Biochimica et Biophysica Acta 856 (1986) 101–108
Elsevier

BBA 72992

Octyl glucoside promotes incorporation of channels into neutral planar phospholipid bilayers. Studies with colicin Ia

James O. Bullock * and Fredric S. Cohen

Department of Physiology, Rush Medical College, Chicago, IL 60612 (U.S.A.)

(Received September 9th, 1985)

Key words: Colicin; Voltage-dependent channel; Phospholipid bilayer; Membrane-detergent interaction; Octyl glucoside

Colicin Ia forms voltage-dependent channels in planar phospholipid bilayers containing acidic phospholipids. Addition of the neutral detergent octyl glucoside, at concentrations three orders of magnitude below its critical micelle concentration (CMC), greatly increases channel-forming activity without altering the properties of the channels themselves. Further, octyl glucoside promotes formation of channels by colicin Ia in membranes containing only neutral lipids, making it possible to study the biophysical properties of the channel undistorted by the effects of negative surface charge. In neutral membranes, the macroscopic currents are biphasic with time, the fast component is voltage-dependent and the slow component voltage-independent. The single-channel conductance in 1 M NaCl is 31 pS and the channel is slightly anion selective. The mechanism by which the detergent facilitates channel formation is discussed.

Introduction

The colicins are bactericidal proteins which are plasmid encoded and secreted by *Escherichia coli* in a water-soluble form. One group of colicins, which includes A, E, K, E1, Ia and Ib, form ion-permeable channels in the plasma membranes of related bacteria, causing an ultimately lethal collapse of the membrane potential of the target cell [1–3]. When these colicins are studied in model membrane systems, negatively charged lipid must be present if channel forming activity is to be expressed. This is true both for planar [1,4] and vesicular [5] systems. We report here that in the presence of very low concentrations of the neutral detergent octyl glucoside, this requirement is relaxed, and substantial colicin Ia channel activity can be observed in planar membranes having no net negative surface charge. The basic properties of colicin Ia in neutral membranes is described.

Materials and Methods

Our purified colicin Ia was the generous gift of Dr. J. Konisky (University of Illinois, Urbana, IL). The lyophilized protein was dissolved in 100 mM Tris (pH 7.5) buffer containing 10% glycerol at a concentration of 0.4 mg/ml. Frozen aliquots of this stock solution were stored at −80°C until needed. However, after several months of storage under these conditions, we noted a loss of activity and smaller single-channel conductances. The data reported here were obtained with the more active material.

Conductance measurements. Planar phospholipid bilayer membranes were formed by the union

* Present address: Department of Physiology, Emory University School of Medicine, Atlanta, GA 30322, U.S.A.

Abbreviations: CMC, critical micelle concentration; DPhPC, diphytanoylphosphatidylcholine; Mes, 4-morpholineethanesulfonic acid.

of two monolayers of phospholipid across a hole in a sheet of Teflon separating two shallow compartments. The monolayers were spread from 5 to 10 μl of 1% phospholipid dissolved in hexane as previously described [4]. Each aqueous compartment bathing the membrane contained 5 ml.

All electrical measurements were made at room temperature under voltage clamp conditions, with a single pair of calomel electrodes contacting the solutions through saturated KCl junctions. After forming a membrane and determining that it had a high resistance ($10^8\ \Omega \cdot cm^2$), an aliquot of colicin Ia was added to one compartment (*cis* side). For single-channel and macroscopic-current measurements, the concentrations of protein used were approx. 30 ng/ml and 200 ng/ml, respectively. The solutions were magnetically stirred.

The voltages (V) indicated below were those of the *trans* compartment; the voltage of the *cis* side was set equal to zero. The conductance of the membrane in symmetrical solutions is defined as the total current divided by the voltage.

The asolectin used was Type IV-S (Sigma), and was washed in acetone [6]. All other phospholipids were purchased from Avanti. Salts and solvents were of reagent grade and used without further purification. Millipore Milli-Q water was used for all solutions (18 M $\Omega \cdot$ cm). Symmetrical solutions of 1 M NaCl, 10 mM Mes, 3 mM $CaCl_2$, 0.1 mM EDTA (pH 6.0) bathed the membrane unless otherwise indicated.

