Identification of a Voltage-Responsive Segment of the Potential-Gated Colicin E1 Ion Channel†

A. R. Merrill and W. A. Cramer*
Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907
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ABSTRACT: The voltage dependence of channel activity of the bactericidal protein colicin E1 was found to be correlated with insertion into the membrane bilayer of a specific segment of the 178-residue COOH-terminal thermolytic colicin channel peptide. The insertion into the bilayer was detected by an increase in labeling by one of two different lipophilic photoaffinity probes or by a decrease in iodination of peptide tyrosines from the external solution. Imposition of a potassium diffusion potential of −100 mV resulted in an increase of 35–60% in the labeling of the peptide by the lipophilic probe in the bilayer and a concomitant decrease in labeling of Tyr residues in the peptide by the iodination reagent in the external solution. The change in labeling decreased upon dissipation of the membrane potential with a half-time of about 1 min. The labeling change was localized to a 36-residue peptide segment bounded by alanine-425 and by tryptophan-460. This segment containing seven positively charged residues at low pH is a voltage-sensitive region that inserts into the membrane bilayer when the channel is turned on by the potential and is extruded from it when the voltage is removed and the channel is turned off.

Protein import is commonly found to require a membrane potential. The bactericidal protein colicin E1 is a voltage-gated channel that is imported into the *Escherichia coli* cell (Cramer et al., 1990). Therefore, it was of interest to inquire whether the voltage gating of the colicin E1 channel involves an extensive translocation of the protein across the membrane and, if so, to identify the translocated segments. The voltage-dependent channel activity of colicin E1 COOH-terminal channel-forming peptides (Dankert et al., 1982) can be reconstituted in membrane vesicles (Peterson & Cramer, 1987), allowing the use of biochemical labeling techniques to assay topographical changes in response to an imposed membrane potential.

Materials and Methods

Preparation of ^{35}S- and ^{3}H-Labeled Thermolytic Peptide. The 174–178-residue COOH-terminal thermolytic channel peptide was prepared as described (Merrill et al., 1990) from colicin E1 labeled by cell growth in the presence of [^{3}H]-L-leucine containing a supplemented minimal medium (Davidson etal., 1985). Cysteine-505, the only cysteine in colicin E1, was labeled with [^{35}S]-L-cysteine by the method of Cleveland et al. (1983).

Preparation of Single Trp Mutants of Colicin E1. Colicin mutants containing a single Trp were prepared (Shiver et al., 1987) by substituting Phe for the natural Trp in two of the three Trp positions (W424, W460, and W495) in the peptide; synthetic oligonucleotides that had a two-base change from 5′-CCA-3′ (Trp) to 5′-AAA-3′ (Phe) were used and verified by sequencing. Each of the mutants was tested for cytotoxicity by a "spot test" on a bacterial lawn, and all were comparable to the wild-type colicin.

Cleavage of Single Trp Mutants with BNPS-skatole.[1] Thermolytic peptides from the mutants possessing a single Trp (W424 and/or W460) were dried in microcentrifuge tubes and subjected to cleavage at the single Trp residue according to Savige and Montana (1977). A solution containing 4 mg/mL BNPS-skatole (Pierce) in 80% acetic acid was added (2 μL/μg of protein) to the dried protein, sonicated for 10 s, and incubated in the dark at 25 °C (18 h). Excess reagent was removed with diethyl ether (4×); peptides were precipitated with TCA and washed twice with diethyl ether. The peptides were dried under vacuum (1 h) and then dissolved in Laemmli (1970) sample buffer (SDS–PAGE) or 70% HCOOH (for HPLC separation).

HPLC Separation of Mutant W424-F460-F495 Digest. A total of 0.01% TFA/H_2O (40 μL) was added to the dried peptide digest (~1 mg/mL) in 70% HCOOH; this mixture (50 μL) was injected (with 50 μL of 0.01% TFA/H_2O, 100-μL total volume) on a C18 reverse-phase column and the peptides were eluted: 0–5 min, 0.01% TFA/H_2O, linear gradient (0.2 mL/min) to 70% acetonitrile/0.008% TFA after 35 min, maintained at 70% acetonitrile/0.008% TFA for 20 min.

