article), [more»](#)

Information about commercial reprint orders: <http://journals.asm.org/site/misc/reprints.xhtml>
To subscribe to to another ASM Journal go to: <http://journals.asm.org/site/subscriptions/>

The *omp2* Gene Locus of *Brucella abortus* Encodes Two Homologous Outer Membrane Proteins with Properties Characteristic of Bacterial Porins

HÉLÈNE MARQUIS† AND THOMAS A. FICHT*

Department of Veterinary Pathobiology, The Texas Veterinary Medical Center, Texas A&M University, College Station, Texas 77843-4467

Received 11 March 1993/Returned for modification 29 April 1993/Accepted 14 June 1993

In *Brucella abortus*, a gene encoding a major cell envelope protein, *omp2*, is duplicated within a short segment of the large chromosomal DNA. Although both genes contain open reading frames, encoding proteins of high identity, expression from only one, *omp2b*, has been detected in laboratory-grown *B. abortus*. In the present study, we wished to determine whether *omp2b* encodes the previously studied *Brucella* porin and to characterize the *omp2a* gene product. Experiments were performed with *Escherichia coli* transformants expressing either *omp2a* or *omp2b*. Our results indicated that both gene products localized to the outer membrane of *E. coli*. Initial rates of transport of [¹⁴C]maltose and growth rates in the presence of maltodextrins of defined size indicated an increased hydrophilic permeability of transformants expressing *omp2a*. These cells were also shown to grow on maltotetraose, a molecule with a molecular mass of 667 Da. Activity consistent with the formation of pores could not be demonstrated in transformants expressing *omp2b*. However, Omp2b formed oligomers resistant to heat denaturation up to 70°C in sodium dodecyl sulfate buffer, a property characteristic of bacterial porins. Overall, these results suggest that the *omp2a* gene product has pore-forming activity and that the *omp2b* gene encodes the previously characterized *Brucella* porin.

In gram-negative bacteria, the outer membrane (OM) serves as a primary permeability barrier through which the passage of solutes is controlled by major OM proteins (2, 25, 27). The hydrophilic permeability of the bacterial OM is mainly a function of pore diameter and the number of porin molecules per cell. Accordingly, bacteria can adapt to changing environmental conditions by modulating porin gene expression. The best-studied example of this adaptation process is the regulation of the major *Escherichia coli* porins, OmpF and OmpC. OmpF, which forms a larger pore than OmpC, is preferentially produced under conditions of low osmolarity, whereas OmpC is most abundant under conditions of high osmolarity (24, 34). Temperature also regulates *ompF* and *ompC* gene expression (19).

In *Brucella* species, there are three major groups of OM proteins, the various components of which are believed to be peptidoglycan-associated forms of the same gene product (7, 8, 35). Group 2 OM proteins appear to oligomerize, and the apparent molecular masses of the monomeric forms range from 35 to 43 kDa. Porin activity has been associated with purified group 2 OM proteins, and the pore size was found to be comparable to that of *E. coli* OmpF (7).

Ficht et al. cloned a gene encoding a major *Brucella abortus* cell envelope (CE) protein suggested to be the *Brucella* porin gene (10). Hybridization studies and DNA sequence analysis of the gene locus revealed the presence of duplicate genes, designated *omp2a* and *omp2b*, encoding proteins of 33 and 36 kDa (10-12). The 5' one-third of both genes is identical, and the 3' one-third exhibits 90% identity, while the middle sections differ mainly by the absence of a 108-bp segment in the *omp2a* sequence. The genes are

situated on opposite DNA strands and are separated by 900 bp. Only expression from the *omp2b* gene has been detected in *B. abortus*. In *E. coli*, expression of *omp2a* was obtained after inverting the *KpnI* restriction fragment which contains the *omp2b* promoter region and the 5' end of both genes (11). Because the *omp2a* gene product has not been identified in *B. abortus*, it is not known if *omp2a* is a silent or an active gene.

As a first step to investigate the potential role for this gene duplication in *B. abortus*, we used *E. coli* transformants to characterize *B. abortus omp2a* and *omp2b* gene products. Our results showed that *B. abortus omp2a* and Omp2b were both transported to the OM of *E. coli* transformants and that Omp2b possessed physicochemical properties characteristic of bacterial porins. Most significant were the observed differences in activity of the Omp2a and Omp2b proteins, notably the increased hydrophilic permeability of an *E. coli* transformant expressing *omp2a*. The potential conditions under which a larger pore would be required for *Brucella* survival are discussed.

