Novel Porin genes and modes of Porin regulation in Salmonella typhi

Irma Martínez-Flores, Ricardo Oropeza, Roxana Cano, Clara L. Sampieri, José Luis Puente, Edmundo Calva

Abstract

S. typhi ompS1 constitutes a novel porin gene. It has seven putative OmpR-binding boxes and three promoters. Our current model for expression contemplates activation of transcription, from P1, by OmpR-binding to boxes II and III (C-type, low affinity), whereas boxes I and IV-VII would be involved in negative regulation. The fact that ompS1 expression increases more than 10-fold upon removal of the cis-acting elements, and that this level is more than 10-fold higher than that for ompC, which codes for a major porin, raises interesting questions on its role and regulation in nature. The analysis of the expression of S. typhi and E. coli ompC: lacZ fusions, in cross-complementation experiments with either the S. typhi or E. coli ompB (ompR/envZ) operons, in either S. typhi or E. coli ompB backgrounds, showed that both S. typhi and E. coli ompC are not regulated by osmolarity when they are under the control of S. typhi ompB in S. typhi. Interestingly, in an S. typhi background, both genes are osmoregulated under E. coli ompB. In contrast, in E. coli, they are both osmoregulated under S. typhi or E. coli ompB. Thus, the lack of osmoregulation of S. typhi ompC expression, is determined both by the S. typhi ompB operon and by other unknown factors present in S. typhi.

Typhoid Fever and other Salmonellosis

Typhoid fever (TF) is still a major health problem throughout the developing world; it is estimated that there are 16.6 million cases annually, with nearly 600 thousand deaths. S. typhi, the causal agent of TF, causes a septicemia in man; while S. typhimurium causes a typhoid-like infection in mice. Thus, new knowledge on S. typhi virulence and on the immune response during TF, should allow the design of improved procedures for the control and prevention of the disease, especially in poor areas of the world. Moreover, the new information should be of use towards a global understanding of the molecular pathogenesis of other salmonellosis, on the rise worldwide. Of great relevance will be the possibility of providing new insights on gene regulation in bacteria.

Porins in Salmonella typhi

The bacterial surface structures are of fundamental importance in these processes, as they provide contact with the host cells and milieu. Porins are pore-forming proteins that reside in the outer membrane of gram-negative bacteria; they are believed to participate in the non-selective passive diffusion of small solutes. Accordingly, one might imagine bacteria as having a single-kind of porin, forming multipurpose pores for exchanging nutrients, waste substances, and other compounds, in-and-out of the cell.

Nonetheless, S. typhi synthesizes two major porins, OmpC and OmpF, which are highly abundant upon growth in standard laboratory media. It also has a third major outer membrane protein (OMP), denominated OmpA, which is thought to have a structural role. Another major porin, PhoE, is synthesized un-
der phosphate limitation, as its counterpart in Escherichia coli (E. Calva et al., unpublished).

In E.coli, the pores formed by OmpC and OmpF are weakly cation-selective; in contrast, the PhoE pores are somewhat anion-selective. These three porins produce a similar pore size, although the OmpC pore seems to be slightly smaller, i.e. 1.0 nm versus 1.1-1.2 nm, a characteristic that is thought to confer a selective advantage under high osmolarity, by restricting the diffusion of potentially harmful molecules⁵. In S. typhimurium, OmpC, OmpF, and OmpD, form weakly cation-selective pores, relatively larger than the E.coli pores, i.e. of 1.2-1.3 nm⁶⁷; while no studies on pore size have been done for S.typhi. Presently, we know that S.typhi OmpC, OmpF, and PhoE contain the conserved amino acid residues that have been implicated in pore function, and that S.typhi OmpC and PhoE are 79% and 98 % identical to their E.coli counterparts, respectively⁸⁹, and A. Torres, MSc. Thesis, UNAM, 1995.

