Ion Selectivity of Colicin E1: II. Permeability to Organic Cations

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Summary. Channels formed by colicin E1 in planar lipid bilayers have large diameters and conduct both cations and anions. The rates at which ions are transported, however, are relatively slow, and the relative anion-to-cation selectivity is modulated over a wide range by the pH of the bathing solutions. We have examined the permeability of these channels to cationic probes having a variety of sizes, shapes, and charge distributions. All of the monovalent probes were found to be permeant, establishing a minimum diameter at the narrowest part of the pore of approximately 9 Å. In contrast to this behavior, all of the polyvalent organic cations were shown to be impermeant. This simple exclusionary rule is interpreted as evidence that, when steric restrictions require partial dehydration of an ion, the structure of the channel is able to provide a substitute electrostatic environment for only one charged group at a time.

Key Words colicin · ion selectivity · lipid bilayers · electrostatic interactions

Introduction

The ability of ion channels to play functional roles in the membranes of particular cells is dependent on their capacity to discriminate among the various ions which may be present. As more kinds of channels have been made available for study and our knowledge of their molecular structures has increased, understanding the basis for the ion selectivity of channels has become a more challenging task. Channels formed in planar lipid bilayers by a group of proteins known as colicins are particularly interesting objects for the study of this process [7, 9, 13, 32, 35, 41, 42]. Not only are these pores permeable to cations and anions alike, they are apparently so wide that no ions have yet been identified which will not pass through them [34]. These channels are not simply featureless conduits, however. The absolute transport rates, even for the most permeant small ions, are quite slow. Furthermore, the relative preference of the channels for cations versus anions is modulated over a very wide range by the pH of the bathing medium [6, 34]. Our aim has been to understand how the interactions of the transported ions with the channel structure create this unexpected intricacy in its selectivity behavior.

The colicins have attracted the attention of investigators with a wide variety of interests. They are secreted by certain strains of Escherichia coli as aqueous proteins and are readily purified in large amounts. A variety of physical techniques have been used to study their properties [5, 26, 33, 42, 43], and site-directed mutants have been produced to examine structure-function relationships in these molecules [2, 19, 21, 37–39]. Because they are produced for the purpose of killing other strains of E. coli, these plasmid-encoded proteins can properly be considered toxins or antibiotics. A single channel formed by one of these molecules in the plasma membrane of the target cell produces a lethal depolarization. To gain access to the plasma membrane, other regions of the colicin molecules bind to specific receptors in the outer membrane and translocate the toxic region into the periplasmic space. The domains of the colicin E1 and A molecules which actually form channels have been shown to lie at the carboxy-terminal ends of the proteins, and all six of the channel-forming colicins show high degrees of homology in the corresponding regions [11, 12, 22, 24, 29, 36, 40, 44]. Both the proteins and their C-terminal peptides have been used as model systems for the study of membrane assembly and protein export [15, 31, 33]. In the present study, we have explored the steric and electrostatic topology of the pore formed by colicin E1 in planar lipid membranes using cationic probes of varying size and charge distribution. A preliminary account of this work has appeared [10].

Materials and Methods

Chemicals and Biochemicals

Inorganic salts, buffer compounds, and solvents were of reagent grade and used without further purification. Conductivity-grade water (18 MΩ-cm) was used for all solutions. Working solutions
contained the chloride salt of the cation under test at a formal concentration of either 1.0 or 0.1 M. All solutions also contained 3 mM CaCl₂ to enhance membrane stability, and each of the following pH buffer compounds at concentrations of 3 mM: glutaric acid, pKₐ = 4.13, 5.03; 2-(N-morpholino)ethanesulfonic acid (MES), pKₐ = 6.15. Asolectin type IV-S (Sigma Chemical, St. Louis, MO) was washed in acetone, and bacterial phosphatidylethanolamine (PE) was obtained from Avanti Polar Lipids (Birmingham, AL). Colicin E1 protein was the generous gift of Dr. W.A. Cramer (Purdue University, West Lafayette, IN). Di(pentafluorophenyl)mercury [(C₆H₅)₂Hg] was obtained from PCR, Gaithersburg, MD, and was generously given to us by Dr. Alan Finkelstein (Albert Einstein College of Medicine, Bronx, NY).

The following compounds, used as test cations, were obtained from Aldrich Chemical (Milwaukee, WI): Tetramethylammonium (TMA); N,N,N',N'-tetramethyl-L-lysine (TMA); 4-(2-hydroxyethyl)morpholine (ME-OH); Tropine; 1-benzyl-4-cyano-4-hydroxypiperidine (BCHP); 1-aza-bicyclo[2.2.2]octane (Quiniclidine); 1,4-bis(2-hydroxyethyl)piperazine (HEPE-OL); 2,2-bis(hydroxymethyl)-2'2'-nitrolitioethanol (Bis-Tris); N-methyl-D-glucamine (NGM); N,N,N',N'-tetramethyl-1,3-propanediamine (Bis-T3); 1,3-bis[tris(hydroxymethyl)ethylamino]propane (Bis-Tris-Propane); 4,4'-bipiperidinomethane; 1,1',methylene-dipiperidinomethane; 4,4'-trimethylene-dipiperidinomethane (TMDP); N,N,N',N'-tetramethyl-1,6-hexanediamine (bis-T6); and Hexamethonium (Bis-Q6). Spermine and Spermidine were obtained from Sigma Chemical (St. Louis, MO). The structures, formula weights, (FW), and molecular dimensions of these compounds are shown in Tables 1 and 2. All measurements were made at room temperature.