Use of detergent. Octyl glucoside (octyl β-D-glucopyranoside, mol. wt. 292.39) was purchased from Calbiochem and was prepared as a stock solution at a concentration of 12 mg/ml (41 mM). The critical micelle concentration (CMC) of this detergent is 7.3 mg/ml (25 mM) [7]. To promote colicin incorporation, a maximum of 2 μl of detergent solution was added to the *cis* compartment of the planar bilayer chamber, resulting in a final detergent concentration of 4.8 μg/ml (16 μM). Alternatively, a stock solution containing 30 μg/ml colicin Ia was pretreated with octyl glucoside at a concentration of 3.5 mg/ml. Aliquots of this solution were added to one side of the bilayer chamber. The final concentrations of both protein and detergent were approximately the same as described for experiments in which they were added separately.

Results

Effect of octyl glucoside on colicin Ia-induced conductance

We examined the properties of the conductance induced by colicin Ia in negatively charged membranes in the presence and absence of detergent to determine whether octyl glucoside alters the behavior of the protein. Fig. 1A shows the changes in macroscopic conductance produced by colicin Ia in an asolectin membrane in response to various applied potentials. The conductance increase elicited by applying negative voltages is clearly biphasic. The initial rise becomes larger and more rapid as the potential is made more negative. The slower phase appears as a linear increase whose rate is voltage-independent. Following application of positive potentials, the conductance rapidly declines to that of bare bilayer. Thus, all the colicin Ia channels will close with positive potentials, even those corresponding to the slower component where the rate of increase is relatively voltage-insensitive. If, after a short interval, negative potentials are restored, a fraction of the original conductance is rapidly reactivated. The fraction of channels in this 'reactivatable' state declines with a time course on the order of tens of seconds as the membrane is held at positive voltages. Between each of the responses shown superimposed in Fig. 1, the membrane was held at positive potentials for several minutes to allow complete restoration of colicin conductance to its original state. The general features of this behavior were noted previously [8,11]. In Fig. 1B, the response of this same membrane after *cis* addition of octyl glucoside to a concentration of 9 μM is shown. While the conductance has increased substantially, the general kinetic features of the colicin are unaltered by the presence of detergent. Octyl glucoside in the absence of colicin has no effect on the conductance of the lipid bilayer.

While it is clear that the voltage dependence of colicin Ia is quite steep, this property is difficult to quantitate. To do this, it is first necessary to make some assumptions about the slower, voltage-independent component, so that it may be subtracted from the total response. This is especially problematic for smaller potentials where the two components are not well resolved. When this correc-