Peptide Sequencing. The mutant W424-F460-F495 cleavage peptide I345-W424 was prepared for sequencing by collection directly from HPLC, followed by removal of acetonitrile under vacuum. Peptides were prepared for solid-phase microsequencing as described by Matsudaira (1987): peptide mixtures were resolved by SDS–PAGE [20% acrylamide and 1.6% bis(acrylamide) as resolving gel; 5% acrylamide and 0.5% bis(acrylamide) as stacking gel; 6 M urea in both], with sodium thioglycolate (10 mM final concentration) in the upper buffer, and then electrophoretically transferred (0.3 A, 1 h) to PVDF paper (Immobilon P, Millipore) with a Bio-Rad minigel transfer system (buffer: 10 mM CAPS, pH 11.0, with 20% methanol), the appropriate bands were excised, and NH_2 termini were sequenced in an Applied Biosystems Model 470A gas-phase microsequencer with a 120A HPLC separation system.

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* To whom correspondence should be addressed.

[1] Abbreviations: BNPS-skatole, 2-[(2-nitrophenyl)sulfenyl]-3-methyl-3-bromoindolenine; CAPS, 3-(cyclohexylamino)-1-propanesulfonic acid; Δψ, membrane potential; DMG, dimethyl glutarate; HPLC, high-performance liquid chromatography; [^{125}I]INA, 5-[^{125}I]-iodonaphthalene 1-azide; PVDF, poly(vinyl difluoride); SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; TFA, trifluoroacetic acid; TFD, 3-(trifluoromethyl)-3-[*m*-(formylamino)-phenyl]diazirine; [^{125}I]TID, 3-(trifluoromethyl)-3-(*m*-[^{125}I]iodophenyl)-diazirine; μ, hydrophobic moment.

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Calibration of UV Light Source and Photolysis of the Lipophilic Photoaffinity Probes TID and INA (Brunner & Semenza, 1981). All manipulations involving light-sensitive compounds were conducted in a dark room under a 25-W red light. A stock solution in ethanol of 3-(trifluoromethyl)-3-[*m*-(formylamino)phenyl]diazirine (TFD), a close analogue of TID, was stored in the dark at 4 °C. The working solution [2.2 mM TFD, ϵ_M = 266 M^{-1} cm^{-1} (355 nm); Brunner et al., 1980] was irradiated with a 450-W xenon light source, heat- and UV- ($\lambda \gtrsim 315$ nm) filtered through 0.2 cm of saturated copper sulfate, and focused on a 1.0-cm cuvette at a distance of 25 cm, resulting in an incident intensity of 420 mW/cm^2. The cuvette was stirred and thermostated at 25 °C. TFD and INA (Loebstraum Laboratories, Gaithersberg, MD) were rapidly photolyzed, with $t_{1/2}$ = 30 s for disappearance of the diazirine absorption at 355 nm and $t_{1/2}$ (filter removed) = 15 s for loss of the INA azido group at 310 nm (ϵ_M = 21 400 M^{-1} cm^{-1}; Bayley & Knowles, 1980).

Photolabeling with TID and INA. Asolectin liposomes [20 mg of lipid/mL loaded with 0.1 M KCl, 2 mM $CaCl_2$, and 10 mM DMG, pH 5.0, average diameter 4000 Å (Peterson & Cramer, 1989)] were diluted 50-fold (2 mL total volume) into 100 mM choline nitrate, 0.5 mM $CaCl_2$, and 10 mM DMG, pH 4.0, buffer and equilibrated (25 °C for 1 min), and 3 μL of [^{125}I]TID (7×10^6 dpm; specific activity 10 Ci/mmol; Amersham) was added and equilibrated for 1 min. Valinomycin was added to the liposome suspension (15 nM final concentration) to generate the membrane potential ($\Delta\psi$ = −100 mV, negative inside, from 50-fold dilution into K^+-free buffer), followed immediately by the addition of 5 μg of channel peptide (5 μL, 1 mg/mL stock), after which the sample was irradiated for 60 s. In samples without a $\Delta\psi$, choline nitrate was replaced with KNO_3 or 2 μL of methanol was added without valinomycin. To dissipate the $\Delta\psi$, 50 μL of concentrated KNO_3 (100 mM final concentration) was added to the liposomes 2–3 s after the addition of peptide. From this time ("zero"), the solution was incubated with stirring for various time intervals, after each of which the photolabeling was accomplished by irradiation for 30 s.