Whether the S. typhi porins form similar pores to those of their analogues in E. coli, is an open question; moreover, the biological significance of having this array of porins is largely unknown. Interestingly, porins appear to play a key role in the permeation of -lactams, as illustrated by an E. coli OmpF-deficient mutant with an increased resistance to certain -lactams⁷¹⁰.

**Immunogenicity of OMPs**

A specific humoral and cellular immune response is mounted against S. typhi OMPs in TF patients; this has allowed the design of novel diagnostic assays¹¹-¹⁶. Moreover, an enzyme-linked immunosorbent assay (ELISA) for the rapid and specific detection of TF patients, based on S. typhi OMPs and designed for the Mexican population, was also found to be useful in Malaysia¹⁴. Furthermore, S. typhimurium and S.typhi OMPs have proven to be relevant immunogens for protection of mice challenged with virulent strains¹⁷-²¹.

In addition, the protective effect was enhanced when porin-lipopolysaccharide (LPS) complexes were used as immunogens, indicating the role of intricate outer membrane structures in eliciting an immune response ¹⁷-²¹. These observations might help explain the dependence of the protective effect on the type of LPS present on the surface of the bacteria²². In this respect, it has been recently shown that neisserial porins can act synergistically with the LPS, to induce proliferation and immunoglobulin secretion by resting B-cells²³, further supporting the notion that the immune response to the cell envelope is towards a complex mixture of antigens, a subject that requires further addressing by researchers.

Furthermore, the stimulation of peripheral lymphocytes, the activation of complement, and the release of several cytokines, namely tumor necrosis factor, interleukin-1-, and IL-6, have been observed in response to S. typhimurium porins²⁴-²⁸. In this regard, immunomodulatory activities, as well as a hypersensitivity reaction, have been recently described for S. typhi porins; so that their use as adjuvants or as immunostimulants towards unrelated antigens, such as those from Mycobacteria, has been proposed²⁷.

**The Interaction of OMPs with Host Cells**

The study of OMPs, including porins, is of relevance to the understanding of the bacterial pathogen-host interaction. Adherence and invasion of mucosal epithelial cells are the first stages in the establishment of infection by enteropathogenic bacteria. Thus, it has been shown that OMPs participate in the invasion of host cells by Yersinia and Shigella²⁸-³². In particular, the lack of OmpC in Shigella, results in lowering of virulence due to a decrease in intracellular spreading³¹. Interestingly, S. typhimurium strains lacking both the OmpC and OmpF porins have lowered virulence³³. A Salmonella OMP, PagC, has been involved in survival within macrophages³⁴. Moreover, it has been found that the S. typhimurium ompR porin regulatory locus is of major importance, for the survival of Salmonella within host macrophages³⁵.

Toxic effects by S. typhimurium porin preparations on Hela, Hep-2, and mouse renal cells have been observed³⁶; as well as a stimulation of platelet-activating-factor biosynthesis, by human endothelial cells and polymorphonuclear neutrophils³⁷.

**Regulation of ompC in S.typhi**

Our group has initially focused its research on the major, abundant, S.typhi porins and their genes⁴⁸,³⁹,⁴⁰, as well as on the ompR and envZ regulatory loci⁴¹. In this respect, we have shown that the expression of OmpC in S.typhi is not influenced by osmolarity, being always expressed at high levels; whereas expression of OmpC in E. coli is indeed higher at high osmolarity. This observation is contrary to the generally accepted view that OmpC has a specific role in high osmolarity environments, such as those found inside
host cells, in the serum, or in the bile tract. On the other hand, OmpF is regulated in the same manner in *S. typhi* as in *E. coli*: it is expressed preferentially at low osmolarity.

Our recent observations point towards the existence of particular factors in *S. typhi* that, together with the EnvZ and OmpR regulatory proteins, determine the particular behavior of OmpC expression. The analysis of the expression of *S. typhi* and *E. coli* ompC-lacZ fusions, in cross-complementation experiments with either the *S. typhi* or *E. coli* ompB (ompR/EnvZ) operons, in either *S. typhi* or *E. coli* ompB backgrounds, showed that both *S. typhi* and *E. coli* ompC are not regulated by osmolarity when they are under the control of *S. typhi* ompB in *S. typhi*. Interestingly, in an *S. typhi* background, both genes are osmoregulated under *E. coli* ompB. In contrast, in *E. coli*, they are both osmoregulated under *E. coli* ompB; and, surprisingly, they are also osmoregulated by *S. typhi* ompB (Martínez-Flores, et al, manuscript in preparation).