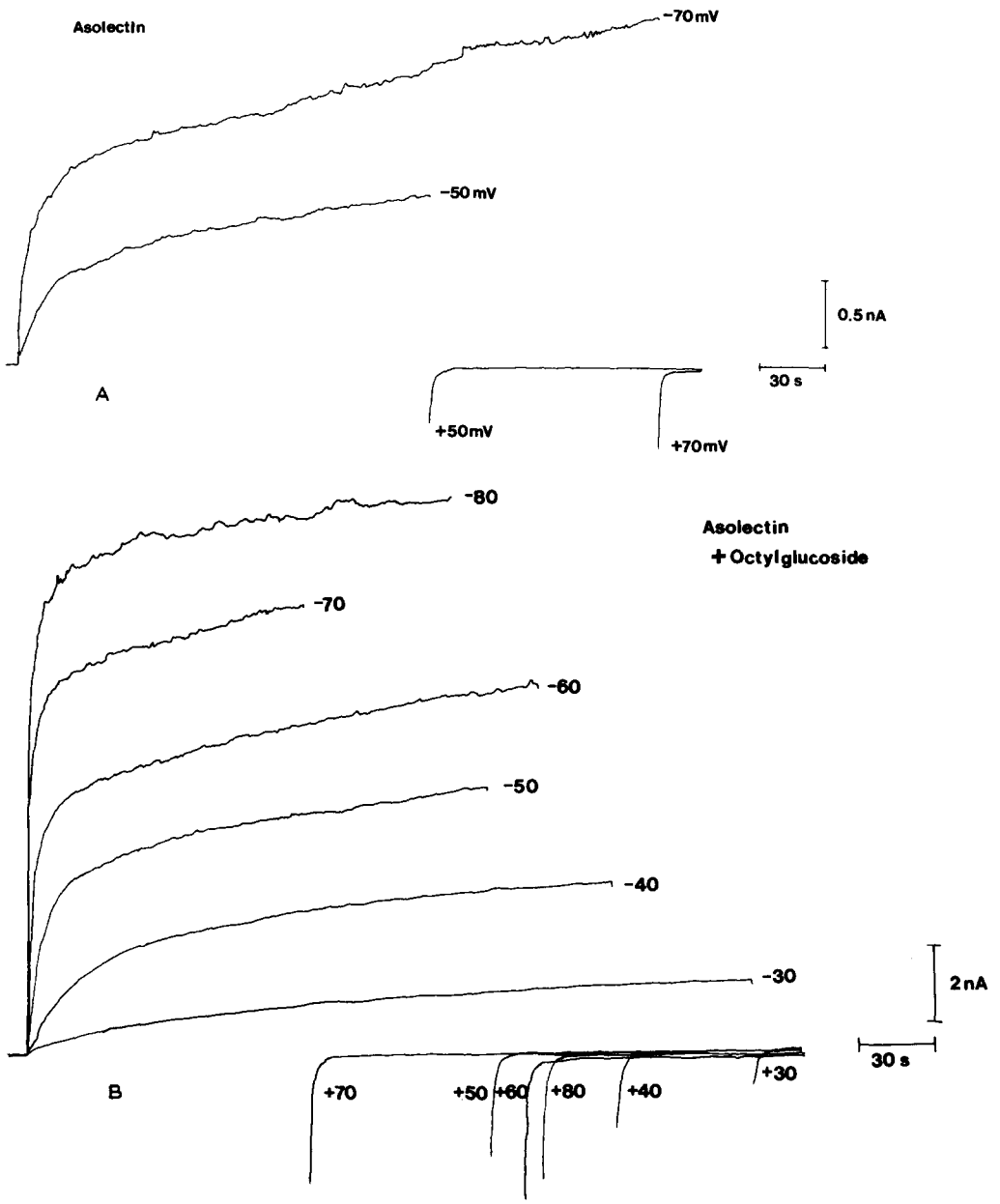

Fig. 1. Effects of voltage on colicin Ia activity. (A) Protein was added to one side (*cis*) of an asolectin membrane held at 0 mV. The currents that resulted when the membrane was abruptly switched to the indicated negative potentials have been superimposed. The slow linear increase is preceded by a rapid rise in conductance which becomes more pronounced at higher potentials. After the slow linear increase in conductance was established, the voltage was switched to its positive value (e.g. −50 mV to 50 mV). As seen, the currents decayed rapidly to that of bare bilayer when these positive potentials were applied. Membranes were held several minutes at positive potentials between application of negative voltages. (B) Currents after addition of octyl glucoside (final concentration ≈ 3 μg/ml) to the *cis* side of the membrane of panel A. Often, in the presence of detergent the conductance of the membrane did not reach a steady state; rather the size of the response increased each time negative voltages were applied. This was not the case for the data of panel B. Responses at a potential of −50 mV elicited before and after the traces shown could be superimposed. As in panel A, the currents rapidly decreased when the potentials were switched to their positive values (e.g. −70 mV to 70 mV). The membrane potential, in mV, is indicated adjacent to each of the appropriate current tracings.

tion was made by assuming the slow component to be voltage independent and linear, the voltage dependence was quantitatively variable from membrane to membrane; even on a single membrane the voltage dependence may vary with time. Because we do not yet have a protocol that results in quantitatively reproducible macroscopic voltage dependence, we have not attempted to compare the voltage dependence of colicin Ia in the presence and absence of detergent on asolectin planar membranes. However, it is apparent that no major shifts in voltage dependence or gross alterations in kinetic behavior are produced by the presence of octyl glucoside.

Octyl glucoside does not alter the channel properties of colicin Ia

The single-channel conductances of colicin Ia, in the presence and absence of detergent, were quantitatively compared. At a potential of -50 mV, where these comparisons were made, the rate of channel closing is negligible. (The voltage dependence of colicin Ia is quite steep, and at potentials more negative than about -40 mV, once channels open they remain open.) As a consequence, each individual channel-forming protein is counted only once in the statistics, in contrast to the situation in which statistics are accumulated from repeated openings and closings of the same channel-forming molecule.