MATERIALS AND METHODS

Growth media and chemicals. Growth media were obtained from Difco Laboratories. Lysozyme and maltodextrins were obtained from Boehringer Mannheim Biochemicals. M63 minimal medium was prepared as described by Misra and Benson (23). Additional chemicals were purchased from either Aldrich Chemical Co., Inc., Fisher Scientific Co., or Sigma Chemical Co.

Bacterial strains and growth conditions. Bacterial strains included *E. coli* MC4100 (6) and ECB611 [MC4100($\Delta lamB ompF::Tn5 ompC::Tn10$)], which was the kind gift of Spencer Benson. ECB611 was transformed with plasmid pAGF201, pAGF211, or pAGF21 (11). ECB611(pAGF201) and ECB611(pAGF211) expressed the *omp2b* and *omp2a*

* Corresponding author. Electronic mail address: TAF4314@TAMVM1.TAMU.EDU.

† Present address: Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104-6076.

genes of *B. abortus*, respectively. ECB611(pAGF21) was a negative control which expressed neither gene. Each bacterial strain was grown in the presence of appropriate antibiotics, including ampicillin ($50 \mu\text{g ml}^{-1}$), kanamycin ($50 \mu\text{g ml}^{-1}$), and tetracycline ($12.5 \mu\text{g ml}^{-1}$). All bacterial strains were stored frozen at -80°C in Luria broth containing 50% glycerol.

Bacterial membrane fractionation. Bacterial membrane fractionation was performed as described by Osborn et al. (28). Briefly, bacterial cells were plasmolyzed in ice-cold sucrose, and spheroplasts were formed after lysozyme digestion of the cell wall. Spheroplasts were lysed in ice-cold distilled water, and CEs were harvested by ultracentrifugation.

CE components were fractionated by isopycnic centrifugation on a 30 to 55% (wt/wt) sucrose step gradient. Differentiation of fractions containing cytoplasmic membrane (CM) and OM components was based on buoyant density, protein content, and NADH oxidase activity. The buoyant density was determined by weighing a specified volume of each fraction. Protein concentration was determined by the bicinchoninic acid protein assay (Pierce). The NADH oxidase assay was performed as described below.

Sucrose fractions containing CM and OM components were individually pooled and washed in 10 mM Tris-HCl (pH 7.8)–5 mM EDTA (pH 8.0)–1 mM β -mercaptoethanol (Lutkenhaus buffer) (20). CM and OM suspended in Lutkenhaus buffer were tested for protein content, NADH oxidase activity, the presence of 2-keto-3-deoxyoctonate (KDO), and protein profiles by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE).

NADH oxidase assay. The NADH oxidase assay was performed as described by Osborn et al. (28). An aliquot of each fraction was diluted in assay buffer to obtain a final concentration of 50 mM Tris-HCl (pH 7.5)–0.2 mM 1,4-dithiothreitol. The NADH solution was prepared fresh at a concentration of 1.2 mM in the same buffer as described above. Immediately after mixing NADH and the sample at a ratio of 1:9 (vol/vol), the decrease in A_{340} was measured. The data were transformed into micromoles of NADH oxidized per minute per milligram of protein.

KDO assay. The KDO assay was performed as described by Karkhanis et al. (16). The lipopolysaccharide was hydrolyzed in sulfuric acid at 100°C and successively reacted with periodate, sodium arsenite, and 2-thiobarbituric acid, forming a red chromophore. Dimethyl sulfoxide was added to stabilize the chromophore, and the A_{548} was recorded. A standard curve was prepared by using pure KDO, and the concentration of KDO in the CM and OM fractions was calculated by using linear regression analysis of the standard curve. The results were transformed into micromoles of KDO per milligram of protein.

Bacterial OM permeability assays. Two types of assays were performed to analyze OM permeability of *E. coli* transformants: (i) an assay of initial rate of maltose transport and (ii) an assay of bacterial growth rates in minimal medium with various maltodextrins.

The assay of initial rate of maltose transport was performed as described previously (1, 5), with some modifications. Briefly, cells were grown in M63–0.4% (wt/vol) maltotriose up to an optical density of 0.5 to 0.6 at 600 nm, washed, and suspended in M63 to an A_{600} of approximately 0.5. Serial dilutions were prepared and plated in duplicate on Luria broth agar to determine viable cell counts. Five minutes before the assay, 0.2% (vol/vol) glycerol was added to the cell suspension. A portion of the cell suspension was

vacuum filtered, and then [^{14}C]maltose was added to a final concentration of $3.3 \mu\text{M}$. Equal portions of cell suspension were vacuum filtered at 10, 20, 40, 60, and 80 s. After liquid scintillation measurement, counts were transformed into picomoles of maltose. The results were adjusted for 10^8 CFU per sample. For each bacterial strain, the assay was performed twice in triplicate.

