

Minireview

Protein uptake into *E. coli* during *Bdellovibrio* infection

A process of reverse secretion?

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Abstract

Bdellovibrio bacteriovorus is a small bacterial parasite that infects other Gram-negative bacteria, resides in the periplasm of the host cell, and utilizes host macromolecules as a source of nutrients. Evidence is summarized suggesting that *B. bacteriovorus* secretes proteases and nucleases synthesized in its own cytoplasm that are targeted to the cytoplasm of the host cell. Possible mechanisms for this trans-trimembrane protein transport process are discussed.

Key words: Protein transport; Secretion; Virulence; *Bdellovibrio bacteriovorus*; *Escherichia coli*; Sec machinery

1. Protein secretion in bacteria

A newly synthesized protein can be secreted across the cytoplasmic membrane of a Gram-negative bacterium to any one of three extracytoplasmic locations: (a) the outer membrane, (b) the periplasmic space between the inner and outer membranes, and (c) the external medium [1–3]. One such process, involving the components of the so-called Sec (secretion) system, is energized by both ATP and the electrochemical membrane potential [4,5]. During and following synthesis of a secretory protein, cytoplasmic chaperone proteins bind to distinct sites within it and maintain it in an export-competent state [6,7]. A variety of peripheral and integral membrane constituents comprise the actual export machinery. These proteins include the peripheral membrane SecA ATPase [8], the integral membrane SecY and SecE proteins which are thought to together comprise a transmembrane channel [9,10], and the SecD and SecF proteins which may form a complex on the periplasmic side of the membrane and act during a late step in the protein export process [11–13]. Other proteins may also be involved [10,14,15]. Export across the outer membrane requires distinct secretory proteins [3].

Extracellular chaperone proteins may also play a role in the export process, facilitating maturation, folding and assembly of the newly exported proteins [16–18]. Some of these extracytoplasmic chaperones catalyze peptidyl-prolyl *cis-trans* isomerization [19] as well as correct disulfide bond formation [20,21]. While other types of secretory systems can export proteins from the cytoplasm where they are made to their final destinations, the Sec system is believed to be the most important export system in *E. coli*, accounting for over 90% of the secretory activity of the cell [3,22–25].

2. *Bdellovibrio*: a unique bacterial parasite

Bdellovibrio bacteriovorus is a small bacterium that preys upon other Gram-negative bacteria as a source of nutrients for reproduction [26]. They utilize few carbohydrates as efficient sources of carbon and energy and instead derive energy primarily from the degradation of host nucleic acids, proteins and lipids [27]. The *Bdellovibrio* cell begins its attack by attaching to the surface of a susceptible bacterium such as *Escherichia coli*. The outer envelope of the *E. coli* cell is quickly penetrated, and the *Bdellovibrio* lodges in the periplasmic space of the invaded cell where it remains throughout its growth and reproductive phase (Fig. 1) [28,29]. The presence of unusual transport systems, such as its nucleoside-phos-

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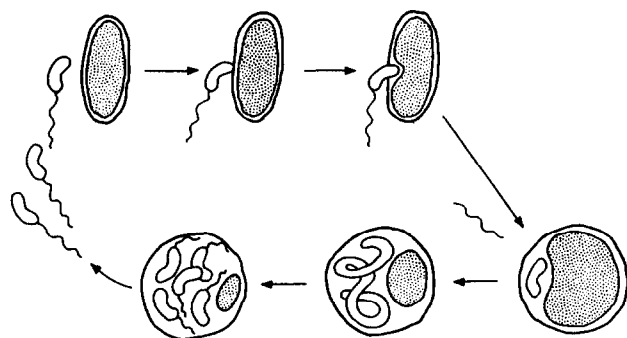


Fig. 1. Schematic representation of the *Bdellovibrio* life cycle. Free living, motile *Bdellovibrio bacteriovorus* (extreme left) attaches to the outer envelope of a Gram-negative bacterium (upper center) and bores into the periplasm (upper right) where it becomes non-motile. It replicates its constituents in the periplasm without cell division while secreting macromolecular degradative enzymes into the host cell cytoplasm, causing the latter compartment to shrink (lower left). Then, the *Bdellovibrio* snakes synchronously divide and differentiate to the motile state prior to the dissolution of the host cell wall and outer membrane, which releases the parasite for another round of infection (Redrawn from [28] with permission of Blackwell Scientific Publications, Ltd.).

phate uptake system, correlates with the unusual, exclusively parasitic lifestyle of *B. bacteriovorus* [30,31].