Fig. 2 illustrates a single-channel record. Note that the channels do not close at -50 mV. At $+50$ mV, however, the channels rapidly close, a response consistent with macroscopic currents (Fig. 1). In 1 M NaCl the single-channel conductance in the absence of detergent at -50 mV is 44.4 ± 0.7 pS (S.E., $n = 39$). With detergent present, the single-channel conductance under the same conditions is 43.5 ± 1.5 pS (S.E., $n = 11$).

It is convenient to obtain the current-voltage (I-V) characteristics of individual channels at negative potentials using low protein concentrations. This normalized I-V relation, shown in Fig. 3, is non-ohmic: the single channel conductance increases with increasing voltage. The shape of the normalized I-V relation is not affected by detergent. Furthermore, the zero-current potential of the macroscopic conductance is unaffected. These measurements were made with 1 M NaCl in the

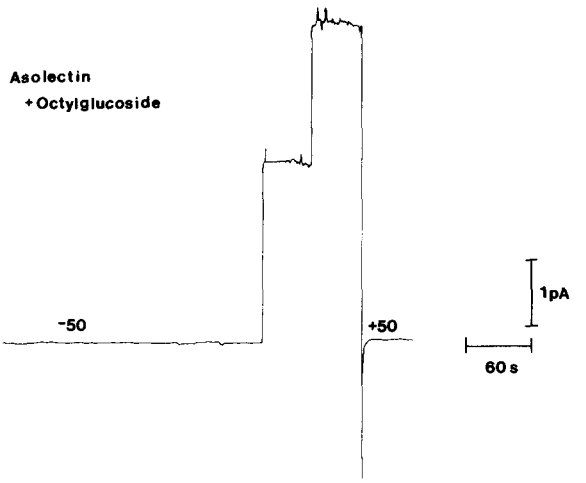

Fig. 2. Single channels of colicin Ia in asolectin membranes. Colicin Ia and octyl glucoside were added to the *cis* side of a planar membrane. When the membrane potential was calmped to -50 mV, channels turned on and at this voltage did not close. This allowed I-V relations (Fig. 3) to be obtained on individual channels as explained in the text. In this figure, two channels turned on. Note the channel conductances are not uniform. When the membrane potential was switched to $+50$ mV, the channels rapidly closed. Identical results were obtained in the absence of detergent.

trans compartment and 0.1 M NaCl in the *cis* compartment at pH 5. The average value was -28.9 ± 1.2 mV ($n = 5$). (The sidedness of the solutions was chosen so that the channels would remain open at the reversal potential. The sign of the reversal potential indicates a selectivity for Na^+ over Cl^-.)

Macroscopic behavior of colicin Ia in neutral membranes

Colicin Ia does not produce macroscopic conductances in membranes composed of neutral lipids, although one can observe single channels at high colicin concentrations. In the presence of octyl glucoside, however, macroscopic conductances are induced. Illustrated in Fig. 4 is the response of protein pretreated with octyl glucoside to negative voltages in a membrane composed of diphytanoylphosphatidylcholine (DPhPC). A biphasic response, similar to that observed in asolectin membranes, is evident. However, the potential required to elicit the smallest observable response (threshold), and to distinguish fast and slow components, are substantially higher in the

neutral lipid. Clearly the voltage dependence of colicin Ia is shifted in the negative direction in DPhPC membranes compared with asolectin membranes. This shift is on the order of 30 mV.

Octyl glucoside was effective in promoting conductance activity of colicin Ia regardless of whether the protein was pretreated with detergent or whether colicin and detergent were added to the chamber separately. In the latter case, the order of addition was also unimportant. For experiments using neutral lipids, the protein was routinely pretreated as a matter of convenience.

The reversal potential of colicin Ia in DPhPC membranes is $+11.7 \pm 3.1$ mV (S.D., $n = 5$) when measured under the same conditions as previously described for asolectin. Therefore, colicin Ia is actually slightly anion-selective, whereas in asolectin membranes it appears to be somewhat cation-selective at pH 5. This alteration of the selectivity of colicin Ia by asolectin is qualitatively consistent with the higher cation and lower anion

