Dependence of the Conformation of a Colicin E1 Channel-forming Peptide on Acidic pH and Solvent Polarity*

(Received for publication, February 21, 1984)

Kurt R. Brunden†, Yoshihiko Uratani§, and William A. Cramer¶
From the Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907

The secondary structure content of the COOH-terminal tryptic peptide of colicin E1 has been measured by analysis of UV circular dichroism spectra as a function of pH in aqueous medium and in the presence of the nonionic detergents octyl glucoside and Triton X-100. The α-helical content of the peptide increased by approximately 10% from 45–47% to 56–57%, in the presence of the nonionic detergents, but not in aqueous medium, as the pH was decreased from 4.5 to 3.5. This pH dependence of conformation is similar to that reported elsewhere for the in vitro activity and binding of this peptide. A smaller increase in helical content was observed for the peptide in aqueous medium or in Triton X-100 as the pH was decreased from 6.5 to 4.5. The letter change in helical content was not seen in octyl glucoside which was present at a detergent concentration of 100 times that of Triton. The mean residue ellipticity measured at 222 nm for peptide added to asolectin vesicles by a freeze-thaw treatment was slightly larger at pH 3.5, and substantially larger at pH 4.5, than found at these pH values in the detergent solutions. Changes in helical content at the former, but not the latter pH, could be attributed to peptide insertion.

It appears that protonation of one or more acidic amino acid residues in the COOH-terminal region of the molecule causes a conformational change that can be attributed to an extra helical domain that is stabilized in nonpolar environments. From these results, the pH dependence of the conformational change and in vitro binding and activity, it is inferred that interaction of this domain with the membrane is essential for binding and insertion.

Colicin E1 is one of a group of colicins that de-energizes the Escherichia coli cell by depolarizing the inner membrane (1–8). Studies of the properties of these colicins in artificial planar membranes (9–11), as well as lipid vesicles (3, 12, 13), have led to the conclusion that depolarization occurs as a result of formation by the colicin of a nonspecific ion channel in the inner membrane. It is agreed that colicin E1 allows relatively free passage of small ions at a rate sufficiently rapid (~10^2 ions/s/channel in 1.0 M salt, see Ref. 7) that the cellular membrane potential is readily dissipated.

An acidic pH (pH < 6) is required for detectable in vitro activity of these colicins added to artificial membrane vesicles (13). A qualitatively similar pH requirement has also been observed in in vitro studies of the activity of colicin A (14), diphtheria toxin (15–17), tetanus toxin (18), and fusion to membranes of enveloped animal viruses (19–21). In the case of the toxins and animal viruses, it has been proposed that the requirement of acidic pH may indicate that the mechanism of uptake involves passage through an acidic organelle compartment (15–21). This mechanism is unlikely to apply to the colicins (7, 8). To understand the mechanism of insertion into membranes of toxins, viral proteins, and colicins, more information is needed on the effect of low pH on these proteins. Studies on the mechanism of channel formation by colicin E1 have been aided by determination of its complete nucleotide sequence (22) and the isolation of channel-containing COOH-terminal peptide fragments of the colicin which have membrane-depolarizing activities (3, 4, 10, 11) and single channel conductances similar to that of the parental molecule (10, 11).

A property shared by colicin E1, its COOH-terminal channel-forming peptides and the toxin proteins, is the ability to change from a water-soluble protein to one that can insert into membranes. The events that trigger this conformational change are not clear. It is, therefore, of interest to compare the secondary structure of the colicin E1 channel-forming domain in aqueous and detergent-containing media and, because of the strong dependence of activity on acidic pH (13), to make this comparison as a function of pH.

MATERIALS AND METHODS

Preparation of Colicin E1—Purified colicin E1 was prepared from strain JC411 (Col E1) by the procedure of Cleveland et al. (11), except for the use of 0.5 μg/ml of mitomycin C for induction. The purified colicin was dialyzed against 100 mM potassium phosphate, pH 7.4, and stored at 4 °C.

Preparation of the COOH-terminal Tryptic Peptide of Colicin E1—Colicin E1 (~15 mg) was digested with a 1:250 molar ratio of trypsin to colicin and purified by gel permeation chromatography as previously described (3). The purity of the tryptic peptide preparations was examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Preparation of Aqueous Solutions of COOH-terminal Tryptic Peptide for Circular Dichroism—Purified tryptic peptide was dialyzed at least 12 h against the following buffers for use in circular dichroism measurements: 10 mM formate, 0.1 M KCl, for pH values of 3.0, 3.5, 4.5; 10 mM acetate, 0.1 M KCl, for pH 6.5. The final protein concentration of the dialyzed samples used for circular dichroism measurements in aqueous medium was 0.20–0.35 mg/ml.