Iodination of Surface-Exposed Tyrosine Residues. The accessible tyrosine residues of the nine in the channel domain (Yamada et al., 1982) were labeled with Na^{125}I by using the mild solid-phase oxidant Iodogen (1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril, Pierce) (Fraker & Speck, 1978). Iodogen, dissolved in $CHCl_3$ (2 mg/mL), was added to 5-mL flat-bottomed glass vials, each containing 500 μL of $CHCl_3$ and 10 μg of catalyst, and the mixture evaporated to dryness with N_2. The iodination mixture contained 2×10^8 cpm of Na^{125}I (NEN), 5 μg of peptide, 0.8 mg of asolectin, 10 mM DMG, pH 4.0, 0.5 mM $CaCl_2$, and 100 mM choline nitrate ($\Delta\psi$ = −100 mV) or 100 mM KNO_3 ($\Delta\psi$ = 0 mV) in a volume of 2.0 mL. The mixture was stirred at 25 °C for 60 s, and the reaction was terminated by transferring the solution to an Eppendorf tube containing 20 μL of 0.1 M DTT.

Extraction of [^{125}I]TID and ^{125}I-Labeled Peptide from Vesicles. The labeled protein was delipidated and quantitatively recovered from the liposomes in $CHCl_3$/MeOH (2:1 v/v) by the method of Xu et al. (1988), except that the peptide was dried under vacuum for 15–20 min to ensure removal of all residual TCA.

SDS–PAGE and Quantitation of [^{125}I]TID Covalently Attached to Peptide. The extracted dried peptide was solubilized and subjected to SDS–PAGE (Laemmli, 1970). Fifteen percent acrylamide gels with 0.8% bis(acrylamide) as cross-linker were used to separate free [^{125}I]TID from that covalently associated with the peptide. After destaining, the gels were scanned with a densitometer (ISCO Model 1312) to quantify the relative amount of protein per lane. Alternatively, when ^{3}H-labeled peptide was used, a double-label scintillation counting procedure was devised to determine the specific photolabeling (per microgram of peptide) by [^{125}I]TID or iodination (^{125}I).

Table I: Dependence of the Labeling of Colicin E1 Channel Peptide by (A) [^{125}I]TID, (B) [^{125}I]INA, and (C) Na^{125}I on Membrane Potential[a]

	membrane potential $\Delta\psi$ (mV)	relative labeling	n[d]
	(A) [^{125}I]TID		
	0	1.00 ± 0.08[b]	10
	−100	1.37 ± 0.07[b]	10
	(B) [^{125}I]INA		
	0	1.00 ± 0.05[c]	3
	−100	1.61 ± 0.21[c]	3
	(C) Na^{125}I		
membrane	0	1.00 ± 0.11[b]	3
	−100	0.64 ± 0.09[b]	3
soln (no liposomes)		1.35 ± 0.07[b]	3

[a] Peptide was labeled with the hydrophobic photoaffinity probe [^{125}I]TID (3 μCi, 10 Ci/mmol), [^{125}]INA (1 μCi, 5 Ci/mmol), or Na^{125}I (50 μCi, 17.4 Ci/mg) and 10 μg of the reagent Iodogen (Pierce) in the presence of asolectin liposomes [lipid:peptide (mol/mol) = 4×10^3] at pH 4.0 suspended in 100 mM choline nitrate, 0.5 mM $CaCl_2$, and 10 mM dimethyl glutarate buffer. [b] These data were found to be significantly different with a confidence level of p = 0.99. [c] These data were found to be significantly different with a confidence level of p = 0.95. [d] n, number of trials.

Results and Discussion

Differential Labeling of the Channel Peptide in the Presence of a Membrane Potential ($\Delta\psi$). The effect of an imposed potassium diffusion potential on insertion into the membrane bilayer of the channel-forming COOH-terminal 178-residue thermolytic peptide of colicin E1 (NH_2 terminus, Ile-345; COOH terminus, Ile-522) was determined by using three labeling reagents as probes: (a) the lipophilic photoaffinity compounds [^{125}I]TID and [^{125}I]INA that generate a reactive carbene (Brunner & Semenza, 1981; Brunner, 1989) and nitrene (Bercovici & Gitler, 1978), respectively, upon irradiation with ultraviolet light and (b) the tyrosine labeling agent Iodogen (Pierce), which labels surface-exposed tyrosines in the solution outside the liposomes.

