# INTERACTION BETWEEN Bdellovibrio bacteriovorus AND THE CYTOPLASMIC MEMBRANE OF Escherichia coli B

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Adsorption of Bdellovibrio bacteriovorus (Bdv) on the surface of Escherichia coli is accompanied by a sharp decrease in the initial rate of entry of $\alpha$-methylglucoside-$C^{14}$ and thiomethylgalactopyranoside-$C^{14}$ into the host cell. Interaction between the parasite and E. coli leads to the rapid departure of previously accumulated labeled glucosides and $\beta$-galactosides from the bacteria. Meanwhile the ATP content in E. coli falls sharply. Adsorption of Bdv on E. coli spheroplasts was established as a fact. The possible mechanisms of interaction between Bdv and the host cell at the cytoplasmic membrane level are discussed.

KEY WORDS: Bdellovibrio bacteriovorus (Bdv); interaction of Bdv with E. coli; cytoplasmic membrane.

Bdellovibrio bacteriovorus (Bdv) parasitizes Gram-negative and Gram-positive bacteria [14]. On account of its motility the parasite can attach itself to a sensitive cell and penetrate into the periplasmic space, where it multiplies. However, the disturbance of the motility of the host bacteria and the increase in permeability and arrest of macromolecular syntheses in the host cell take place at different stages of interaction with Bdv, probably in connection with damage to the cytoplasmic membrane [4, 12, 16]. The study of the mechanism of bacteriolysis has shown that Bdv possesses several enzymes to enable it to penetrate into the host cell and undertake its subsequent lysis. Muramidase, protease, and lipase have been isolated from different strains of Bdv and described [14]. In its ability to inhibit biosynthesis and also to disturb permeability in E. coli, Bdv very closely resembles phage "ghosts" and colicins [1, 5, 17].

However, in the accessible literature there are only isolated descriptions of the action of the parasite on the membrane of the host cell [4, 12]. These investigations established the fact that the permeability of the E. coli cell is disturbed after attachment of the Bdv (release of certain chemical components of the cell from the bacteria, increased intake of 8-aniline-1-napthosulfonic acid).

The object of this investigation was to study transport processes in E. coli cells infected with Bdv under conditions excluding its penetration into the periplasmic space and to obtain evidence of the possibility of direct interaction between the parasite and the cytoplasmic membrane of the host cell.

## EXPERIMENTAL METHOD

Bdv "OP" was used, with E. coli B as the host bacteria. The E. coli cells were grown in DNB medium [13] for 3.5 h at 37°C up to a concentration of $2 \times 10^8 - 5 \times 10^8$ cells/ml, the Bdv was added, and the cultures were incubated on a shaker for 18 h at 28°C. The cells of the parasite were separated from the unlysed E. coli cells by passing the mixture in succession through Aufs and DA Millipore filters with a pore size of 0.65 $\mu$. The Bdv was concentrated by centrifugation at 17,000 rpm for 30 min in the TsVR centrifuge.

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Cultures of E. coli B were grown for 3.5 h in medium M-9 with the addition of 0.4% glycerol (for the experiments to study the accumulation of TMG-$C^{14}$* and to determine the ATP content) or of 0.4% glucose (for the experiments to study the accumulation of $\alpha$-MG-$C^{14}$). In the experiments to study the accumulation of $\alpha$-MG-$C^{14}$ and TMG-$C^{14}$ in E. coli 0.0001 M IPTG was added to the growth medium to induce TMG permease. The cells were washed, suspended in DNB medium to a concentration of $2 \times 10^8 - 5 \times 10^8$ cells/ml, and mixed with Bdv so that the multiplicity of infection was 20.

The accumulation of labeled carbohydrates was investigated at 35°C. $\alpha$-MG-$C^{14}$ and TMG-$C^{14}$ were added to the culture in final concentrations of 0.2 and 0.12 $\mu$Ci/ml, respectively. The bacteria were harvested on HA millipore filters with a pore size of 0.45 $\mu$ and washed with DNB medium, dried, and the radioactivity was measured in a type SL-20 liquid scintillation counter.

To obtain spheroplasts of E. coli a culture was grown in Hottinger's broth for 3.5 h, after which the frozen cells were treated with lysozyme [8] in DNB medium with 0.5 M sucrose. Since the Bdv lose their motility in 0.5 M sucrose solution and have no lethal action on intact cells, the resulting spheroplasts were diluted with DNB medium to a final sucrose concentration of 0.2 M, when the spheroplasts remained osmotically stable. Adsorption of Bdv was carried out in this same medium with a multiplicity of infection of 20. The percentage of spheroplast formation and the lethal activity of Bdv on the E. coli cells were tested by seeding on dishes with nutrient agar. The mean level of spheroplast formation was 98%. The rate of survival of E. coli after treatment with Bdv and after incubation for 20 min at 35°C with the parasite, with a multiplicity of infection of 20, was less than 10%. IPTG in a concentration of 0.001 M was used as the inducer of $\beta$-galactosidase in the experiments with the spheroplasts. The inducer was added after incubation of the spheroplasts with Bdv at 35°C and the cultures were incubated for 30 min.

$\beta$-Galactosidase activity was determined in spheroplasts disintegrated by toluene from the rate of splitting of ONPG [9]. The quantity of free o-nitrophenol was determined spectrophotometrically at 420 nm.

The ATP content was determined by the luciferin-luciferase method [2]. E. coli cells treated for 20 min in DNB medium and intact E. coli cells were harvested on the centrifuge and suspended in a small volume of bidistilled water. Extracts were obtained by boiling the bacterial suspension for 10 min on a water bath and centrifuging. The ATP content in the samples was expressed per milligram protein, determined by the method of Lowry et al. [10].