Preparation of Detergent Solutions of Tryptic Peptide—Tryptic peptide dialyzed against the aqueous buffers listed above was diluted with these same buffers containing either 1.0% octyl glucoside (prepared by Drs. W. Baehr and W. Widger, Purdue University) or 0.02% Triton X-100 (Sigma, T-6878), such that the final concentration of peptide was typically 0.15 mg/ml. These detergent concentrations are both close to their respective critical micelle concentrations (23).
TABLE I

<table>
<thead>
<tr>
<th>pH</th>
<th>Aqueous Avg</th>
<th>Octyl glucoside Avg</th>
<th>Triton X-100 Avg</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>3.5</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>4.5</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>6.5</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
</tbody>
</table>

Preparation of Sonicated Asolectin Vesicles—Asolectin (Associated Concentrates, Woodsise, Long Island, NY) was purified by the method of Kagawa and Racker (24) and suspended in the buffers used for dialysis of the COOH-terminal peptide at the appropriate pH values, as noted in the figure legends, at a final concentration of 20 mg/ml of asolectin. The asolectin was then sonicated to clarity using a bath-type Branson 12 sonicator.

Preparation of Freeze-Thaw Vesicles Containing Tryptic Peptide—Dialyzed tryptic peptide was added to a suspension of sonicated vesicles that had been diluted to 2 mg/ml with the appropriate buffer, such that the final protein concentration was typically 0.10 mg/ml. This suspension was allowed to incubate for 15 min at room temperature, frozen in ethanol-dry ice, thawed, and sonicated for 15 s. This freeze-thaw procedure (25) was repeated a second time prior to the circular dichroism measurements.

Binding of Tryptic Peptide to Freeze-Thaw Vesicles—Vesicles were prepared by the above freeze-thaw method in 10 mM acetate, 0.1 M KC1, pH 6.5. 30-μl aliquots of these vesicles were mixed with 2 ml of the following buffers containing 35 μg of H-peptide fragment (26): 10 mM acetate, 0.1 M KC1, pH 6.5, or 10 mM formate, 0.1 M KC1, for pH 3.5 and 4.5. These mixtures were subjected to two freeze-thaw steps as described above, and 0.5-ml samples were filtered (0.1 μm, Millipore type VC). The filters were dried and dissolved in scintillation fluid for counting, and background activity due to nonspecific binding of labeled peptide to the filters was subtracted from the total activity of the sample.

Circular Dichroism Measurements—All measurements were made with a Cary 60 spectropolarimeter equipped with a Cary 6002 circular dichroism attachment that had been calibrated with 4-10-camphorsulfonic acid (27). Measurements were done at room temperature (25 °C) with a 1.0-mm path length cell, a sensitivity setting of 0.1 °C full scale, and a time constant of 1 s. The dynode voltage of the spectropolarimeter was not allowed to exceed 0.5 kV. All spectra were corrected for the system response of the instrument by employing blank samples containing all of the assay components except the tryptic peptide. The mean residue molecular weight used for calculation of mean residue ellipticity of the tryptic peptide was corrected for this factor. Protein concentrations determined with this extinction coefficient were, within experimental error, identical to those obtained by the method of Lowry et al. (31).

RESULTS

Effect of pH on the Circular Dichroism Spectra of the Tryptic Peptide Measured in Aqueous Solution—The circular dichroism spectra of the COOH-terminal tryptic fragment of colicin E1 were examined in aqueous solution at pH values of 3.5, 4.5, and 6.5. As can be seen in Fig. 1, there was a shift to larger negative ellipticities in the measured spectral range as the pH of the aqueous medium was lowered from 6.5 to 4.5. As the pH was lowered further to 3.5, little change in the shape or amplitude of the spectra was observed (Fig. 1). The α-helical content, determined by the method of Provencher and Glockner (28), also increased by a small amount as the pH was lowered from 6.5 to 4.5. At pH 6.5, the secondary structure content of the peptide was found to be 41% α-helix, 16% β-sheet, and 43% remainder and changed somewhat at pH 4.5 to 47% α-helix, 9% β-sheet, and 44% remainder. The calculated change in secondary structure is consistent with the change in the spectra as the pH is decreased from 6.5 to 4.5, since larger negative ellipticities at 208 and 222 nm generally indicate a larger α-helical content (32). The secondary structure content at pH 3.5 was found to be 43% α-helix, 16% β-sheet, and 41% random coil. Although the average α-helical content at pH 3.5 is slightly less than that at 4.5, this may not be significant since the variability in α-helical content about the mean is ±1.3% for measurements in aqueous solution of colicin E1 COOH-terminal peptide fragment.

