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In vivo properties of colicin A: channel activity and translocation across the envelope of *Escherichia coli*

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Introduction

Colicins are toxins of high molecular mass (40 to 70 kDa) produced by and active against *Escherichia coli* and closely related bacteria. Colicin A belongs to the group of colicins which form voltage-dependent ionic channels in planar lipid bilayers [1,2]. To exert its lethal activity colicin A first binds specifically to proteins (BtuB/OmpF) located on the outer membrane of *E. coli*, then it is translocated through the envelope; this translocation involves the participation of bacterial proteins encoded by the *tol* Q, R, A, B gene cluster [3]. The primary effects of the pore-forming colicins are a leakage of internal K^+ and a collapse of the electrochemical proton gradient ($\Delta\mu H^+$) [4]. On the basis of these in vitro and in vivo properties, it is generally believed that the killing activity of these colicins results from the formation of channels in the inner membrane. The first part of this review summarizes experiments devoted to the characterization of a putative channel-forming activity of colicin A in *E. coli* cells [5]. The second part focuses on the translocation mechanism of colicin A: we show that colicin A unfolds during its translocation and spans the whole cell envelope when its pore has formed [6]; furthermore, this translocation appears to take place at contact sites between the inner and outer membranes, domains where the translocation machinery of the 'Tol' proteins is also found.

Evidence suggesting that colicin A forms channels in vivo

Kinetic analysis of the net efflux of cytoplasmic K^+ induced by colicin A [5] (measured with a K^+ selective electrode) [7] indicates that colicin A forms channels in the cell envelope. The efflux of K^+ occurs independently of the functioning of the K^+ transport systems and is likely to be channel-mediated since a single colicin molecule added to one bacteria induced an efflux of $3 \cdot 10^5$ K^+ ions s^{-1}. This is fast compared to the turnover rate of mobile carriers. The linear relationship between the initial rate of efflux and the amount of added colicin suggests that there is no cooperativity between colicin molecules: presumably, each colicin molecule forms a single channel.

Addition of colicin A results in a partial depolarization of the inner membrane which occurs with the same kinetics as the K^+ efflux. The same steady state of $\Delta\Psi$ (about 70 mV, negative inside) was attained whatever the amount of colicins added suggesting that the channel closes when this threshold is attained. Closing of the channel could also be induced by decreasing $\Delta\Psi$ below 70 mV after the onset of the K^+ efflux by addition of the protonophore TCS (3,3',4'-tetrachlorosalicylanilide); the channel could then be opened at any time after its closing by addition of bovine serum albumin (BSA) which by desorbing TCS from the membranes allows $\Delta\Psi$ to rise.

Relationship between channel activity and cell killing

The fact that the channel closes once $\Delta\Psi$ falls below 70 mV raises the question of how colicins kill bacteria. The decrease of $\Delta\Psi$ cannot alone explain the lethality since bacteria can retain viability with a reduced $\Delta\mu H^+$ [8]. Colicins belonging to the same group as colicin A are known to induce a depletion (but not a leakage) of 80% of the internal ATP in less than 2 min [9]. It has been proposed that ATP levels were diminished by the action of the H^+-ATPase attempting to reenergize the membrane [10]. However, recent observations are not consistent with this proposal: first, a decrease of $\Delta\Psi$ in normal cells (in the absence of colicin) to 80 mV by the addition of a protonophore

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only resulted in a 10% decrease in internal ATP concentration after 15 min; second, colicin A induced a loss of 80% of the ATP from a H^+-ATPase deleted strain [11]. We have demonstrated that the decrease of ATP is the consequence of the efflux of inorganic phosphate through the colicin channel (unpublished data). It is likely that this massive drop in the internal ATP concentration is one the major events causing cell death.

Unfolding of colicin A accelerates its translocation

The K^+ efflux caused by colicin A is preceded by a lag time. During this period, the colicin binds to its receptor and is translocated through the envelope [12,13,5]; this lag time was reduced by a factor of two when colicin A was first caused to unfold in 8 M urea and then diluted in the cell suspension (under these conditions the time elapsed between dilution and binding is short compared to the time required for refolding of colicin A). Unfolding, however, had no effect on the characteristics of the channel. These results suggest that one of the limiting steps in the translocation of colicin A may be the partial unfolding of the polypeptide chain [6].

