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The effect of nonreceptor adsorption on the lethal action of colicin E1

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The survivability of *Escherichia coli* K12s cells has been studied after treatment with ^{125}I-labeled colicin E1. It has been shown that for low amounts of adsorbed colicin the survivability follows single-hit kinetics. When the number of colicin molecules adsorbed exceeds approx. 50 per cell, deviation from single-hit kinetics occurs towards higher survivability. Colicin E1 adsorbed nonreceptorwise by the cell's surface has been shown to inhibit the lethal action of colicin E1 molecules adsorbed at specific receptors. This fact has been used in accounting for the elevated survivability of cells at high colicin doses. The functional significance of the phenomenon is discussed.

Colicin E1; Adsorption; Single-hit kinetics; *(E. coli)*

1. INTRODUCTION

Colicins are protein toxins produced by, and active against, *Escherichia coli* and closely related bacteria [1–3]. The lethal action of colicin can be divided into three stages [2]: (i) adsorption of a colicin molecule at a specific receptor of the outer membrane of a sensitive cell; (ii) translocation of the colicin molecule (or active fragment thereof) from the receptor to the biological target; (iii) modification of the target causing death of the cell. Many of the colicin receptors have been shown to be involved in outer membrane-mediated nutrient uptake [4]. For example, the polypeptide that serves as the receptor for colicins E1, E2, and E3 functions in the uptake of vitamin B12 [5]. A total of six distinct modes of action of colicin have been identified [3]. Colicin E1, studied here, belongs to the major group of colicins (A, Ia, Ib, K), which form pores in the cytoplasmic membrane and allow the free diffusion of ions across the membrane, thereby destroying the cell's energy potential [1].

It is well known that the survivability of sensitive cells treated with low doses of colicins follows single-hit kinetics [6,7,9]. The single-hit kinetic curve also describes the specific biochemical changes occurring in colicin-treated cells [10–12]. In contrast, at high colicin doses, the dependence of the fraction of surviving cells on the amount of colicin added or adsorbed deviates from single-hit kinetics towards higher survivability [7,9]; however, the biological interpretation of this phenomenon remains unclear.

Here, we have studied the survivability of the *E. coli* K12s cells treated with ^{125}I-labeled colicin E1 and report on the reasons for the observed deviation of the survivability curve from single-hit behavior: this deviation has been shown to be due to nonreceptor adsorption of colicin molecules by the sensitive cell's membrane and its resulting effect on the process of translocation of colicin (or the active fragment thereof) from the receptor to the biological target.

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2. MATERIALS AND METHODS

2.1. *Extraction and iodination of colicin E1*

Colicin E1 was induced and purified as described [8], using the colicinogenic strain *E. coli* K12s (ColE1), and iodinated with lactoperoxidase. 100 μl reaction mixture contained 1 μl of 10^{-4}% H_2O_2, 100 μl colicin E1, 10 μg lactoperoxidase (Sigma), and the appropriate amount of $Na^{125}I$ [label was normally introduced to yield a specific activity of (1–2) × 10^8 cpm/mg]. The reaction was carried out at 0°C for 20 min, the incorporation efficiency being 40–60%. Free label was removed by gel filtration on Sephadex G-25.

3. RESULTS AND DISCUSSION

3.1. *Killing activity of colicin E1*

Fig.1 shows the survival of *E. coli* K12s plotted as a function of bound ^{125}I-colicin E1 molecules. One can see that for low numbers of adsorbed colicin molecules (of the order of several dozens) the survivability follows single-hit kinetics, i.e. the logarithm of the survivability depends linearly on dose of colicin.

This result is in agreement with previous data [7,9] and can be obtained theoretically by treating the adsorption process in terms of probability theory. Let p be the average number of colicin molecules adsorbed per cell with a representing the probability of an adsorbed colicin molecule killing the cell. According to the Poisson distribution, the fraction of cells containing no 'lethal' colicin molecules is $A/A_0 = \exp(-ap)$. The parameter $1/a$, referred to the killing unit, depends on the quality of the colicin preparation and is always greater than unity, which appears to be due to nonreceptor adsorption of colicin. It was shown earlier that, although the number of receptor copies is only several hundred per cell (for colicin E1, about 200 [16]), the adsorption capacity of the cell reaches several thousands of colicin molecules [6,7,9,15]. Since in vitro experiments with purified colicin E1 receptor indicate the equimolar nature of the interaction of the receptor with colicin [16] and because receptorless resistant cells also possess a high adsorption capacity [9], nonreceptor adsorption of colicin molecules at the outer membrane of the cell takes place. Thus, the parameter a also depends on the probability of adsorption of a colicin molecule at the receptor rather than nonspecific adsorption on the cell membrane.

Agreement between the mathematical description of the above model and experimental data on

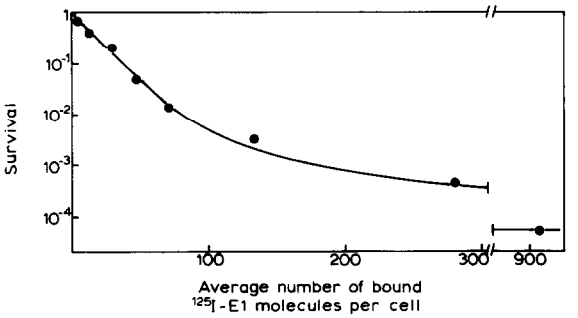

Fig.1. Bactericidal activity of ^{125}I-colicin E1 as a function of colicin adsorption. K12s cells were grown in LB broth to A_{536} = 1.0. The culture was chilled, washed twice with cold physiological saline, and resuspended in the initial volume of LB broth containing 1 mg/ml BSA. Aliquots of the culture were incubated for 10 min at 37°C, then mixed with various amounts of ^{125}I-colicin E1, and incubated again for 20 min. Survivability of cells was measured by seeding on an agar-plate. The amount of adsorbed colicin was determined by measuring the radioactivity of cells washed once by centrifugation.

low-colicin-dose cell death was used as a basis for drawing the conclusion of the noncooperative nature of the process of colicin-induced killing of cells. Concerning the colicin-receptor complexes, these were treated in mathematical models [13,14] as being lethal complexes, i.e. those leading in one way or another to the death of sensitive cells.