The labeled peptide was extracted from the liposomes in $CHCl_3$/MeOH (2:1 v/v) (Xu et al., 1988). Because most of the [^{125}I]TID and [^{125}I]INA label was found in the lipid fraction, it was important to demonstrate that all of the label associated with the peptide after electrophoresis was covalently attached to the channel peptide. This was documented by the absence of labeled peptide extracted from liposomes containing the TID photoaffinity probe irradiated before addition of peptide, compared to the results when the liposomes were irradiated in the presence of peptide (not shown). The pH dependence of labeling by the TID reagent displayed the same increase at acidic pH values, with a monotonic increase of approximately 20-fold from pH 5.0 to pH 3.0 (not shown), as that previously documented for binding and activity of the colicin E1 channel peptide (Davidson et al., 1985).

All three probes showed changes in labeling of the colicin channel peptide indicative of insertion of the peptide into the membrane bilayer in response to the −100 mV potential ($\Delta\psi$). The increase in colicin peptide labeling by [^{125}I]TID and [^{125}I]INA in the presence of the $\Delta\psi$ (Table IA,B) was approximately 37% and 61%. The measured labeling changes may be smaller than the true values because the membrane

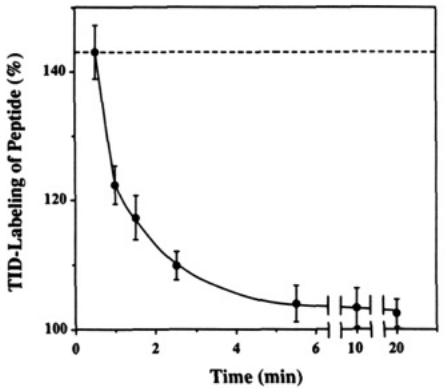

FIGURE 1: Time course for the reversibility of insertion of colicin channel peptide upon dissipation of $\Delta\psi$. Conditions were as described under Materials and Methods except that at time = 0 an aliquot of concentrated KNO_3 (100 mM final concentration) was added to dissipate the $\Delta\psi$, followed by a 30-s irradiation initiated at the different time points indicated in the graph. Values are the mean of three experiments.

potential is dissipated in ~5–10 s, the apparent half-time for formation of the channel population (Peterson & Cramer, 1987; unpublished fluorescence probe data). The labeling changes are only measurable because the $t_{1/2}$ for extrusion after dissipation of the $\Delta\psi$ is ~1.5 min (Figure 1). The iodination of the tyrosine residues of the peptide by the Iodogen reagent decreased by 26% when the peptide was bound to membrane vesicles in the absence of the $\Delta\psi$ (Table IC) compared to peptide labeling in solution. Imposition of the $\Delta\psi$ caused a further decrease of 36% in labeling of bound peptide (Table IC), ascribed to a decrease in accessibility of some or all of these Tyr residues upon insertion of a segment of the peptide into the bilayer. The membrane potential caused no detectable change in protein binding to the liposomes, as 82 ± 13% and 83 ± 6% of the channel peptide was bound to liposomes in the absence and presence of the $\Delta\psi$ (not shown).

It is possible that the increase in peptide labeling by TID and INA, and the decrease of tyrosine labeling by Iodogen, was not due to its insertion into the membrane bilayer but due to labeling at the membrane surface (Meister et al., 1985) and a change from loose to tight binding at the surface in the presence of the voltage. However, labeling at the aqueous interface is less probable because (a) TID labeling is inefficient in the presence of H_2O (Brunner & Semenza, 1981; Bayley & Knowles, 1978) and (b) the binding of the COOH-terminal 140 amino acids (I383-I522) to the membrane surface is tight enough to make this segment trypsin resistant (Xu et al., 1988) in spite of the presence of 1 Arg and 16 Lys residues.

Time Course of Decrease of Labeling upon Dissipation of $\Delta\psi$. The insertion of the channel peptide into the membrane bilayer measured with [^{125}I]TID was reversible when the $\Delta\psi$ was dissipated by adding K^+ to the external medium (Figure 1). The degree of photolabeling by [^{125}I]TID, initially 43% above the value in the absence of a potential, decreased with a half-time of approximately 1 min to the baseline value corresponding to zero potential. Although the voltage dependence of channel activity is smaller in membrane vesicles (Peterson & Cramer, 1987) compared to planar bilayers, this half-time is similar to that measured for the decay of colicin E1 channel peptide activity in planar bilayers upon reversal of the transmembrane voltage (Davidson et al., 1984; Raymond et al., 1986).