Preparation of Freeze-Thaw Vesicles—Vesicles were prepared by the above freeze-thaw method in 10 mM acetate, 0.1 M KC1, pH 6.5. 30-μl aliquots of these vesicles were mixed with 2 ml of the following buffers containing 35 μg of H-peptide fragment (26): 10 mM acetate, 0.1 M KC1, pH 6.5, or 10 mM formate, 0.1 M KC1, for pH 3.5 and 4.5. These mixtures were subjected to two freeze-thaw steps as described above, and 0.5-ml samples were filtered (0.1 μm, Millipore type VC). The filters were dried and dissolved in scintillation fluid for counting, and background activity due to nonspecific binding of labeled peptide to the filters was subtracted from the total activity of the sample.

Circular Dichroism Measurements—All measurements were made with a Cary 60 spectropolarimeter equipped with a Cary 6002 circular dichroism attachment that had been calibrated with 4-10-camphorsulfonic acid (27). Measurements were done at room temperature (25 °C) with a 1.0-mm path length cell, a sensitivity setting of 0.1 °C full scale, and a time constant of 1 s. The dynode voltage of the spectropolarimeter was not allowed to exceed 0.5 kV. All spectra were corrected for the system response of the instrument by employing blank samples containing all of the assay components except the tryptic peptide. The mean residue molecular weight used for calculation of mean residue ellipticity of the tryptic peptide, calculated from the nucleotide and amino acid sequence of Yama
du et al. (22). The secondary structure content of the peptide fragment was analyzed by the revised procedure of Provencher and Glockner (28), employing data points at 1-nm intervals and not discriminating between remainder and β-turn in the secondary structure analysis. All spectra shown are the average of two or more individual spectra obtained from separate COOH-terminal fragment preparations. The numerical values of α-helical and secondary structure content cited in the text were averages of the values calculated from the spectra of two or more individual peptide preparations, as seen in Table I. The α-helical content of these individual preparations is also summarized in Table I.

Protein Determination—The concentration of the tryptic peptide fragment was determined spectrophotometrically, using a value of ε_{280} = 3.06 × 10^{4} for the molar extinction coefficient of the fragment. This extinction coefficient was determined using the molar extinction coefficients of tryptophan and tyrosyl residues (29) and the known content of these residues in the fragment (3, 22). The extinction coefficient calculated for the colicin E1 molecule was within 6–7% of that previously measured (30). The extinction coefficient actually used for calculation of mean residue ellipticity of the tryptic peptide was corrected by this factor. Protein concentrations determined with this extinction coefficient were, within experimental error, identical to those obtained by the method of Lowry et al. (31).

FIG. 1. Circular dichroism spectra of the COOH-terminal tryptic fragment of colicin E1 in aqueous solution. Aqueous solutions of tryptic peptide were prepared as described under "Materials and Methods." The spectra shown, and secondary structure contents, are: pH 6.5, α-helix = 41%, β-sheet = 16%, remainder = 43% (-----); pH 4.5, α-helix = 47%, β-sheet = 9%, remainder = 44% (-----); pH 3.5, α-helix = 43%, β-sheet = 16%, remainder = 41% (-----).
pH-dependent Conformation of Colicin E1 Peptide

It should also be noted that slight differences in the secondary structure content calculated at any pH result (Ref. 33, data not shown) from use of other methods of spectral analysis (e.g. Ref. 34). The Provencher and Glockner method (28) was chosen over that of Chen et al. (34) because the theoretical spectra generated by the former method fit the observed spectra more closely. The nature of the fit to data obtained with the latter method can be seen in Ref. 35.

Effect of Detergents on the Circular Dichroism Spectra of the Tryptic Peptide—The nonionic detergent, octyl glucoside, has been employed as a replacement for lipid bilayers in determining the conformation of integral membrane proteins (36,37). Detergent/protein solutions offer an advantage over membrane vesicle/protein systems in that the light scattering and absorption flattening problems often observed in the latter preparations are avoided. In addition, background absorbance due to lipid at ultraviolet wavelengths below 210 nm is absent.