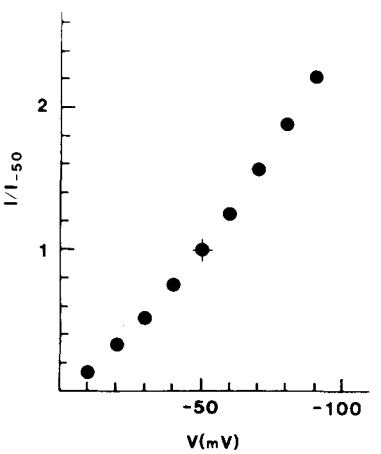

Fig. 3. Normalized current-voltage relation of colicin Ia channels in asolectin membranes. Current-voltage relations were obtained using membranes containing only a single open channel. To obtain the shape of this relation independent of the size of the single-channel conductance, whose distribution exhibits a large variance, the current through a particular channel at each voltage was normalized to its value at -50 mV. Measurements at potentials more positive than -10 mV could not be obtained because of the rapid closure of the channels in this range. The point at -10 mV represents the average of three measurements; all other points are derived from at least six measurements. Standard errors of the mean are smaller than the size of the symbols in all cases.

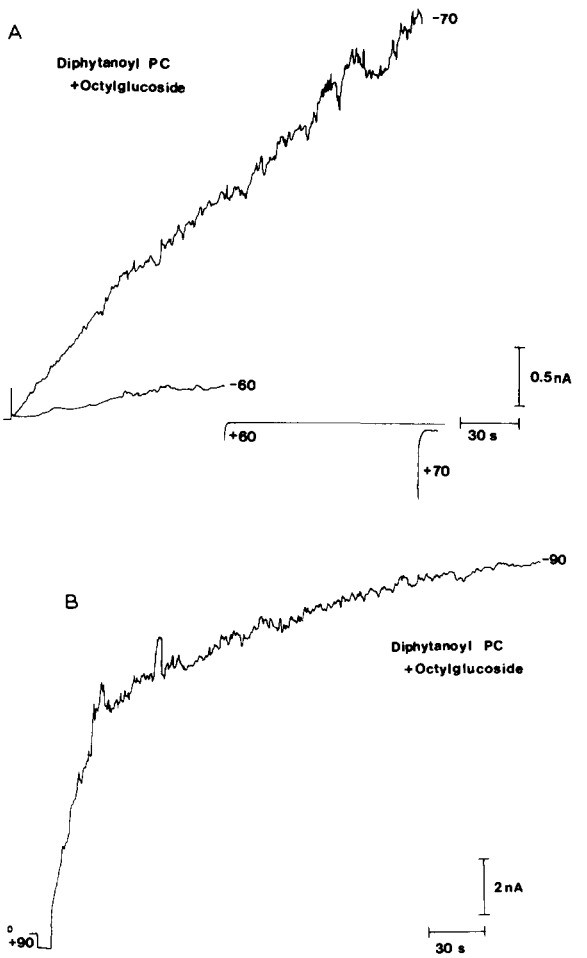

Fig. 4. Biphasic response of colicin Ia in neutral membranes. Colcicin Ia, pretreated with octyl glucoside, was added to the *cis* side of a DPhPC membane. (A) For voltages of -60 mV and -70 mV, the current response was voltage-dependent, but the slow and fast components were not well resolved. (B) For -90 mV, the current response is biphasic; the fast and slow components are easily observed. This is analogous to the results of Fig. 1: a voltage-dependent response, but clearly biphasic only for larger voltages. Also the currents rapidly decrease when positive potentials are applied.

concentrations at the membrane-solution interface which result from the negative surface potential of this lipid mixture.

Single-channel measurements in neutral membranes

The average conductance of single channels of octyl glucoside-treated colicin Ia was 30.6 ± 1.2 pS (S.E., $n = 20$) at -50 mV in 1 M NaCl. The *I-V* relation of single channels in DPhPC shows non-

linear behavior similar to that observed with asolectin membranes (Fig. 3). In membranes made from phosphatidylcholine in 1 M Na_2SO_4, the single-channel conductances were not affected by detergent [10].