The assay of bacterial growth rates was performed as described by Misra and Benson (23). Cells were grown in M63 with 1 mM of either maltose (342 Da), maltotriose (504 Da), or maltotetraose (667 Da) as the sole carbon source. Growth was monitored by reading the A_{600} at regular intervals over 8 h. Growth rates were calculated for exponential growth phases and reported as the reciprocal of the doubling time in hours.

Bacterial CE preparation. Bacterial CEs were prepared as described by Sowa et al. (30). Bacteria were lysed by sonication. Intact cells were discarded after centrifugation at $10,000 \times g$ for 10 min at 4°C , and CEs were harvested from the supernatant by ultracentrifugation at $100,000 \times g$ for 45 min at 4°C .

Protein electrophoresis and Western blot (immunoblot). Proteins were fractionated by SDS-PAGE (17). Proteins were stained with Coomassie brilliant blue R250 or electroblotted to nitrocellulose. The following Bio-Rad protein markers were used to determine the molecular masses of unknown proteins: myosin, 200 kDa; β -galactosidase, 116 kDa; phosphorylase B, 97 kDa; bovine serum albumin, 66 kDa; ovalbumin, 45 kDa; carbonic anhydrase, 31 kDa; soybean trypsin inhibitor, 21 kDa; and lysozyme, 14 kDa.

Fractionated proteins were transferred to nitrocellulose as described previously (30, 33). For immunodetection, the membrane was reacted with rabbit anti-*B. abortus* Omp2b hyperimmune serum preadsorbed with *E. coli* MC4100 (λ gt11) (10). Goat anti-rabbit immunoglobulin G conjugated with alkaline phosphatase (Kirkegaard & Perry Laboratories, Inc.) was used as the secondary antibody, and the enzymatic reaction was induced with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as described by Blake et al. (3).

RESULTS

Cellular localization of Omp2a and Omp2b produced in *E. coli*. In a previous study, it was shown that the *omp2b* gene product was present in the CE of *B. abortus* but the *omp2a* gene product could not be detected (11). To determine the subcellular localization of both gene products, we used transformants of *E. coli* ECB611 expressing either *omp2a* or *omp2b*. After fractionation, bacterial CE components were clearly differentiated. Sucrose fractions containing CM had a mean buoyant density of 1.17 g ml^{-1} and high levels of NADH oxidase activity, while those containing OM had a mean buoyant density of 1.24 g ml^{-1} and an elevated protein content. CM- and OM-containing fractions were then individually pooled, washed, concentrated in Lutkenhaus buffer, and retested for NADH oxidase activity, KDO content, and protein concentration. High levels of NADH oxidase activity were detected in the CM fractions, while levels on the order of 5 to 10% of the CM values were detected in the OM fractions (Table 1). KDO measurements indicated that CM fractions had levels on the order of 30 to 50% of the OM values. These results indicated that, while the CM fractions were substantially contaminated with OM, the OM fractions were 90 to 95% pure.

Membrane fractionation was also monitored by comparing

TABLE 1. Evaluation of CM and OM purification by detection of specific markers

Bacterial strain	NADH oxidase activity ($\mu\text{mol min}^{-1}$ mg of protein $^{-1}$)		KDO concn ($\mu\text{mol mg of protein}^{-1}$)	
	CM	OM	CM	OM
ECB611(pAGF211)	0.521	0.055	0.116	0.365
ECB611(pAGF201)	0.910	0.040	0.124	0.250

SDS-PAGE protein profiles of each fraction. Protein profiles of OM and CM were distinct, as shown for ECB611 (pAGF201) (Fig. 1a). The OM fraction contained a small number of proteins, including a major protein of 34 kDa, OmpA, and other less abundant protein species. The CM fraction was much more complex, containing a high number of proteins at low copy number and contaminating OM proteins. The amount of Omp2a and Omp2b produced in *E. coli* was too low to be visualized on stained gels.

The cellular location of Omp2a and Omp2b in *E. coli* was established by Western blot (Fig. 1b). The bulk of Omp2a and Omp2b was observed in the OM fractions, whereas the reduced amount copurifying with the CM fractions was undoubtedly due to OM contamination, as determined above. These results suggested that Omp2a and Omp2b are OM proteins. However, because fractionation methods may in some instances be misleading (32), it was important to complement these results with additional assays.

Omp2a increases the hydrophilic permeability of transformed *E. coli*. Because *omp2a* and *omp2b* are the apparent result of a gene duplication, it was logical to infer that the functions of their gene products could be highly similar. It

was hypothesized by Ficht et al. (11) that *omp2b* encodes the *Brucella* porin characterized by Douglas et al. (7). Thus, the hydrophilic permeability of *E. coli* transformants expressing either *B. abortus omp2a* or *omp2b* was investigated. Results from two types of assays evaluating OM permeability are described below.