The addition of octyl glucoside at pH 4.5 caused only small changes in the spectra of the tryptic peptide compared to that measured in the absence of detergent at this pH (Fig. 2A). The helical content in both the aqueous and detergent environments at pH 4.5 was 47%. The calculated secondary structure changed only with respect to the content of β-sheet. The calculated β-sheet content, which is known to have a larger uncertainty than that of α-helix (32,38), indicated an increase from aqueous medium to octyl glucoside of 9 to 29%. In contrast to the situation at pH 4.5, the addition of octyl glucoside at pH 3.5 caused a large change in the amplitude of the CD spectra (Fig. 2B). Relative to the spectrum obtained at pH 3.5 in aqueous solution, the amplitude of the mean residue ellipticity increased by approximately 2000 degrees-cm²/dmol at both 222 and 208 nm. The average α-helical content increased from 43 to 57%, an increase of 14% for the average of two measurements in aqueous medium compared to that of five obtained in octyl glucoside. The comparison of the α-helical content at pH 3.5 in aqueous medium with each of the five measurements in octyl glucoside shows a positive change in the detergent in all cases, with the increase ranging from 7 to 21%. The α-helical content of the COOH-terminal fragment determined in octyl glucoside was also approximately 10% larger at pH 3.5 (57%) than at pH 4.5 (47%) and pH 6.5 (46%). The increase in α-helical content in the three preparations for which CD spectra were measured at both pH 3.5 and 4.5 was 6, 10, and 20% (Table I). This increase in α-helical content in octyl glucoside solutions at low pH can be seen directly by comparison of the individual spectra obtained at the different pH values (Fig. 3). It is of interest to compare this effect of acidic pH on the conformation of the tryptic peptide with changes in its activity and binding. The α-helical content of the fragment in 1% octyl glucoside is plotted as a function of pH in Fig. 4, along with the pH dependence of channel-forming activity in membrane vesicles measured previously (13). The helical content did not increase until the pH was decreased below 4.5, and the rate of change of helical content seemed to decrease near pH 3.0. The functional dependence of activity on pH was similar to that of the prepared as described under "Materials and Methods." The spectra shown, and calculated secondary structure contents, are: aqueous, pH 4.5, α-helix = 47%, β-sheet = 9%, remainder = 44% (--.--), with octyl glucoside, pH 4.5, α-helix = 47%, β-sheet = 29%, remainder = 24% (--.--). β, tryptic peptide spectra at pH 3.5. The spectra shown are: aqueous, pH 3.5, α-helix = 43%, β-sheet = 16%, remainder = 41% (--.--); with octyl glucoside, pH 3.5, α-helix = 57%, β-sheet = 16%, remainder = 27% (--.--).
pH-dependent Conformation of Colicin E1 Peptide

Fig. 3. Circular dichroism spectra of the tryptic peptide in octyl glucoside (1%). Octyl glucoside solutions of peptide were prepared as described under "Materials and Methods." The spectra shown, and calculated secondary structure contents, are: pH 6.5, α-helix = 46%, β-sheet = 20%, remainder = 34% (--.--.--); pH 4.5, α-helix = 47%, β-sheet = 29%, remainder = 24% (--.--.--); pH 3.5, α-helix = 57%, β-sheet = 16%, remainder = 27% (--.--.--).

Fig. 4. α-Helical content of the COOH-terminal fragment in octyl glucoside and tryptic peptide activity as a function of pH. The average α-helical content listed in Table I for the peptide in octyl glucoside is plotted as a function of pH. ○, average α-helical content (per cent); O, previously reported ionophoretic activity (13).

Conformational change (Fig. 4), as is the pH dependence of binding (26).

Since octyl glucoside caused a definite change in the circular dichroism spectra of the tryptic fragment at pH 3.5, it was of interest to determine whether a similar change occurred in the presence of another nonionic detergent, Triton X-100. This detergent has been used in circular dichroism studies of bacteriorhodopsin, where it was noted that a relatively low concentration of Triton must be used because of its strong UV absorbance (35) and to avoid exceeding the critical micelle concentration (23). For this reason, the amount of Triton used in the present work was much smaller (molar ratio of detergent:fragment = 40:1) than that of octyl glucoside (4500:1). The helical content (Fig. 5 and Table I) and mean residue ellipticity (Table II) showed a marked increase at pH 3.5 in the presence of Triton X-100 (Fig. 5 and Table I) relative to that obtained in the absence of detergent (Fig. 1 and Table I) and was similar to the change described above for octyl glucoside. The α-helical content increased from 45% at pH 4.5 to 56% at pH 3.5 in the presence of Triton (Table I), an average increase of 11% resulting from 7, 9, and 15%