At the same time, as shown in fig.1, when the number of adsorbed colicin molecules exceeds 50–100 per cell, the survival curve deviates appreciably from single-hit behavior towards higher survivability. Mathematical simulation of colicin adsorption has shown [13,14] that such a dependence of survivability on colicin dose can be attributed to heterogeneity of the cell population with respect to the number of receptors per cell. However, in drawing such a conclusion, it was necessary to assume a wide variance in the number of copies of the receptor, which is unlikely.

Therefore, we suggest the following mechanism to account for the elevated survivability of cells at high colicin doses: colicin molecules adsorbed nonreceptorwise inhibit the lethal action of those adsorbed on receptors. This being the case, it becomes clear that with increase in the amount of colicin adsorbed on the cell, more colicin becomes adsorbed nonspecifically by the membrane, a more pronounced inhibition effect occurs, and greater deviation of the survival curve takes place from single-hit behavior towards higher survivability.

3.2. *Can colicin inhibit itself?*

To prove the above hypothesis, we undertook the following experiment. K12s cells were treated with colicin E1 in the presence of vitamin B12 (see legend to fig.2). With such a proportion of vitamin B12 and colicin E1, which have a common receptor, colicin E1 was adsorbed predominantly nonreceptorwise [5]. Cells treated thus and washed in order to remove unbound vitamin B12 and colicin E1 but carrying nonspecifically adsorbed colicin molecules, in conformity with the hypothesis, should be more resistant to the lethal action of freshly added colicin. This is illustrated in fig.2, showing the dependence of survivability of K12s cells that do (curve 1) and do not (curve 2) carry the preliminarily nonreceptorwise-adsorbed colicin on the amount of newly absorbed ^{125}I-colicin. Thus, colicin molecules adsorbed nonspecifically by the sensitive cell's membrane do inhibit the lethal action of those adsorbed at the receptors.

The question therefore arises as to the mechanism responsible for the observed inhibition effect. The colicin molecule is known to contain three functional domains [1], one of which is located in the N-terminal portion of the molecule and is responsible for translocation of the colicin molecule (or its active fragment) from the receptor to the biological target. On the other hand, there is a very large class of tolerant mutants with a defective second stage [step (ii)] in the lethal action of colicin [17,18]. Thus, colicin-sensitive cells possess certain systems that are used by colicin to enter the cell. This process is likely to involve the interaction of the N-terminal domains of colicin with the membrane components of those systems. In terms of this notion, the inhibition effect could be explained as follows. In order to produce the lethal effect, the colicin molecule adsorbed at the receptor must interact with some translocative membrane component (MC), i.e. it appears that it is not the colicin-receptor complex, but rather the ternary colicin-receptor-MC complex that is lethal. This is supported, albeit indirectly, by the fact that cells having adsorbed colicin can be saved by treatment with trypsin [19]. If one supposes that nonreceptor-adsorbed colicin molecules are capable of interacting with the MC (although this interaction would not cause cell death because of the absence of receptors from the complex that has formed), then such an interaction should lead to a reduction in the free MC, thus providing an explanation for the reduced efficiency of stage (ii) of the lethal action of colicins and the elevation of cell survivability.

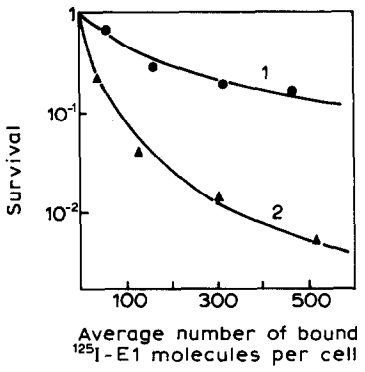

Fig.2. Effect of nonreceptor-adsorbed colicin on bactericidal activity of colicin E1. K12s cells were grown and washed as described in fig.1. After 5 min incubation at 37°C, vitamin B12 was added at 200 μg/ml. After a further 5 min incubation, the culture was divided into two aliquots, one of which was modified by the addition of unlabeled colicin E1 to give 10^4 molecules per cell and incubated for a further 10 min. Thereafter, cultures were chilled, washed twice with cold physiological saline containing 1 mg/ml BSA, and resuspended in the initial volume of LB broth with BSA. The lethal effect of ^{125}I-colicin E1 on cells treated with unlabeled colicin E1 (curve 1) and untreated cells (curve 2) was measured as described in fig.1.

It is difficult to account for the abnormally high survivability of cells at high colicin doses if one considers as being valid the supposition that the functional role of colicin in the microbial antagonism of a mixed population of colicinogenic and noncolicinogenic cells consists of killing sensitive cells with maximum efficiency. However, we believe that nonreceptor adsorption, which is responsible for the elevated survivability of sensitive cells, can serve as a regulator that operates according to the feedback principle, typical of biological systems, and maintains the heterogeneity of the population even at high titres of colicin.

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