Thus, the lack of osmoregulation of *S. typhi* ompC expression, is determined both by the *S. typhi* ompB operon and by other unknown factors present in *S. typhi*. Since the OmpR regulator proteins are identical in *S. typhi* and in *E. coli*, it seems likely that this differential behavior is determined, at least in part, by the EnvZ sensor protein, which differs in 21 out of 450 amino acid residues between both bacteria. Interestingly, 16 of these differences lie towards the carboxy-terminus, between residues 303 and 450.

Hence, this project has lured us into probing structure-function relationships in EnvZ, as well as into reflecting on a general and broad question: what is the significance of osmoregulation in bacterial gene expression?

Regulation of *S. typhi* ompS1

Contributing towards an increasingly complex view of the matter, we made the unexpected finding of two novel *S. typhi* porin genes, namely *ompS1* and *ompS2*. This happened while searching for ompF by heterologous hybridization with the *E. coli* ompF gene as probe. The *ompS1* and *ompS2* genes code for OMPs with the conserved amino acid motifs, characteristic of enterobacterial and neisserial non-selective porins; and are not expressed at major levels under standard laboratory conditions.

By assaying the activity of a reporter gene, linked to various lengths of the *ompS1* 5′ regulatory regions, we observed that expression augmented several-fold, reaching and surpassing the levels observed for *ompC*, when defined portions of the regulatory segment were absent, pointing towards regions for the binding and prompt action of negative regulatory elements (Orópeza et al., manuscript in preparation). In addition, expression was not influenced by changes in osmolarity, temperature, pH; nor the presence of polymyxin or H2O2; nor by anaerobiosis or growth in DME epithelial culture media (Sampieri et al., unpublished observations). Furthermore, *ompS1* has three promoters: one depends on the OmpR transcriptional activator, and the other two are OmpR-independent; moreover, there are seven putative OmpR-binding boxes in the 5′ regulatory region (Orópeza et al., manuscript in preparation).

Our current model for *ompS1* expression contemplates activation of transcription, from P1, by OmpR-binding to boxes II and III (C-type, low affinity); whereas boxes I and IV-VII would be involved in negative regulation. The fact that expression increases more than 100-fold upon removal of the cis-acting elements, and that this level is more than 10-fold higher than that for *ompC*, which codes for a major porin, raises interesting questions on its role and regulation in nature. Thus, we are confronted by a new mode of regulation by OmpR and of porin regulation, in response to unknown physicochemical parameters.

These findings open several questions, such as: why does *Salmonella* require such porin diversity? Is there a yet unknown pore selectivity that warrants such diversity? Moreover, do the OmpS1 and OmpS2 porins constitute relevant antigens for vaccination and diagnosis?

It is particularly intriguing why the *ompS1* and *ompS2* genes are expressed at very low levels under standard laboratory conditions: are the OmpS proteins always expressed at such low levels, and hence fulfill their biological role? or is there a condition, unknown up to now, that allows expression at much higher levels? In addition, we are interested in determining whether the OmpS1 and OmpS2 proteins have a role in the adherence, invasion, and survival of *S. typhi* in tissue culture cells, as well as in the virulence of *Salmonella* in a mouse typhoid model.
Corollary

All these observations evidence the fact that the role of porins and of their regulators, in bacterial physiology, is more complex than initially conceived. Therefore, we are barely starting to pose questions about structure-function relationships and environmental cues, that govern porin expression and function.

References

29. Miller VL, Falkow S. Evidence for two genetic loci from Yersinia enterocolitica that can promote invasion of epithelial...