In the experiments to study the accumulation of labeled carbohydrates and to determine $K_m$ for $\beta$-galactoside permease, the Bdv were adsorbed on E. coli cells in the presence of streptomycin in a concentration of 50 $\mu$g/ml (as was shown previously [15], this concentration depresses invasion but does not prevent adsorption of Bdv on the surface of the bacterial cells).

## EXPERIMENTAL RESULTS

Adsorption of Bdv on the surface of E. coli caused a sharp decrease in the initial rate of intake of $\alpha$-MG-$C^{14}$ and TMG-$C^{14}$ into the host cells (Fig. 1). Attachment of the parasite to the E. coli cells led to the rapid liberation of previously accumulated labeled $\beta$-galactosides and glucosides from the bacteria (Fig. 2). Consequently, interaction of Bdv with E. coli cells under conditions permitting only adsorption of the parasite on the surface of the host bacteria without penetration inside the cells led to a disturbance of transport processes in the host cell. These disturbances of transport processes could be due either to a change in the properties of the carrier (a decrease in its affinity for the substrate) or to a decrease in the rate at which the intact carrier transported the corresponding carbohydrates. Determination of $K_m$ by the Lineweaver–Burke method showed that in fact it was not changed for the hydrolysis of ONPG by the adsorption of Bdv on the E. coli cells, and its value was of the order of $10^{-3}$ M. Consequently, the affinity of $\beta$-galactoside permease for the substrate was unchanged in the presence of Bdv. The results correlate with available data on the infection of E. coli cells by phage "ghosts" [17] and it can be concluded from them that the disturbances of transport processes were due to a decrease in the rate of carbohydrate transport by the intact carriers.

The adsorption of Bdv on the surface of the bacteria was accompanied by a decrease of more than 90% in the ATP content in the cell (most probably on account of the outflow of this compound from E. coli).

*The following abbreviations are used: IPTG – isopropylthiogalactopyranoside; TMG – thiomethylgalactopyranoside; $\alpha$-MG – $\alpha$-methylglucoside; ONPG – o-nitrophenyl-$\beta$-D-galactopyranoside.

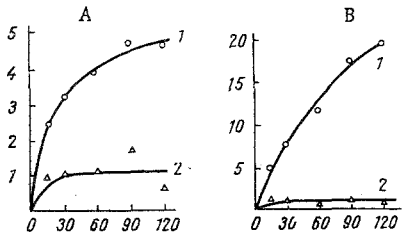


Fig. 1

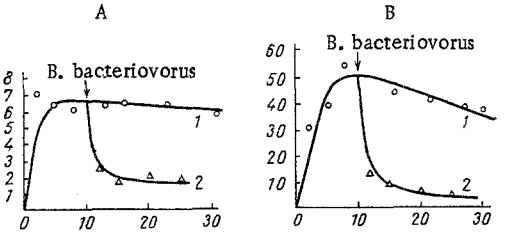


Fig. 2

Fig. 1. Effect of Bdellovibrio bacteriovorus on initial rate of accumulation of TMG-$C^{14}$ (A) and $\alpha$-MG-$C^{14}$ (B) by E. coli B cells: 1) intact E. coli B cells; 2) E. coli B cells previously incubated with Bdv for 20 min. Abscissa, time (in sec); ordinate, number of pulses per minute ($\times 10^3$).

Fig. 2. Effect of Bdellovibrio bacteriovorus on TMG-$C^{14}$ (A) and $\alpha$-MG-$C^{14}$ (B) accumulated by E. coli cells: 1) intact E. coli B cells; 2) E. coli B cells infected with Bdv. Abscissa, time (in min); ordinate, number of pulses per minute ($\times 10^3$).

Data in the literature [12, 14] suggest that Bdv damage the cytoplasmic membrane of the bacteria. This hypothesis was confirmed by the observed change in the rate of carbohydrate transport, for the carrier is localized in the membrane. In addition, as was pointed out above, Bdv possess several enzymes that act on the cytoplasmic membrane [14]. The final stage in this investigation was therefore to study the possibility of adsorption of Bdv on E. coli spheroplasts, from which most of the cell wall has been removed. Bdv are known to disturb inducible processes [16], and for that reason the attachment of Bdv to the surface of the spheroplasts was judged from the rate of synthesis of $\beta$-galactosidase. The results of these experiments showed that in the presence of Bdv $\beta$-galactosidase synthesis in the spheroplasts was reduced by 70%; consequently, the parasite most probably interacted with the cytoplasmic membrane of the bacterial cell. These data correlate with the lethal activity of Bdv against whole cells in the medium containing 0.2 M sucrose.

To sum up, it must be emphasized that Bdv disturb the transport of materials in E. coli cells. These changes, in turn, may be caused by interaction of Bdv with the bacterial membrane and by lowering of the membrane potential – the universal source of energy for the transport of materials into the cell [3, 11]. As stated above, Bdv lead to a sharp decrease in the ATP reserves in the host cell. Bdv also are known to block the oxidative activity of bacteria [12]. The bacterial cell forms its membrane potential either by oxidation or by the energy of ATP [6]. All the phenomena described in this paper (at least, those concerned with TMG transport) most probably therefore take place in the following order: 1) interaction between Bdv and the cytoplasmic membrane of E. coli; 2) disturbance of the ability of the bacterial cell to carry out oxidation and a reduction in the intracellular ATP reserves; 3) equalization of the membrane potential; 4) disturbance of transport processes.

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