Discussion

The colicins [4,8] and diphtheria toxin [9,12] exhibit channel-forming activity in planar lipid bilayers only when negatively charged lipid is present in the membrane. The work reported here shows that very low concentrations of octyl glucoside facilitate the incorporation of colicin Ia into membranes composed only of neutral lipids. The number of open channels obtained is on the order of thousands, comparable to that seen in charged membranes. Thus, whatever role negative charges on membrane lipids play in the process of incorporation, they are not essential to the formation of ion channels. In addition, the detergent markedly enhances colicin activity in membranes that do contain anionic lipids. Our studies with asolectin membranes demonstrate that the conversion of colicin Ia to open channels in response to applied potential is not affected by detergent. We do not observe, for example, any alteration in voltage dependence or kinetics, or any evidence of channels which are assembled backwards (i.e., channels that open in response to positive rather than negative voltages). Instead, the response to detergent can be described by a simple scaling factor. The properties of the ion translocation pathway are likewise unaltered by the presence of detergent. Although the statistical distribution of conductance values for single colicin Ia channels is quite broad, our data show that the mean value is not altered by the presence of detergent. The shape of the current-voltage relation for single channels and the permselectivity of the channel are likewise not influenced.

Differences in the behavior of colicin Ia in PC as opposed to asolectin membranes can be qualitatively explained by the presence of negative surface charges on the latter. Cations are enriched at the membrane-solution interface by such charges, and anions depleted. The observed increase in both single-channel conductance and cation selectivity are thus expected in membranes containing anionic lipids. The negative surface charges also affect the concentrations of protons and hydroxyl ions at the interface, resulting in a substantial lowering of the pH in that region. Since lowering the bulk pH enhances colicin Ia activity (personal observations), the lower pH at the membrane-solution interface, may be the basis for the difference in voltage needed to activate channels in the two lipids. Although the effects of surface charge on the interfacial ionic conditions are readily calculated, the effects of ionic conditions on the colicin Ia protein must be experimentally studied in detail in order to determine whether surface charge effects alone can quantitatively account for the differences observed in the two lipid mixtures. In any case, measurements in PC membranes represent the intrinsic properties of the protein, whereas the use of asolectin introduces a series of complications.

Detergents are widely used for the solubilization [13,14] and reconstitution [15] of membrane proteins. Both procedures are thought to rely upon the stability of such proteins in detergent-lipid mixed micelles. In contrast, colicins do not require detergent for stability, but are quite stable in aqueous solution. Furthermore, the concentrations of detergent in our studies in planar bilayers were three orders of magnitude below the CMC, which avoided higher concentrations which would tend to remove lipid from the membrane by forming mixed micelles, or to produce leaky or unstable membranes by some other mechanism.

Studies of colicin E1 in our laboratories indicate that this protein is bound to asolectin membranes in a voltage-independent step, and that the amount of bound colicin is in large excess compared with the amount existing as open channels in the membrane [4]. The binding of colicin A to lipid monolayers has been measured directly [16]. This process, clearly not involving any voltage, results in significant penetration of the protein into the monolayer, and is markedly facilitated by the presence of anionic lipids in the monolayer. Because the subsequent voltage-dependent steps are unaffected by the presence of detergent, we believe the octyl glucoside promotes activity by enhancing the binding of colicin to the lipid bilayer.

We further conclude that the enhanced binding

of the protein to the membrane is via the detergent monomers binding to the colicin rather than the monomers binding to the phospholipid bilayer. Following Nichols and Pagano [17] the rate of transfer of detergent between the membrane and aqueous phase is given by

$$\frac{d[D]}{dt} = k_+ [D]_m (S_D[D] + S_P[P]) - k_- [D] \quad (1)$$

where [D] and [P] are the concentrations of detergent and phospholipid in the membrane, S_D and S_P their respective molar surface areas, $[D]_m$ the concentration of free detergent monomers, k_+ is the on-rate constant of detergent into the membrane and k_- is the corresponding off-rate constant.

At equilibrium $d[D]/dt = 0$ and by setting $S_D = S_P \equiv S$, we obtain

$$K_{eq} \equiv \frac{k_+ \cdot S}{k_-} = \frac{1}{[D]_m(1+[P]/[D])} \quad (2)$$

In the membrane the molar ratio of phospholipid to detergent is

$$\frac{[P]}{[D]} = \frac{1}{K_{eq}[D]_m} - 1 \quad (3)$$

Assuming that soluble detergent monomers interact with phospholipid bilayers in a manner similar to their interactions with their own micelles, K_{eq} is approximately the reciprocal of the CMC [18].

For our experimental conditions $[P]/[D] \approx 2 \cdot 10^3$, a negligible amount of detergent in the planar membrane. We therefore envision that octyl glucoside monomers bind to the colicin, rendering the protein more hydrophobic, thereby favoring binding to the membrane.