(i) **Assay of initial rate of transport of [^{14}C]maltose.** The transport of maltose in *E. coli* is highly regulated. When maltose is the only carbon source available, a series of genes involved in maltose uptake is expressed (4). Maltose normally enters the cell through LamB, a specific channel with high affinity for maltodextrins (9). It is subsequently transported through the periplasmic gel bound to the maltose-binding protein and through the CM by a specific active uptake system (4). Because ECB611 has a deletion in *lamB*, maltose has to enter the cell through nonspecific diffusion channels. In that case, the number of maltose molecules entering the cell should be a function of pore diameter and the number of porin molecules present in the OM. Subsequent transport of maltose through the periplasm and the CM should proceed as usual. Results from this assay indicated that Omp2a, but not Omp2b, increased the hydrophilic permeability of the OM (Fig. 2).

(ii) **Assay of bacterial growth rates.** Bacterial growth rates in minimal medium supplemented with maltodextrins of defined size were determined. The internal diameter of the largest pore in the bacterial OM should limit the size of hydrophilic nutrients entering the cells, thereby influencing growth rates. When the only source of carbon available was maltose (342 Da), growth rates were similar for the three ECB611 strains tested (Table 2). However, when the only source of carbon available was maltotriose (504 Da), the mean growth rate of ECB611(pAGF211) was 2.9 and 2.2 times higher than that observed for ECB611(pAGF21) and

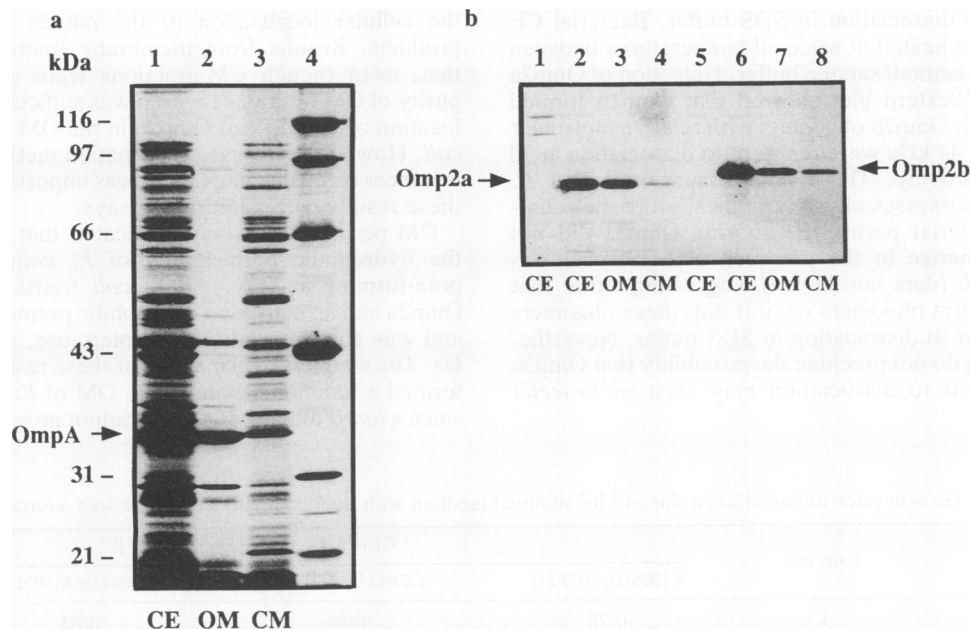


FIG. 1. CE fractionation and localization of *B. abortus* Omp2a and Omp2b in transformed *E. coli* strains. Proteins were heated at 100°C in Laemmli sample buffer and fractionated by SDS-PAGE. (a) Coomassie brilliant blue R250-stained gel. Lanes: 1 to 3, ECB611(pAGF201) CE, OM, and CM proteins (20, 8, and 12 μg), respectively; 4, Bio-Rad SDS-PAGE markers. Molecular masses are indicated in kilodaltons. Omp2b could not be detected on the stained gel. (b) Western blot probed with preadsorbed Omp2b rabbit antiserum. Lanes: 1 and 5, ECB611(pAGF21) CE proteins (10 and 2 μg), respectively as a negative control; 2 to 4, ECB611(pAGF211) CE, OM, and CM (10, 8, and 12 μg), respectively; 6 to 8, ECB611(pAGF201) CE, OM, and CM (2, 1.6, and 2.4 μg), respectively.