The compounds we have abbreviated as BCHP and TMDP yielded highly colored solutions which were decolorized with bipiperidine dihydrochloride and Bis-T3; 1,3-bis[tris(hydroxymethyl)ethylamino]propane (Bis-Tris-Propane); 4,4'-bipiperidinomethane; 1,1',methylene-dipiperidinomethane; 4,4'-trimethylene-dipiperidinomethane (TMDP); N,N,N',N'-tetramethyl-1,6-hexanedi­amine (bis-T6); and Hexamethonium (Bis-Q6). Spermine and Spermidine were obtained from Sigma Chemical (St. Louis, MO). The structures, formula weights, (FW), and molecular dimensions of these compounds are shown in Tables 1 and 2. The dimensions given are the maximum overall length and minimum overall width as determined from CPK models. This is equivalent to the size of the rectangular slot having the narrowest width and longest length which could be made to tightly enclose the molecule. In all cases, the measurements were made on the fully extended conformation of the ion.

The compounds we have abbreviated as BCHP and TMDP yielded highly colored solutions which were decolorized with carbon prior to use; all other compounds were used without further purification. Because BCHP was available only as the hydrochloride salt, HEPE-OL was used as a base to adjust the pH of the buffers in the working solutions. Similarly, Bis-Tris propane base was used with bipiperidine dihydrochloride and Bis-T6 base with Bis-Q6 dichloride. Aqueous titration was used to determine the formal concentration of the stock solutions of the test compounds and the pKₐ values of compounds for which no reports could be found in the literature (see Tables 1 and 2). All of these compounds were soluble to concentrations of at least 1 mM at room temperature and were impermeant with respect to bare bilayers. None was found to destabilize or disrupt the planar membranes used in this study. The selectivity of the colicin E1 channel for a particular cation was determined as the value of the zero current potential of the conductance induced by the protein in a membrane exposed to a 1.0 versus 0.1 M gradient of the cation as a chloride salt (see below). In order to assess the ability of the channel to transport a cation, its zero current potential was compared to the liquid junction potential (Eₗjunc) and chloride equilibrium potential (Ecl) of the experimental solutions used to produce the 10-fold gradients. The values of Eₗjunc shown in Tables 1 and 2 were determined using an agar bridge and a high impedance digital multimeter (Hewlett Packard). Ion-selective electrodes in combination with a pH/Ion meter (Orion Research, Cambridge, MA) were used to determine Ecl. A double junction reference electrode filled with 10% KNO₃ was used to avoid contamination of the solutions by additional chloride.

MEMBRANE CONDUCTANCE MEASUREMENTS

Planar phospholipid bilayer membranes of the solvent-free type [28] were formed across apertures in Teflon septa as previously described [9]. The volume of aqueous solution bathing each side of the membrane was either 1.5 or 5 mL. The apertures used in these experiments were either 100 or 150 μm in diameter. The smaller sized holes offered considerable improvement in the stability of the bilayers, especially those composed of PE, but they required the use of higher protein concentrations to achieve the levels of colicin conductance necessary for the accurate determination of zero current potentials, as described below.

Voltage-clamp conditions were established across the bilayers by means of a Burr Brown 3523L operational amplifier configured as a current-to-voltage converter. Electrical contact with the two aqueous compartments was established by means of a single pair of miniature calomel electrodes, one of which was connected to the system ground and the other to the inverting input of the converter amplifier. Command signals were applied to the noninverting input of this amplifier and subtracted from its output using a unity gain differential amplifier (Burr Brown 3627BM). The resulting signal, which was proportional to transmembrane electric current, was electronically filtered at 3 Hz by an 8-pole, low-pass Bessel filter (Frequency Devices, Haverhill, MA) and monitored using an oscilloscope and a chart recorder. DC command voltages were supplied by mercury batteries in combination with digital voltage dividers (Digitran, Pasadena, CA). The aqueous compartments were magnetically stirred, so that only a few seconds were required for complete mixing in the bulk phase. All measurements were made at room temperature.

After a membrane had been formed, small aliquots of aqueous stock solutions of purified colicin E1 protein were added to one compartment, defined as the cis side of the membrane. Colicin E1 was present at final concentrations ranging from 0.5 to 5.0 μg protein/mL of aqueous solution. The extremely low activity exhibited by the colicins in bilayers composed of PE was enhanced by the addition to the cis compartment of the uncharged detergent octylglucoside at a final concentration of 24 μg/mL, as previously described [8]. Only membranes exhibiting a high resistance (>10⁷ Ω-cm) and a low level of noise were considered suitable for the introduction of protein. Membranes which became unstable or noisy after the introduction of the protein were likewise discarded.

MEASUREMENT AND CONTROL OF pH

The pH in the cis compartment was monitored by means of a miniature glass electrode (Model 407B. Microelectrodes, Londonderry, NH) and a small, battery-powered pH meter. The calomel electrode in contact with the solution in that compartment provided the reference potential for pH measurement as well as the ground return path for transmembrane currents. The pH of the cis compartment could be altered during the course of conductance measurements by titration with aliquots of solutions of HCl or the appropriate test cation as the free amine.

Results

DETERMINATION OF SELECTIVITY

Channels formed by colicin E1 have been reported to be significantly permeable to a wide variety of