Use of octyl glucoside in solubilization of membrane proteins [19] is now widespread. The properties which recommend octyl glucoside for the purpose described here are precisely those for solubilization and reconstitution: its CMC is high, it is chemically well-defined, and it is nondenaturing for most proteins. It was found to produce no alterations in the electrically observable behavior of colicin Ia. Octyl glucoside has also been shown to promote incorporation of colicin E1 (unpublished observations) and diphtheria toxin (Ehrlich, B., personal communication) into neutral membranes. We believe this detergent may prove to be generally useful in facilitating the incorporation of other proteins into planar bilayer membranes. Octyl glucoside may also be useful in improving the efficiency of protein (e.g. colicins, diphtheria toxin) incorporation into vesicular systems. Such an action would be particularly helpful for studies in which a biochemical characterization of membrane-incorporated protein is attempted.

Cholate was also effective in promoting incorporation of colicin into neutral membranes without major alterations in behavior. However, even at the low pH employed here, a significant amount of cholate is unprotonated. The likelihood of negative charges being associated either with the membrane or the protein made it an unsuitable choice for biophysical studies. Treatment of colicin with Triton X-100 resulted in subconductance states and rapid flickering on the single-channel level in asolectin membranes that were not observed with the nude colicin. In addition, Triton X-100 can itself produce channels [20]. We did not further pursue the use of Triton X-100, even though it also resulted in colicin Ia activity in neutral membranes. Therefore, in this as in other applications, caution is advised in the choice of detergent.

Acknowledgements

We wish to thank Dr. Jordan Konisky for a generous gift of purified colicin Ia and Drs. Bruce Kagan and J. Wylie Nichols for helpful discussions. This work was supported by grant NIH GM 31039. J.O.B. was supported by NSF BNS 8317990 (C.L. Schauf, principal investigator).

References

1 Schein, S.J., Kagan, B.L. and Finkelstein, A. (1978) Nature (Lond.) 276, 159–163
2 Tokuda, H. and Konisky, J. (1979) Proc. Natl. Acad. Sci. USA 76, 6167–6171
3 Cramer, W.A., Dankert, J.R. and Uratani, Y. (1983) Biochim. Biophys. Acta 737, 173–193
4 Bullock, J.O., Cohen, F.S., Dankert, J.R. and Cramer, W.A. (1983) J. Biol. Chem. 258, 9908–9912
5 Kayalar, C., Düzgünes, N. and Erdheim, G.R. (1982) Biophys. J. 37, 256a
6 Kagawa, Y. and Racker, E. (1971) J. Biol. Chem. 246, 5477–5487

7 Shinoda, K., Yamagucii, T. and Hori, R. (1961) Bull. Chem. Soc. Jpn. 34, 237–241
8 Kagan, B.L. (1981) Ph.D. dissertation, Albert Einstein College of Medicine
9 Kagan, B.L., Finkelstein, A. and Colombini, M. (1981) Proc. Natl. Acad. Sci. USA 78, 4950–5954
10 Bullock, J.O. and Cohen, F.S. (1982) Biophys. J. 37, 255a
11 Weaver, C.A., Kagan, B.L., Finkelstein, A. andKonisky, J. (1981) Biochim. Biophys. Acta 645, 137–142
12 Donovan, J.J., Simon, M.I., Draper, R.K. and Montal, M. (1981) Proc. Natl. Acad. Sci. USA 78, 872–876
13 Tanford, C. and Reynolds, J.A. (1976) Biochim. Biophys. Acta 457, 133–170
14 Helenius, A., McCaslin, D.R., Fries, E. and Tanford, C. (1979) Methods Enzymol. 56, 734–749
15 Racker, E. (1979) Methods Enzymol. 55, 699–711
16 Pattus, F., Martinez, M.C., Dargent, B., Cavard, D., Verger, R. and Lazdunski, C. (1984) Biochemistry 22, 5698–5703
17 Nichols, J.W. and Pagano, R.E. (1982) Biochemistry 21, 1720–1726
18 Tanford, C. (1973) The Hydrophobic Effect, Wiley, New York
19 Baron, C. and Thompson, T.E. (1975) Biochim. Biophys. Acta 382, 276–285
20 Benz, R. and Hancock, R.E.W. (1981) Biochim. Biophys. Acta 646, 298–308