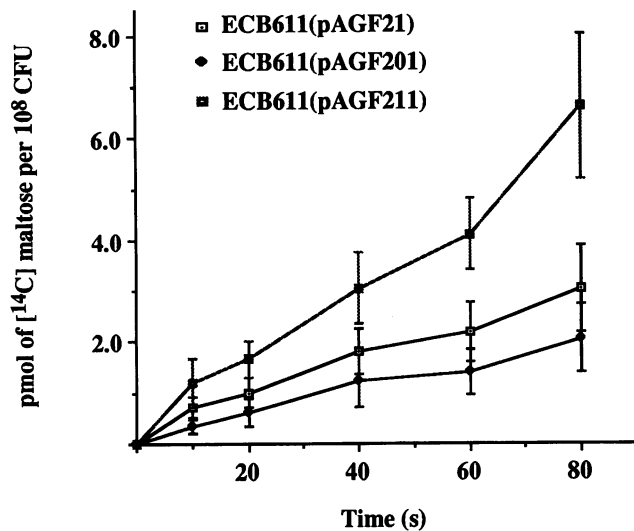


FIG. 2. Initial transport of [¹⁴C]maltose by transformed *E. coli* strains. Six assays were performed with each transformant. Vertical bars indicate standard deviations.

ECB611(pAGF201), respectively. In M63-maltotetraose (667 Da), only ECB611(pAGF211) grew. MC4100 was used as a positive control, and its growth rate was not affected by the size of the maltodextrin, because of the presence of a specific maltoporin, LamB. These results suggested that Omp2a increases the permeability of ECB611 to larger maltodextrins.

Omp2b is resistant to heat denaturation in SDS buffer. The majority of bacterial porins form trimers resistant to heat denaturation in SDS buffer (15, 18). We investigated the possibility that *B. abortus* Omp2a and Omp2b form oligomers resistant to dissociation in SDS buffer. Bacterial CE preparations were heated at selected temperatures between 25 and 100°C in Laemmli sample buffer. Detection of Omp2a and Omp2b by Western blot showed that Omp2b formed oligomers (Fig. 3). Omp2b oligomers with relative molecular masses of 63 and 44 kDa were resistant to dissociation at 70 and 60°C, respectively. These results suggested that *B. abortus* Omp2b possesses physicochemical properties characteristic of bacterial porins. *B. abortus* Omp2a did not appear to oligomerize in the presence of SDS when expressed in *E. coli* (data not shown), suggesting either that Omp2a did not form oligomers or, if it did, these oligomers were not resistant to dissociation in SDS buffer. Nevertheless, these results do not preclude the possibility that Omp2a oligomers resistant to dissociation may exist in *Brucella* species.

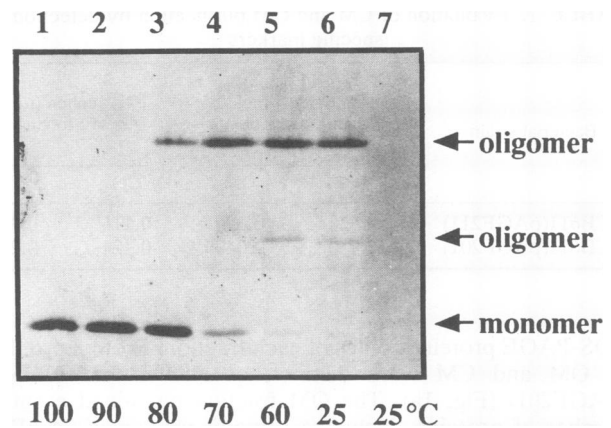


FIG. 3. Resistance of *B. abortus* Omp2b to heat denaturation in SDS buffer. CEs in Laemmli sample buffer were heated at the temperatures indicated on the figure and fractionated by SDS-PAGE. Omp2b was detected by Western blot with a preadsorbed Omp2b rabbit antiserum. Lanes: 1 to 6, ECB611(pAGF201) CE; 7, ECB611(pAGF21) CE. Protein content is 1 µg per lane.

DISCUSSION

Results from the present investigation suggested that Omp2a has pore-forming activity. When *B. abortus* Omp2a was present in the OM of *E. coli*, the hydrophilic permeability of the cell increased, allowing diffusion of molecules as large as 667 Da. Although we were unable to demonstrate porin activity directly for Omp2b, we identified oligomers having resistance to heat denaturation in SDS buffer up to 70°C, a physicochemical property consistent with bacterial porins.

Because the *omp2a* gene product has not been detected in *B. abortus*, we used transformed *E. coli* strains to determine the cellular localization of the *omp2a* and *omp2b* gene products. Results from membrane fractionation indicated that, even though CM fractions were contaminated, the purity of OM fractions (>90%) was sufficient to establish the location of Omp2a and Omp2b in the OM of transformed *E. coli*. However, because fractionation methods may in some instances be misleading (32), it was important to complement these results with additional assays.