Identification of Cleavage Peptides of Trp Mutants. The increase in photoaffinity labeling and the decrease in the iodination of tyrosines could be localized by using the tryptophan

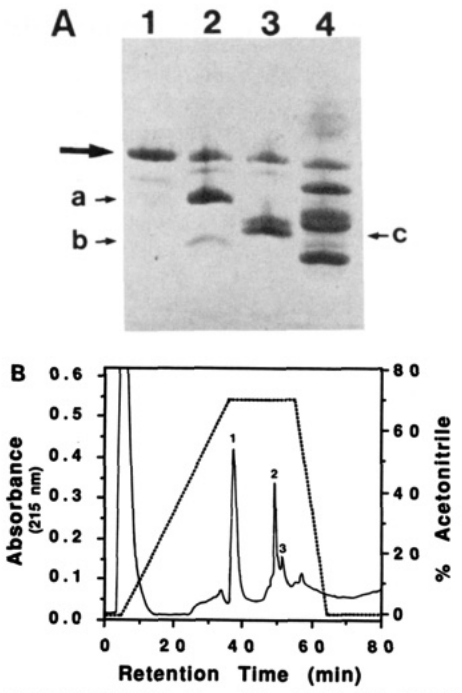

FIGURE 2: (A) SDS–PAGE of peptides obtained by BNPS-skatole cleavage of the single Trp in F424-W460-F495 and W424-F460-F495 mutants. Lanes: 1, wild-type peptide (uncleaved); 2, Trp cleavage of F424-W460-F495 mutant peptide [M_r 10000 (a) and M_r ≈3000 (b)]; 3, Trp cleavage of W424-F460-F495 mutant peptide [I345-W424, lower peptide (c)]; 4, standards (top to bottom) myoglobin polypeptide backbone (17000), myoglobin fragment I–II (14000), myoglobin fragment I (8100), myoglobin fragment II (6200), and myoglobin fragment III (2500). (B) HPLC separation of Trp cleavage digest of mutant W424 (F460-F495) peptide: absorbance (215 nm, —); % acetonitrile (---). Peak 1 was identified as the NH_2-I345-W424 cleavage peptide and was collected in a scintillation vial for determination of the extent of photolabeling ([^{125}I]TID) or iodination (^{125}I). Peak 2 is the uncleaved peptide I345-I522. Peak 3 is peptide A425-I522.

cleavage reagent BNPS-skatole (Hunziker et al., 1980) to obtain cleavage products of the extracted channel peptide after labeling ($\Delta\psi$ = 0 and −100 mV, respectively). The thermolytic channel peptide contains three Trp residues at positions 424, 460, and 495. Each Trp was changed to a Phe by site-directed mutagenesis, and two double mutants containing a single Trp at positions 424 (F460-F495) and 460 (F424-F495) were used to allow separation of the cleavage products. The cytotoxicity and in vitro channel activity of the mutants were comparable to wild type. After cleavage at the only Trp residue in the peptides W424-F460-F495 and F424-W460-F495, the digests were subjected to SDS–PAGE. The cleavage peptides migrated faster on the gels than expected, which necessitated their identification by other means (Figure 2A, lanes 2 and 3). The M_r 10000 and 3000 peptides obtained by cleavage of the mutant F424-W460-F495 were identified from their NH_2-terminal sequences, Ile-Lys-Asp-Ala-Val/His and Lys-Pro-Leu-Phe-Leu, respectively, as the NH_2-terminal peptide I345-W460 of the thermolytic channel peptide and the 62-residue peptide K461-I522-COOH. Autoradiography of the [^{35}S]Cys_{505}-labeled F424-W460-F495 mutant peptide revealed (data not shown) that the smaller peptide contained Cys_{505}, which is 17 residues from the COOH terminus (Yamada et al., 1982). Resolution of the digestion products of mutant peptide W424-F460-F495 by SDS–PAGE was not as effective because the two cleavage products migrated very similarly (Figure 2A, lane 3). Consequently, HPLC was used to separate the peptides (Figure 2B). The sequence of the first eluted and best resolved peak (peak 1) was Ile/Ala-Lys/Val-Asp/

Table II: Dependence on Membrane Potential of the Labeling of the Channel Peptide from Two Colicin E1 Mutants Containing a Single Tryptophan [(1) W424-F460-F495 and (2) F424-W460-F495] by (A) [^{125}I]TID in the Membrane Bilayer and (B) Na^{125}I in Solution