<table>
<thead>
<tr>
<th>pH</th>
<th>Aqueous</th>
<th>Octyl glucoside</th>
<th>Triton X-100</th>
<th>Vesicles</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td>-13,000</td>
<td>-15,800</td>
<td>-15,700</td>
<td>-13,900</td>
</tr>
<tr>
<td>4.5</td>
<td>-12,500</td>
<td>-12,900</td>
<td>-13,200</td>
<td>-12,200</td>
</tr>
<tr>
<td>6.5</td>
<td>-11,200</td>
<td>-13,500</td>
<td>-11,300</td>
<td>-13,400</td>
</tr>
</tbody>
</table>
two pH values, were similar. This would be expected if the peptide in detergent solutions indicated that the \(\alpha\)-helical tryptic peptide did not bind Triton strongly at pH values >4, as was found with diphtheria (17) and tetanus toxin (18), and thus behaved at these higher pH values as though it was in aqueous medium. The absence of change in helical content in the presence of octyl glucoside between pH 6.5 and 4.5 was probably a consequence of octyl glucoside binding to the peptide at the 100-fold higher detergent:peptide ratio used here relative to Triton.

Circular Dichroism Properties of Tryptic Peptide Incorporated into Asolectin Vesicles—The data obtained with the peptide in detergent solutions indicated that the \(\alpha\)-helical content increased markedly as the fragment encountered a low pH and a hydrophobic environment. Although a detergent can serve as a faithful replacement of the lipid bilayer in eliciting characteristic conformations of membrane proteins (35, 37), it is often preferable to examine the correlation between spectra obtained in the presence of detergent and in membrane vesicles. As previously mentioned, the interpretation of CD spectra of proteins incorporated into artificial vesicles is often difficult (e.g. Ref. 39). To avoid possible artifacts of this nature, spectra of the tryptic peptide in vesicles were measured to a wavelength of 210 nm, and the mean residue ellipticity at the 222-nm trough was determined. The possibility of scattering artifacts at 222 nm was examined by measuring the mean residue ellipticity of the tryptic peptide at this wavelength as a function of phospholipid concentration (Fig. 6). The amplitude was approximately constant until the phospholipid concentration was decreased below 0.5 mg/ml, indicating that light scattering due to the presence of vesicles was not a problem.

The mean residue ellipticity at pH 3.5 of the peptide incorporated into vesicles, measured at 222 nm, appeared to be similar, although slightly larger, than that obtained in detergent solutions at this pH. Using the mean residue ellipticities at 222 nm to obtain the approximate \(\alpha\)-helical content of the detergent-solubilized and membrane-inserted peptide at pH 3.5 (e.g. Ref. 40), it was found that the \(\alpha\)-helical contents differ by <4%.

\[^3\]The abbreviation used is: MES, 2-(N-morpholino)ethanesulfonic acid.

**Table III**

<table>
<thead>
<tr>
<th>pH</th>
<th>Membrane-bound fragment (cpm)</th>
<th>Relative binding*</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td>3550</td>
<td>100</td>
</tr>
<tr>
<td>4.5</td>
<td>777</td>
<td>22</td>
</tr>
<tr>
<td>6.5</td>
<td>665</td>
<td>19</td>
</tr>
</tbody>
</table>

* Relative binding is normalized to that at pH 3.5. The absolute binding of the peptide at pH 3.5 was at least 42% of added peptide.

The vesicle preparation showed a much more negative ellipticity at pH 4.5 than either of the detergent preparations at this pH, although the binding of tryptic peptide added directly to vesicles (26) or inserted by freeze-thaw (Table III) is much less at pH 4.5 than at 3.5. Thus, the larger ellipticity at 222 nm of the peptide added to vesicles at pH 4.5 may have been a consequence of a weak peripheral association, but was not due to insertion of the peptide into the vesicle membrane.

**DISCUSSION**

The pH dependence of activity and binding has recently been determined for colicin E1 and the COOH-terminal tryptic peptide (13, 26). This peptide shows an apparent pK for binding and insertion of about 3.8-4.0 (26), as well as maximum ionophoretic activity below pH 4.0 (13). The circular dichroism data shown above suggest that these pH-dependent functions may occur concomitantly with a conformational change in the channel-forming domain of the molecule.