OM permeability assays indicated that Omp2a increased the hydrophilic permeability of *E. coli*, consistent with pore-forming activity. An *E. coli* transformant producing Omp2a had an increased hydrophilic permeability to maltose and was able to grow on maltotetraose, a molecule of 667 Da. The simplest explanation for these results is that Omp2a formed a functional pore in the OM of *E. coli*. In addition, since a *lamB* mutant of *E. coli* cannot grow on maltotetraose

TABLE 2. Growth rates of bacterial strains in M63 minimal medium with defined maltodextrins as sole sources of carbon

Carbon source ^a	Expt no.	Growth rate (doubling time [h ⁻¹])			
		ECB611(pAGF21)	ECB611(pAGF201)	ECB611(pAGF211)	MC4100
Maltose (342)	1	0.78	0.86	0.83	0.95
	2	0.80	0.83	0.88	0.88
Maltotriose (504)	1	0.17	0.20	0.68	0.96
	2	0.30	0.43	0.69	0.87
Maltotetraose (667)	1	0.00	0.00	0.41	0.97
	2	0.00	0.00	0.25	0.88

^a Sole carbon source available at 1 mM concentration. Molecular weight is shown in parentheses.

(23), it may be speculated that the Omp2a pore is larger than the OmpF pore. The observation that Omp2b did not increase the hydrophilic permeability of *E. coli* will be discussed further below.

A large number of bacterial OM pore-forming proteins exist as oligomers in SDS buffer, and a limited number have been shown to exist as trimers (14, 15). These properties are characteristic of pore-forming proteins and were observed with Omp2b. Omp2b formed oligomers resistant to dissociation up to a temperature of 70°C in SDS buffer. The SDS-PAGE migration pattern of Omp2b oligomers was comparable to that of *E. coli* porin trimers and dimers, which migrate as proteins of approximately 66 and 50 kDa (29). Considering that porin oligomerization occurs in the OM (26), the above results are consistent with Omp2b oligomers in the OM of transformed *E. coli*, supporting the results of membrane fractionation.

The observation that Omp2a was not detected in an oligomeric form is by no means conclusive, and future experiments are designed to analyze the protein in *B. abortus*. Porins can be monomeric or oligomers can be unstable in SDS. For example, the Tsx porin of *E. coli* and OprE of *Pseudomonas aeruginosa* have only been detected as monomers by SDS-PAGE (15, 21).

There is strong evidence indicating that the characterized *Brucella* porin and the *omp2b* gene product are the same: (i) both proteins are a major component of the *Brucella* CE (7, 10), (ii) they have similar molecular masses (7, 11), (iii) their amino acid compositions are almost identical (11, 35), and (iv) they both form oligomers resistant to dissociation in SDS (7). However, Omp2b porin activity was not observed in ECB611. This may be consistent either with the complete absence of porin activity in *E. coli* despite oligomerization or a porin activity which is masked by the presence of other pore-forming proteins. ECB611 does not express the major *ompF* and *ompC* porin genes and has a deletion in *lamB*. The contribution of Tsx or PhoE to the basic hydrophilic permeability of ECB611 can be eliminated on the basis that Tsx has a small internal diameter and a strong affinity for nucleosides (21) and that *phoE* expression is repressed by inorganic phosphate (18), which was present in the growth medium. However, a recent report suggests that OmpA has pore-forming activity (31) and that additional pore-forming proteins are expressed in *E. coli* (14a). The sizes of the channels formed by OmpA, the CE1248 porin, and the *Brucella* porin are all similar to those of OmpF and OmpC porins (7, 14a, 31). Therefore, expression of these proteins may have masked Omp2b pore activity and thus represents a limitation of the methods employed.

What would be the utility of a duplicated porin gene? Bacterial evolution is often associated with adaptation because novel adaptive capabilities increase the chances of survival in hostile environments. Adaptation is frequently associated with duplication of essential chromosomal determinants of pathogenicity. One of the best-studied examples of adaptive evolution and gene duplication is the *ompF* and *ompC* porin genes of *E. coli*. Levels of expression of *ompF* and *ompC* are primarily dependent on the osmolarity of the extracellular milieu (24, 34). OmpC forms a smaller pore than OmpF does and is the predominant porin under conditions of high osmolarity, whereas the amount of OmpF is greater under iso- or hypoosmotic conditions. This is thought to help *E. coli* to survive environmental osmotic fluctuations.