		(A) [^{125}I]TID			
	$\Delta\psi$ (mV)	relative labeling			n
(1) F424-W460-F495		uncleaved[a]	I345-W460[b]	K461-I522[b]	7
	0	1.00 ± 0.07	1.00 ± 0.08	1.00 ± 0.11	
	−100	1.30 ± 0.08	1.57 ± 0.10	0.92 ± 0.12	
(2) W424-F460-F495		uncleaved[a]	I345-W424		3
	0	1.00 ± 0.09	1.00 ± 0.06		
	−100	1.34 ± 0.08	1.06 ± 0.13		
		(B) Na^{125}I			
	$\Delta\psi$ (mV)	relative labeling			n
(1) F424-W460-F495		uncleaved[b]	I345-W460[b]	K461-I522[c]	4
membrane	0	1.00 ± 0.07	1.00 ± 0.06	1.00 ± 0.09	
	−100	0.76 ± 0.04	0.62 ± 0.05	1.14 ± 0.12	
soln (no liposomes)		1.46 ± 0.10	1.88 ± 0.09	1.07 ± 0.08	
(2) W424-F460-F495		uncleaved[d]	I345-W424[e]		3
membrane	0	1.00 ± 0.07	1.00 ± 0.12		
	−100	0.65 ± 0.07	0.95 ± 0.15		
soln (no liposomes)		1.80 ± 0.12	2.16 ± 0.44		

[a] Uncleaved refers to intact peptide that was not fragmented during the tryptophan cleavage reaction. This was typically 20–25% of the total peptide subjected to cleavage. [b] The data in these columns were found to be significantly different with a confidence level of $p = 0.99$ for n trials. [c] The data are not significantly different: confidence level $p = 0.70$–0.80. [d] $p = 0.98$ indicates that these differences are significant. [e] $p = 0.95$ indicates that these differences are significant.

Asp-Ala/Ala-Val/Thr. This ragged amino terminus has been observed for the wild-type thermolytic peptide (Merrill et al., 1990). Collection of fractions after HPLC separation of the Trp digest of the [^{35}S]Cys-labeled mutant W424-F460-F495 revealed that peak 1 had no label (I345-W424) and that peaks 2 (uncleaved peptide I345-I522) and 3 (COOH-terminal peptide A425-I522) contained the Cys_{505} label. Peak 3 could not be further separated from peak 2, and thus labeling changes were not measured in the peptide A425-I522.

Localization of the Voltage-Dependent Labeling Change in the Channel Peptide. Three peptide fragments were isolated from the two double mutants after labeling and extraction from liposomes: (i) The COOH-terminal peptide Lys-461–Ile-522-COOH is the most hydrophobic segment. In contrast to the uncleaved channel peptide measured in the same experiment (1.30 ± 0.08 vs 1.00 ± 0.07), it showed no increase in labeling by the TID reagent (0.92 ± 0.12 vs 1.00 ± 0.11) in the presence of $\Delta\psi = -100$ mV (Table IIA). There was also no significant decrease in labeling of the two tyrosine residues (Tyr-478 and Tyr-507) located in the hydrophobic peptide by Iodogen upon imposition of the $\Delta\psi$ (Table IIB). The small decrease in labeling of the membrane-bound K461-I522 peptide relative to the peptide in solution is consistent with these two Tyr residues being buried in the soluble peptide to the same extent as in the membrane. (ii) The peptide NH_2-Ile-345–Trp-424 also did not show an increase in labeling by TID (1.06 ± 0.13 vs 1.00 ± 0.06) or a decrease in iodination (0.95 ± 0.65 vs 1.00 ± 0.12) in the presence of the $\Delta\psi$ (Table IIA). The iodination of the five Tyr residues in this peptide decreased by a factor of 2 upon binding to the membrane (Table IIB). (iii) The peptide NH_2-Ile-345–Trp-460 was the only one of the peptide fragments that was labeled to a much larger extent (1.57 ± 0.10 vs 1.00 ± 0.08) by TID and to a significantly smaller extent by Iodogen (0.62 ± 0.05 vs 1.00 ± 0.06) in the presence of the potential. As observed for the 345–424 peptide, the labeling of the seven Tyr residues in the I345-W460 peptide fragment by Iodogen decreased substantially (1.00 ± 0.06 vs 1.88 ± 0.09) upon binding to the membrane in the absence of the $\Delta\psi$.