Upon invasion of the periplasmic space of a host *E. coli* cell by a *Bdellovibrio* species, a number of changes occur in the host. Messenger RNA and protein syntheses are inhibited within 3 and 6 min, respectively [32], and the capacity of the host cell to accumulate nutrients in processes driven by the proton motive force is gradually lost thereafter [29,33]. Respiratory capacity is lost more slowly (50% loss in about 30 min), and a gradual increase in non-specific membrane permeability, as measured by the unmasking of β -galactosidase activity, has been reported to occur after a lag period [33]. Eventually the host cells lyse, releasing the parasite for attack of other Gram-negative bacteria. The molecular events responsible for the progressive loss of host cell functions culminating in cell lysis are not well understood.

3. Appearance of *Bdellovibrio* macromolecular degradative enzymes in the host cell cytoplasm

In a recent report we demonstrated that *E. coli* cells gradually lose their sugar uptake activities as catalyzed by the phosphoenolpyruvate:sugar phosphotransferase system (PTS) over a period of 2 h following entry of *Bdellovibrio bacteriovorus* into the periplasm of the former bacterium [34]. In vitro complementation assays revealed that the *E. coli* PTS enzymes, Enzyme I, HPr, and the glucose- and mannitol-specific Enzymes II, were all lost in parallel with the disappearance of uptake activity. Thus, loss of activity in vivo was not due to membrane leakiness, energy depletion, or preferential inhibition or inactivation of any one protein component of the PTS.

Instead, loss of PTS activity was attributed to digestion of the protein constituents of the system by proteases present in the cytoplasm of the host cell after *Bdellovibrio* entry. β -galactosidase was degraded at approximately the same rate as were the PTS proteins. Ethylene diaminetetraacetate and phenylmethylsulphonyl fluoride individually provided partial protection against inactivation in vitro, and the two inhibitors together gave full protection against this in vitro proteolysis, suggesting that both metallo- and seryl-proteases were involved. Protease activity increased progressively with time following *Bdellovibrio* infection and appeared to degrade the *E. coli* PTS enzymes in vivo. Evidence was presented suggesting that the proteases responsible for PTS enzyme degradation were encoded by genes on the *B. bacteriovorus* chromosome [34]. These findings correlated with those of Rosson and Rittenberg [35], suggesting that *Bdellovibrio* nucleases appeared in the cytoplasm of the invaded cell following infection.

The observations noted above strongly suggest that macromolecular degradative enzymes are translocated across the two membranes of the periplasmic *B. bacteriovorus* as well as the cytoplasmic membrane of the host cell. The final destination of the proteins transported by this trimembrane translocation process is the host cell cytoplasm. There, the parasitic enzymes degrade host proteins and nucleic acids (and possibly other host cell macromolecules), and the degradation products then leak out of the cell into the periplasm where they serve as sources of nutrients for the *Bdellovibrio*.

4. The mechanism of protein import in *E. coli*

No corresponding prokaryotic protein translocation process has been characterized to date in any other cell system, and the molecular mechanism by which *E. coli* takes up foreign proteins into its cytoplasm is at present a total mystery. The presence of *Bdellovibrio* in the host cell periplasm might conceivably induce synthesis of novel host cell proteins which mediate uptake of the degradative enzymes synthesized by its parasite (Fig. 2A). If so, the host cell brings about its own demise. Alternatively, *Bdellovibrio* might secrete channel-forming proteins that insert into the cytoplasmic membrane of the host as do certain colicins [36–38] and certain bacterially synthesized animal toxins [39]. One might then need to propose the existence of two distinct types of *Bdellovibrio*-synthesized channel proteins, one concerned with uptake into the host cell of the macromolecular degradative enzymes that generate cytoplasmic nutrients, and a second that functions in the release of these nutrients to the periplasm where *B. bacteriovorus* resides.

A third alternative would involve the use of an existent host secretory system for uptake of the enzymes secreted