B. abortus-duplicated genes, *omp2a* and *omp2b*, may also be the consequence of adaptive evolution. This is best

explained by the formation of a pore, Omp2b, the permeability of which is optimal to allow good growth and protection against harmful compounds such as antimicrobial agents. The larger pore formed by Omp2a may be advantageous for intracellular growth of *Brucella* species which compete with the host for nutrients, the concentration of which may be particularly low within the phagosome (13). Under these conditions, a large pore would be advantageous for growth, accelerating nutrient assimilation by increasing the permeability coefficient. It is interesting that the sequence missing from *omp2a* is reminiscent of the situation in *E. coli* *ompC* mutants isolated by Benson and Decloux (1) which were selected for growth on large maltodextrins. These mutants were shown to have a deletion of the L3 loop which functions to narrow the channel diameter (6a). Final characterization of *omp2a* and *omp2b* gene products awaits purification directly from *B. abortus* or genetic analysis of the appropriate mutants. The former requires determination of growth conditions which will induce the expression of *B. abortus omp2a*. On the basis of the hypothesis that Omp2a forms a porin with a larger internal diameter than Omp2b does, one can speculate on a large variety of conditions that may induce *omp2a* expression, the simplest being osmotic regulation. However, induction of *omp2a* gene expression in *B. abortus* was not detected under hypoosmotic conditions (22). Experiments investigating porin gene expression under environmental conditions directly associated with *Brucella* pathogenicity, such as intracellular growth, and genetic analysis via construction of appropriate mutants are in progress.

ACKNOWLEDGMENTS

We thank Howard Goldfine, Jeff Weiser, and Daniel A. Portnoy from the University of Pennsylvania for critically reviewing the manuscript. This research was supported by U.S. Department of Agriculture grants 84-CRSR-2-2503 and 86-CRSR-2-2806 and grants from the Texas Advanced Research Technology Program.

REFERENCES

- Benson, S. A., and A. Decloux. 1985. Isolation and characterization of outer membrane permeability mutants in *Escherichia coli* K-12. *J. Bacteriol.* **161**:361-367.
- Benz, R. 1988. Structure and function of porins from Gram-negative bacteria. *Annu. Rev. Microbiol.* **42**:359-393.
- Blake, M. S., K. H. Johnston, G. J. Russell-Jones, and E. C. Gotschlich. 1984. A rapid, sensitive method for detection of alkaline phosphatase-conjugated antibody on Western blots. *Anal. Biochem.* **136**:175-179.
- Brass, J. M. 1986. The cell envelope of Gram-negative bacteria: new aspects of its function in transport and chemotaxis. *Curr. Top. Microbiol. Immunol.* **129**:1-92.
- Brass, J. M., W. Boos, and R. Hengge. 1981. Reconstitution of maltose transport in *malB* mutants of *Escherichia coli* through calcium-induced disruptions of the outer membrane. *J. Bacteriol.* **146**:10-17.
- Casadaban, M. J. 1976. Transposition and fusion of the *lac* genes to selected promoters in *Escherichia coli* using bacteriophage lambda and Mu. *J. Mol. Biol.* **104**:541-555.
- Cowan, S. W., T. Schirmer, G. Rummel, M. Steiert, R. Ghosh, R. A. Pauptit, J. N. Jansonius, and J. P. Rosenbusch. 1992. Crystal structures explain functional properties of two *E. coli* porins. *Nature (London)* **358**:727-733.
- Douglas, J. T., E. Y. Rosenberg, H. Nikaido, D. R. Verstrete, and A. J. Winter. 1984. Porins of *Brucella* species. *Infect. Immun.* **44**:16-21.
- Dubray, G., and C. Charriaut. 1983. Evidence of three major polypeptide species and two major polysaccharide species in the *Brucella* outer membrane. *Ann. Rech. Vet.* **14**:311-318.
- Ferenci, T. 1989. Selectivity in solute transport: binding sites