The differential labeling by TID and Iodogen of the three fragments of the 178-residue COOH-terminal channel domain of colicin E1 localizes the voltage-responsive region of the channel domain to the region centered around the segment Ala-425 → Trp-460 whose sequence is AKHLDQFAK-YLKITGHVSFGYDVVSDILKIKDTGDW. This segment contains 5 Lys and 2 His residues as 7, or 8 (including Lys-461), positive charges at low pH and consists of two potentially amphipathic membrane-spanning helical segments, Lys-420–Thr-438 ($\mu_{max} = 0.54$, periodicity 110°) and Asp-446–Lys-461 ($\mu_{max} = 0.42$, 117°), calculated according to Shiver et al. (1989). The voltage-dependent translocation inferred by Raymond et al. (1986) was thought to occur outside the channel domain, and its relation to the voltage gating of channel conductance was not clear. However, Ala-425–Trp-460 is part of the channel domain. The voltage-dependent translocation of this peptide segment is therefore most easily interpreted as part of the voltage-gating mechanism of the channel.

Channel Model. Figure 3 presents a hypothesis for the effect of membrane potential on insertion of the Ala-425–Trp-460 segment into the bilayer. (i) One main feature is a helical conformation based not only on measurements of the channel peptide in solution (Brunden et al., 1984; Parker et al., 1989) but also on infrared spectra of the peptide in the membrane (Rath et al., 1990). (ii) Another is an even number of helices with both NH_2 and COOH termini on the cis side, because of the necessity of connecting to the remainder of the colicin E1 molecule. (iii) The absence of $\Delta\psi$-dependent labeling of the 461–522 peptide in liposomes is consistent with the latter segment containing a hydrophobic helical hairpin anchor extending from approximately Ala-471 to Ile-508 whose insertion into the membrane, with the COOH terminus on the cis side (Cleveland et al., 1983; Xu et al., 1988), occurs in the absence of $\Delta\psi$ (Raymond et al., 1986; Parker et al., 1989; Song & Cramer, 1990). (iv) In the absence of $\Delta\psi$, the last 140 residues of the channel peptide are bound closely to the membrane surface as indicated by the absence of an accessible protease site downstream of Lys-382 (Xu et al., 1988) in spite of the presence of 17 basic residues. A large net (+) charge is a consequence of the neutralization of the negative