Possible Mechanisms of *Bdellovibrio* Protein Import into Infected Host Cells

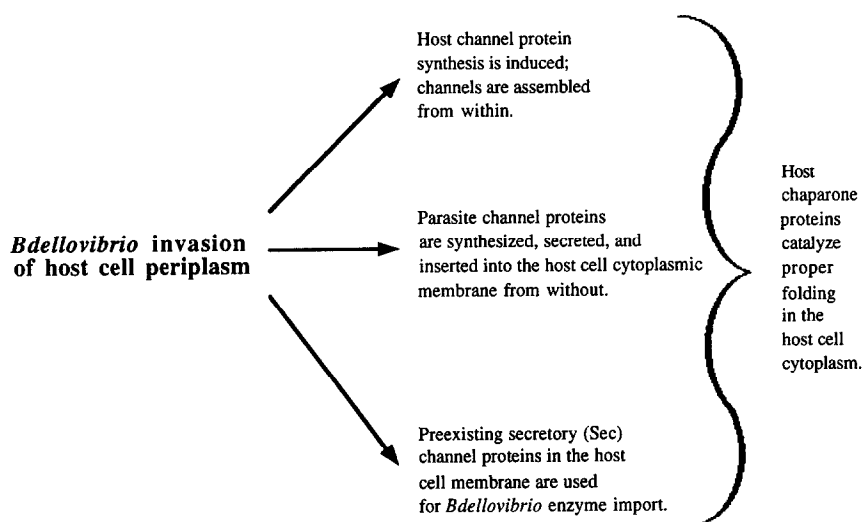


Fig. 2. Three possible mechanisms for the secretion of proteases and nucleases across the host cell membrane into its cytoplasm. Mechanism A presupposes the existence of cryptic host genes that when activated by the presence of *Bdellovibrio* secretory products in the periplasm, possibly detected by a transmembrane sensor kinase-response regulator system, result in the synthesis of a protein import channel in the cytoplasmic membrane. Mechanism B involves the secretion by the parasite of proteins that can spontaneously insert into the cytoplasmic membrane of the host to form a protein translocating channel. Such an insertion process might be analogous to the mechanism by which certain transmembrane channel-forming colicins insert into the *E. coli* membrane or various bacterial toxins insert into animal cell membranes to allow entry of associated proteins or protein domains into the host cytoplasm [38,39]. Mechanism C, the one suggested to be operative in the uptake *Bdellovibrio* hydrolytic enzymes, utilizes the existent major secretory pathway involving the SecAYEDF complex. Regardless of the mechanism employed (A, B, or C), cytoplasmic chaperone proteins of the host may be required to allow proper folding of the degradative enzymes during or following their entry into the cell.

by the parasite into the host periplasm. This process would be analogous to that of 'reverse secretion', a process recently discussed by Jan Tommassen and Jonathan Beckwith at the International Symposium on Cellular and Molecular Biology of Phosphate and Phosphorylated Compounds in Microorganisms (Woods Hole, Mass, Sept. 12–17, 1993). It seems that the secretory process involving host proteins may be a reversible one in the absence of processing of the secreted protein. The Sec system therefore provides a potential pathway for the entry of *B. bacteriovorus*-synthesized proteins during its quest to dominate and then destroy its host while utilizing its cellular constituents as sources of nutrients.

5. Are chaperones involved?

Bdellovibrio degradative enzymes must not only be released into the host cytoplasm, they must also be properly folded to give the enzymatically active conformations of these proteins. The question must therefore be posed – do chaperone proteins play a role in this process? And if so, do they function in the periplasm as well as

the cytoplasm? If the overall process involves two steps, including release of the enzyme from the *Bdellovibrio* cell in the periplasm, the involvement of periplasmic chaperones [16–21] might be required. If, on the other hand, a one step process, involving concerted secretion across all three membranes occurs, then an involvement only of cytoplasmic chaperones [6,7] needs to be proposed. Any or all of the SecB, GroELES, and DnaJK proteins as well as the cytoplasmic protein disulfide reduction enzymes might play a role.

6. Prospects for the immediate future

As *E. coli* mutants defective for most of the Sec and chaperone proteins are available, a straightforward molecular genetic approach to this novel process of protein uptake can be initiated. The key initial question concerns whether the parasitic *Bdellovibrio* species utilize the secretory machinery of their bacterial host or generate their own protein translocation system. Regardless of the answer to this question, it seems likely that novel mechanistic aspects will be revealed. Unexpected parallels

with protein import into eukaryotic cells may be discovered. Thus, *Bdellovibrio* may provide an all bacterial model system for examining the detailed molecular mechanisms for specific aspects of bacterial and viral virulence in plants and animals [40].