- and channel structure in maltoporin and other bacterial sugar transport proteins. *Bioessays* 10:3-7.
10. Ficht, T. A., S. W. Bearden, B. A. Sowa, and L. G. Adams. 1988. A 36-kilodalton *Brucella abortus* cell envelope protein is encoded by repeated sequences closely linked in the genomic DNA. *Infect. Immun.* 56:2036-2046.
 11. Ficht, T. A., S. W. Bearden, B. A. Sowa, and L. G. Adams. 1989. DNA sequence and expression of the 36-kilodalton outer membrane protein gene of *Brucella abortus*. *Infect. Immun.* 57:3281-3291.
 12. Ficht, T. A., S. W. Bearden, B. A. Sowa, and H. Marquis. 1990. Genetic variation at the *omp2* porin locus of the brucellae: species-specific markers. *Mol. Microbiol.* 4:1135-1142.
 13. Garcia-del Portillo, F., J. W. Foster, M. E. Maguire, and B. B. Finlay. 1992. Characterization of the micro-environment of *Salmonella typhimurium*-containing vacuoles within MDCK epithelial cells. *Mol. Microbiol.* 6:3289-3297.
 14. Hancock, R. E. W. 1987. Model membrane studies of porin function, p. 187-225. *In* M. Inouye (ed.), *Bacterial outer membranes as model systems*. John Wiley & Sons, Inc., New York.
 - 14a. Hancock, R. E. W., C. Egli, R. Benz, and R. J. Siehnel. 1992. Overexpression in *Escherichia coli* and functional analysis of a novel PP_i-selective porin, OprO, from *Pseudomonas aeruginosa*. *J. Bacteriol.* 174:471-476.
 15. Hancock, R. E. W., R. Siehnel, and N. Martin. 1990. Outer membrane proteins of *Pseudomonas*. *Mol. Microbiol.* 4:1069-1075.
 16. Karkhanis, Y. D., J. Y. Zeltner, J. J. Jackson, and D. J. Carlo. 1978. A new and improved microassay to determine 2-keto-3-deoxyoctonate in lipopolysaccharide of Gram-negative bacteria. *Anal. Biochem.* 85:595-601.
 17. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* 227:680-685.
 18. Lugtenberg, B., and L. Van Alphen. 1983. Molecular architecture and functioning of the outer membrane of *Escherichia coli* and other Gram-negative bacteria. *Biochim. Biophys. Acta* 737:51-115.
 19. Lundrigan, M. D., and C. F. Earhart. 1984. Gene *envY* of *Escherichia coli* K-12 affects thermoregulation of major porin expression. *J. Bacteriol.* 157:262-268.
 20. Lutkenhaus, J. F., B. A. Moore, M. Masters, and W. D. Donachie. 1979. Individual proteins are synthesized continuously throughout the *Escherichia coli* cell cycle. *J. Bacteriol.* 138:352-360.
 21. Maier, C., E. Bremer, A. Schmid, and R. Benz. 1988. Pore-forming activity of the Tsx protein from the outer membrane of *Escherichia coli*. *J. Biol. Chem.* 263:2493-2499.
 22. Marquis, H., and T. A. Ficht. Unpublished data.
 23. Misra, R., and S. A. Benson. 1988. Isolation and characterization of *OmpC* porin mutants with altered pore properties. *J. Bacteriol.* 170:528-533.
 24. Mizuno, T., and S. Mizushima. 1990. Signal transduction and gene regulation through the phosphorylation of two regulatory components: the molecular basis for the osmotic regulation of the porin genes. *Mol. Microbiol.* 4:1077-1082.
 25. Nikaido, H. 1992. Porins and specific channels of bacterial outer membranes. *Mol. Microbiol.* 6:435-442.
 26. Nikaido, H., and J. Reid. 1990. Biogenesis of prokaryotic pores. *Experientia* 46:174-180.
 27. Nikaido, H., and M. Vaara. 1985. Molecular basis of bacterial outer membrane permeability. *Microbiol. Rev.* 49:1-32.
 28. Osborn, M. J., J. E. Gander, E. Parisi, and J. Carson. 1972. Mechanism of assembly of the outer membrane of *Salmonella typhimurium*. Isolation and characterization of cytoplasmic and outer membrane. *J. Biol. Chem.* 247:3962-3972.
 29. Reid, J., H. Fung, K. Gehring, P. E. Klebba, and H. Nikaido. 1988. Targeting of porin to the outer membrane of *Escherichia coli*. *J. Biol. Chem.* 263:7753-7759.
 30. Sowa, B. A., K. A. Kelly, T. A. Ficht, M. Frey, and L. G. Adams. 1991. SDS-soluble and peptidoglycan-bound proteins in the outer membrane-peptidoglycan complex of *Brucella abortus*. *Vet. Microbiol.* 27:351-369.
 31. Sugawara, E., and H. Nikaido. 1992. Pore-forming activity of *OmpA* protein of *Escherichia coli*. *J. Biol. Chem.* 267:2507-2511.
 32. Tommassen, J. 1986. Fallacies of *E. coli* cell fractionations and consequences thereof for protein export models. *Microb. Pathog.* 1:225-228.
 33. Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA* 76:4350-4354.
 34. Van Alphen, W., and B. Lugtenberg. 1977. Influence of osmolarity of the growth medium on the outer membrane protein pattern of *Escherichia coli*. *J. Bacteriol.* 131:623-630.
 35. Verstrete, D. R., M. T. Creasy, N. T. Caveney, C. L. Baldwin, M. W. Blab, and A. J. Winter. 1982. Outer membrane proteins of *Brucella abortus*: isolation and characterization. *Infect. Immun.* 35:979-989.