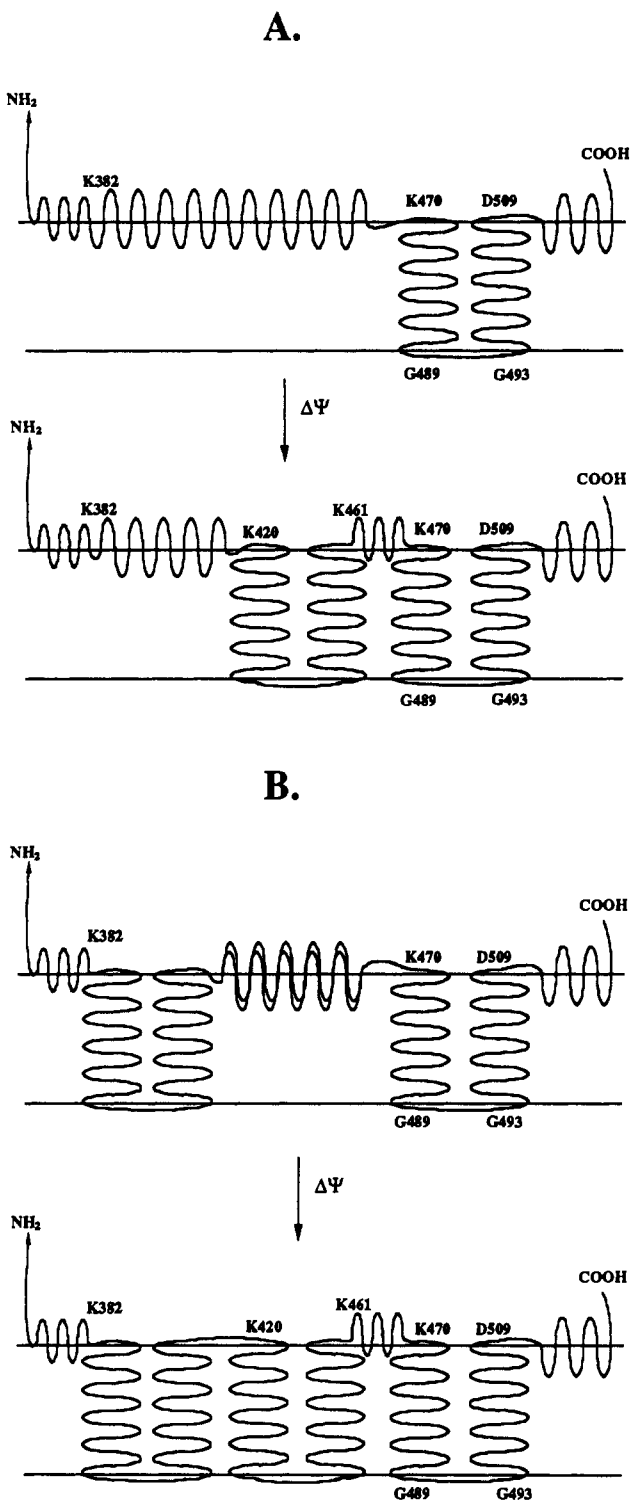

FIGURE 3: Depiction of potential-dependent insertion of the Ala-425–Trp-460 segment into the membrane bilayer as an α-helical hairpin in a four- (A) or six- (B) helix model. When the peptide is bound to vesicles without $\Delta\psi$, K382 is the tryptic cleavage site closest to the COOH terminus.

charges at pH 4.0. The ability of the membrane potential to provide energy for translocation of part of the channel peptide into the bilayer presumably arises from the action of the potential on particular positively charged basic residues or segments and, in the second order, possibly on the dipole moment of α-helical segments of the peptide. (v) The labeling change of 35–60% would be consistent with insertion of one additional helical hairpin segment, including Ala-425–Trp-460, upon imposition of the $\Delta\psi$ in the presence of two such hairpins previously formed in the absence of a potential (Figure 3B). These data would favor a model consisting of a total of six membrane-spanning helices. However, a four-helix model (Figure 3A) would be simpler and would include only segments for which there is evidence for a membrane-spanning or -inserted structure. Four helices with no membrane-spanning segments extending further upstream than residues 425–430 would also be consistent with the proposal of Liu et al. (1986) for a channel as short as 94 residues from the COOH terminus. However, it would be difficult for such a short channel polypeptide to include a large lumen (Raymond et al., 1985) if the channel is monomeric, as seems likely (Cramer et al., 1990). In addition, a significant amount of label ($\sim$25% of the total in the presence of $\Delta\psi$) was found in the 345–424 peptide (data not shown). Thus, a tentative model is drawn with a helical hairpin upstream of the 420–460 region whose boundaries are not precisely specified. This upstream hairpin is proposed to be mostly inserted in the membrane in the absence of a potential (Figure 3B), but the data of Raymond et al. (1986) suggest that it may also insert more deeply into the membrane in response to a $\Delta\psi$. (vi) This model takes into account the existence of a major cleavage site (trypsin, clostripain) at K382, accessible from the cis side only, and a significant degree of amphipathicity (μ = 0.40) in the segment 385–403 (Shiver et al., 1989). (vii) The channel is made of three pairs of helical hairpins. Each membrane-spanning helix would include one of the short helical spans (helices 3–4, 5–6, and 8–9) determined in the soluble channel peptide of the similar colicin A (Parker et al., 1989). It is noted that the resulting transmembrane helices shown in Figure 3 and the transmembrane hydrophobic hairpin of colicin A (Parker et al., 1989) that is analogous to the hairpin of residues 471–508 (Song & Cramer, 1990) in Figure 3 are somewhat short (15–18 residues) for membrane-spanning α-helices. However, the shorter of the two voltage-sensitive amphipathic segments (D446-K461) may not include all of the transmembrane helix, as the peptide segment that inserts into the bilayer in the presence of the $\Delta\psi$ may be somewhat longer than defined by the tryptophan cleavage sites.

Other Implications. The ability of the membrane potential to drive the translocation of a specific peptide segment of the colicin E1 channel peptide mass into the bilayer provides greater insight into the process of voltage gating of such channels. The role of potential-dependent protein translocation in the gating process suggests that there may be some mechanistic overlap with processes of protein import and secretion.

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