NOTES

Mode of Action of Vibriocin: Initial Interaction with *Vibrio comma* Cells

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Certain metabolic requirements, upon initial interaction of sensitive cells with vibriocin, were a prerequisite for lethal activity. Transient "immunity" of these cells, manifested under suboptimal growth conditions, differed from the immunity of vibriocinogenic cells.

It was earlier observed that interaction of vibriocin with sensitive *Vibrio comma* cells (V_{ind}) under optimal conditions (37 C, pH 7, exponential-phase aerobic cells) caused the cells to leak potassium, leak ultraviolet light-absorbing material, and lose their colony-forming ability (5, 6). It was also shown that cells grown under anaerobic conditions or to the stationary phase, or pretreated with certain metabolic inhibitors (sodium azide, 2,4-dinitrophenol, chloramphenicol), were much less sensitive to the lethal effects of vibriocin (5, 6). This paper is concerned with some requirements of V_{ind} cells during the initial interaction with vibriocin for manifestation of the lethal effect, the transient protection in their absence, and comparison to the immunity of the vibriocinogenic strain.

Adsorption of vibriocin occurs in presence of sodium azide, 2,4-dinitrophenol, and at low temperature (5). By similar techniques, we have found that adsorption occurs in presence of puromycin, a protein synthesis inhibitor (2). Figure 1 demonstrates that, if vibriocin (multiplicity, $m = 1$) was adsorbed under suboptimal growth conditions (at 0 C or in presence of puromycin), the cells were protected and seemed "immune" to this vibriocin even when optimal conditions were restored. However, this protection was only transient, since a second hit under optimal conditions produced lethal effects. There was no detectable desorption of the vibriocin, since extracellular fluids were devoid of vibriocin activity. Furthermore, as shown in Fig. 1, V_{ind} cells saturated with vibriocin at 0 C [$m = 50$; there are 35 to 40 receptor sites/cell (5)] and then returned to 37 C were viable and resistant to additional vibriocin. This also indicated that receptor sites were not

FIG. 1. Susceptibility of V_{ind} at optimal growth conditions, to vibriocin adsorbed at suboptimal growth conditions. Exponential-phase cells grown in Heart Infusion (Difco) were centrifuged and suspended in 0.5% saline. The cells were (a) prechilled to 0 C or (b) incubated with puromycin (PU) (30 μ g/ml of bacteria) at 37 C for 5 min before addition of vibriocin [prepared as described earlier (4)]. After 10 min, samples were removed (a) to 37 C or (b) removed, spun down at low speed, and suspended in PU-free saline. The cells were then treated with either vibriocin ($m = 1$) or saline (control) for 15 min at 37 C before being assayed for survivors by viable count. Cells returned to optimal conditions (37 C or no PU) and mixed with saline ($\square$) or vibriocin ($m = 1$; hatched). Note: The leakage of ultraviolet absorbing material was invariably detected as previously described (6) in the extracellular fluids of mixtures with a low proportion of survivors. No such material was detected in mixtures with a high proportion of survivors.

FIG. 2. Effect of postadsorption chilling of V_{ind} cells on vibriocin activity. Exponential-phase cells, grown in Heart Infusion (Difco), were suspended in 0.5% saline, and a viable count was made. Cells treated with vibriocin ($m = 1$) or saline (control) were incubated at 37°C. At suitable intervals, samples were removed from each system. A plate count was made immediately and again after 10 min at 0°C. Cells treated with vibriocin: $\blacktriangle$, no chilling; $\bullet$, with 10-min chilling. Control cells: $\triangle$, no chilling; $\circ$, with 10-min chilling.

unmasked by desorption and hence not available for the secondary adsorption.

In summary, vibriocin adsorbs under unfavorable growth conditions, does not desorb, and yet is not lethal to the cells. It is also known that 0°C does not inactivate vibriocin (it is stored at 0°C). Nevertheless, vibriocin lost its lethal effect. This system is unlike the reversible step following phage adsorption at 0°C, which required desorption or phage inactivation (3, 9, 10). However, there is a similarity in our observations with Stent's postulate (10) of the requirement for "another reaction which must play an important role in phage adsorption" to make it effective.

Figure 2 shows that, if the incubation of vibriocin and the V_{ind} cells occurs at 37°C and then is interrupted for 10 min at 0°C, even within the first minutes of adsorption, there is no reversal of the lethal effect of the vibriocin at 37°C, resulting in a typical killing curve. Thus, the first interaction between vibriocin and sensitive cells must occur at a temperature suitable for cell growth and under conditions favorable to protein synthesis for the lethal effect to be manifested. Since anaerobic or stationary-phase cells are also less sensitive and appear "immune" to vibriocin (5), we can

regard this transient immunity of sensitive cells to be linked to some function essential to normal cell growth. According to Nomura (8), following bacteriocin adsorption to a sensitive cell, a transmission system is activated which affects the biochemical target. We suggest that the suboptimal conditions specified above were detrimental to this transmission system and thus neutralized the potential lethal effect of vibriocin, despite adsorption to sensitive cells and lack of desorption. In this vein, mere attachment of the bacteriocin to a

FIG. 3. Effect of vibriocin on vibriocinogenic cells (V_{prod}). Exponential-phase V_{prod} cells grown in Heart Infusion (Difco) containing ^{42}K ($0.1 \mu Ci/ml$) were centrifuged, washed, and resuspended in 0.5% saline; a viable count was made. a, Equal volumes of cells were mixed with vibriocin ($m = 1$) or saline (control). Leakage of ultraviolet absorbing material was measured as previously described (5): $\blacktriangle$, cells plus vibriocin; $\triangle$, cells plus saline. Survivors were assayed by the plate count method: $\bullet$, cells plus vibriocin; $\circ$, cells plus saline. b, ^{42}K efflux was measured as previously described (5). Radioactivity of bacteria treated with vibriocin: $\blacksquare$, cellular; $\blacklozenge$, extracellular. Radioactivity of bacteria treated with saline (control): $\square$, cellular; $\lozenge$, extracellular.

receptor of a sensitive cell seems not adequate to affect the conformational transition of the membrane protomers and trigger the lethal event(s), as was suggested by Changeux and Thiéry (1). It seems that a cooperative effort is required upon the initial interaction, which involves at least aerobic oxidative phosphorylation (5), protein synthesis (6), and an optimal temperature for enzyme function, in maintaining an effective transmission mechanism.

The protective immunity afforded the V_{ind} cells by vibriocin attachment under suboptimal conditions differs from the immunity of the vibriocinogenic strain (V_{prod}) which was shown to adsorb vibriocin (4). When the latter cells interact with vibriocin, even under optimal conditions, there is no efflux of potassium, no leakage of ultra-violet-absorbing material, and no loss of colony-forming ability (Fig. 3). This immunity is probably afforded by the bacteriocinogenic factor which may code for some "immunity substance" as postulated by Nomura for colicins (8) and as shown to function in V_{prod} cells as long as they are not derepressed by appropriate inducers (7).

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