References

- [1] Saier Jr., M.H., Werner, P.K. and Müller, M. (1989) *Microbiol. Rev.* 53, 333–366.
- [2] Schatz, P.J. and Beckwith, J. (1990) *Annu. Rev. Genet.* 24, 215–248.
- [3] Pugsley, A.P. (1993) *Microbiol. Rev.* 57, 50–108.
- [4] Geller, B.L. (1991) *Mol. Microbiol.* 5, 2093–2098.
- [5] Driessen, A.J.M. (1992) *Trends Biochem. Sci.* 17, 219–223.
- [6] Randall, L.L., Topping, T.B. and Hardy, S.J.S. (1990) *Science* 248, 860–863.
- [7] Ellis, R.J. and van der Vies, S.M. (1991) *Annu. Rev. Biochem.* 60, 321–347.
- [8] Oliver, D.B. (1993) *Mol. Microbiol.* 7, 159–165.
- [9] Schatz, P.J., Bieker, K.L., Ottemann, K.M., Silhavy, T.J. and Beckwith, J. (1991) *EMBO J.* 10, 1749–1757.
- [10] Ito, K. (1992) *Mol. Microbiol.* 6, 2423–2428.
- [11] Gardel, C., Johnson, K., Jacq, A. and Beckwith, J. (1990) *EMBO J.* 9, 3209–3216.
- [12] Nishiyama, K., Mizushima, S. and Tokuda, H. (1992) *J. Biol. Chem.* 267, 7170–7176.
- [13] Sugai, M. and Wu, H.C. (1992) *J. Bacteriol.* 174, 2511–2516.
- [14] Ribes, V., Rörmich, K., Giner, A., Dobberstein, B. and Tollervey, D. (1990) *Cell* 63, 591–600.
- [15] Brundage, L., Fimmel, C.J., Mizushima, S. and Wickner, W. (1992) *J. Biol. Chem.* 267, 4166–4170.
- [16] Ang, D., Liberek, K., Skowrya, D., Zylicz, M. and Georgopoulos, C. (1991) *J. Biol. Chem.* 266, 24233–24236.
- [17] Langer, T., Lu, C., Echols, H., Flanagan, J., Hayer, M.K. and Hartl, F.U. (1992) *Nature* 356, 683–689.
- [18] van Rosmalen, M. and Saier Jr., M.H. (1993) *Res. Microbiol.*, in press.
- [19] Trandinh, C.C., Pao, G.M. and Saier Jr., M.H. (1992) *FASEB J.* 6, 3410–3420.
- [20] Noiva, R. and Lennarz, W.J. (1992) *J. Biol. Chem.* 267, 3553–3556.
- [21] Bardwell, J.C.A. and Beckwith, J. (1993) *Cell* 74, 769–771.
- [22] Pugsley, A.P., Poquet, I. and Kornacker, M.G. (1991) *Mol. Microbiol.* 5, 865–873.
- [23] Lory, S. (1992) *J. Bacteriol.* 174, 3423–3428.
- [24] Wandersman, C. (1992) *Trends Genet.* 8, 317–322.
- [25] Salmond, G.P.C. and Reeves, P.J. (1993) *Trends Biochem. Sci.* 18, 7–12.
- [26] Stolp, H. (1973) *Annu. Rev. Phytopathol.* 11, 53–76.
- [27] Hespell, R.B., Rosson, R.A., Thomashow, M.F. and Rittenberg, S.C. (1973) *J. Bacteriol.* 113, 1280–1288.
- [28] Thomashow, M.F. and Rittenberg, S.C. (1979) in: *Developmental Biology of the Prokaryotes* (Parish, J.H., Ed.) pp. 115–138, Blackwell Scientific, Oxford.
- [29] Ruby, E.G. (1989) in: *Intracellular Parasitism* (J.W. Moulder, Ed.) pp. 17–34, CRC Press, Boca Raton, FL.
- [30] Ruby, E.G., McCabe, J.B. and Barke, J.I. (1985) *J. Bacteriol.* 163, 1087–1094.
- [31] Ruby, E.G. and McCabe, J.B. (1986) *J. Bacteriol.* 167, 1066–1070.
- [32] Varon, M., Drucker, I. and Shilo, M. (1969) *Biochem. Biophys. Res. Commun.* 37, 518–525.
- [33] Rittenberg, S.C. and Shilo, M. (1970) *J. Bacteriol.* 102, 149–160.
- [34] Romo, A.J., Ruby, E.G. and Saier Jr., M.H. (1992) *Res. Microbiol.* 143, 5–14.
- [35] Rosson, R.A. and Rittenberg, S.C. (1979) *J. Bacteriol.* 140, 620–623.
- [36] Cramer, W.A., Cohen, F.S., Merrill, A.R. and Song, H.Y. (1990) *Mol. Microbiol.* 4, 519–526.
- [37] Cramer, W.A., Cohen, F.S., Stauffacher, C.V., Zhang, Y.L., Merrill, A.R., Song, H.Y. and Elkins, P. (1992) in: *Bacteriocins, Microcins, and Antibiotics* (James, R., Lazdunski, C. and Pattus, F., Eds.) (Proceedings of a workshop entitled Bacterial Plasmid-Coded Toxins) Springer-Verlag, Berlin, New York.
- [38] Parker, M.W., Tucker, A.D., Tsernoglou, D. and Pattus, F. (1990) *Trends Biochem. Sci.* 15, 126–129.
- [39] London, E. (1992) *Mol. Microbiol.* 6, 3277–3282.
- [40] Thomashow, M.F. and Cotter, T.W. (1992) *J. Bacteriol.* 174, 5767–5771.