A membrane potential is not required for the translocation of colicin A in the inner membrane

The possible role of the membrane potential in the translocation of colicin A was studied by comparing the lag time preceding efflux in energized and deenergized cells [5]. Translocation, was shown to occur in deenergized cells. However, it is not possible to conclude from these experiments whether or not colicin A is translocated and inserted into the deenergized membrane and $\Delta\Psi$ is necessary for channel opening or if $\Delta\Psi$ serves both for insertion and channel opening.

Colicin A spans the whole envelope upon pore formation

Addition of trypsin to the external medium after the onset of the colicin A-induced K^+ efflux (i.e., under conditions where all colicins have reached their target in the inner membrane) caused immediate closing of the channels (as judged from the arrest of the K^+ efflux) [6]. A similar result was obtained with the pore-forming colicin B, which shares neither the same receptor nor the same translocation machinery with colicin A [12]. Control experiments indicated that trypsin does not damage any bacterial component necessary for binding or translocation and does not gain access either to the periplasmic space or to the inner membrane. Thus, the pore-forming colicins remain accessible from the external medium when forming the pore in the inner membrane. Although colicins are elongated molecules [3] they are not long enough to traverse the whole envelope, it is thus likely that they are active in an unfolded state.

What triggers colicins to unfold? The polypeptide chain of colicins is linearly organized into three domains: the C-terminal domain forms the channel, the central domain binds to the receptor. Indirect evidence suggests that the N-terminal 'domain is involved in translocation. It is likely that the interactions of colicins with their receptor trigger the first step of unfolding in the external membrane [3,13–15]. Further unfolding may be favoured by interactions with the translocation machinery. It has been recently shown [16] that the N-terminal domain of colicin A interacts in vitro with the C-terminal domain of TolA, one of the proteins of the translocation machinery [17]. This interaction may be relevant to the in vivo translocation mechanism.

Localization of the translocation machinery and of colicin A in the cell envelope

The sites of colicin A insertion and the location of the translocation machinery were determined by fractionation of the cell envelope into the inner, outer membrane and a third fraction which is likely to correspond to contact sites between the membranes [18]. A strain overproducing the Tol machinery was used, in which the synthesis of the Tol proteins was under the control of a T7 promoter [17], allowing specific radioactive labelling of the Tol proteins. The specific activity of the Tol proteins was twice as high in the contact sites as either in the inner or outer membrane. Furthermore, the presence of colicin A increased the specific activity of the Tol proteins in the contact sites by a factor of two and decreased it in the outer membrane. Finally, colicin A was identified by monoclonal antibody and radioactive labelling and found essentially in these contact sites (unpublished data).

The origin of these contact sites is still obscure. Several experiments suggest that the number of contact sites increases after infection by phages or upon expression of a phage-encoded proteins [19–21]. It is thus likely that such events create new contact sites or stabilize pre-existing ones. In view of the data on the location of the Tol proteins and of colicin A and on the finding that colicin A spans the whole envelope, it is tempting to speculate that some of these contact sites are formed by the interaction of colicin A with the Tol machinery.

Are there common pathways for the translocation of colicins and of the DNA of phages?

Colicins and bacteriophages share common receptors and translocation mechanisms: phage T1 and F80

and type 'B' colicins use the TonB pathway whereas type 'A' colicins and filamentous phages use the TolQRAB pathway [22,23]. We have proposed, on the basis of the analysis of the K^+ efflux induce by several T phages that the transfer of their DNA through the envelope takes place through specific channels [24]. It has been recently proposed that phage T5 tail proteins participate in the formation of these channels. These proteins were found mainly in the contact sites [21]. It is tempting to speculate that the contact sites formed at least by the Tol or Ton machinery may be main pathways for the import of foreign macromolecules.

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