Addition of the detergents octyl glucoside or Triton X-100 to the peptide resulted in an increase in \(\alpha\)-helix of approximately 10% as the pH was lowered from 4.5 to 3.5. This increase in helical content presumably occurred as a consequence of protonation of 1 or more acidic residues that allowed or generated structural changes of the peptide in a hydrophobic environment. As can be seen in Fig. 4, the \(\alpha\)-helical content of the tryptic peptide in octyl glucoside has a functional dependence on pH similar to the titration of activity (13) as a function of pH and is also similar to the pH titration of peptide binding (26).

The average \(\alpha\)-helical content determined at pH values of 3.5 and 4.5 in the presence of Triton X-100 is similar within 1–2% to that found at the respective pH values with octyl glucoside. This observation seems to eliminate the possibility of artificial conformation changes due to interaction with a particular detergent. It should be noted that both detergents used were nonionic, thus eliminating possible effects of detergent protonation and ionization with the tryptic peptide.

When the mean residue ellipticity at 222 nm of the COOH-terminal peptide incorporated into vesicles was compared to that of the peptide in detergent (Table II), the ellipticities at pH 3.5 were found to be similar, although the vesicle system showed a slightly more negative value. At pH 4.5, the fragment added to membranes by freeze-thaw treatment had a much more negative ellipticity value than in either of the detergents. The binding studies showed that the increase in ellipticity was not due to irreversible binding and insertion of the fragment into the membrane at pH 4.5 (Table III), but rather must have been a result of a loosely associated surface-bound species since it was readily washed off the vesicles at this pH. The conformational change that occurred in the absence of detergent between pH values 6.5 and 4.5 (Fig. 1) may allow this loose surface association to occur at the latter pH.

Recently, models of the colicin E1 channel have been proposed (8, 11, 41) that contain predictions of the conforma-
tional state of the channel-forming region, as well as the amino acid sequences of the respective conformational domains (8, 11, 41). The secondary structure content predicted by circular dichroism studies of the tryptic peptide in nonionic detergents will allow further evaluation of these models. The tryptic peptide showed averages of approximately 55-60% α-helix and 5-16% β-sheet in detergent at pH 3.5, which is close to the pH for maximum ionophoretic activity (13). The α-helical content increases by ~10% in the hydrophobic environment of the detergents as the pH is decreased from 4.5 to 3.5. This 10% increase would correspond to 19 residues of the COOH-terminal fragment that contains 187 residues based on comparison of its NH2-terminal sequence with that of the parental molecule and its mobility (Mf = 20,000) on gels (3). Such a sequence of 19 residues corresponds to approximately what is needed to form one membrane-spanning α-helix, as it takes ~20 residues to span a bilayer in an α-helical conformation (42, 43). This change in the α-helical content within the tryptic peptide requires protonation between pH 4.5 and 3.5 of at least 1 acidic amino acid residue. The possibility that glutamic acid 468 in the colicin sequence is one such residue has been discussed recently (8). Protonation of Glu 468 in the hydrophobic face of an amphipathic helix, proposed to be part of the colicin channel, would allow insertion of the helix into the membrane due to a lowering of the free energy of insertion. In addition, the prediction of 55–60% α-helix at pH 3.5 in a detergent environment indicates the involvement of 103–113 residues in α-helical structures. 95–100 residues were required to form five trans-membrane amphipathic helices proposed to be involved in formation of the colicin channel (8).

Finally, it should be noted that the general idea of the requirement of a pH ≤4–5 for binding of the diphtheria and tetanus toxins to membranes (17, 18) and membrane fusion activity of enveloped animal viruses (19–21) also has been proposed to involve conformational changes and exposure of hydrophobic groups (17–20), perhaps triggered by protonation of particular acidic residues (20). In these cases, the physiological significance of the low pH is proposed to arise from the acidic organelle compartments proposed to be involved in the uptake mechanism. It is unlikely that any cellular compartment as acidic as pH 4.0 is involved in the uptake mechanism of colicin (7, 8). The significance of the in vitro pH titration of colicin and its tryptic fragment is that it provides evidence for protonation of acidic residues as an essential step in the binding and insertion that precedes channel formation. The effective pK of the critical amino acid(s) when colicin inserts into the cell envelope in vivo must either be shifted to much higher values or, alternatively, the fraction of active molecules in the colicin population exposed to a periphasmic pH of 6–7 would have to be small. The acidic pH requirement may, in fact, provide an explanation for the well known observation that the fraction of active colicin E1 molecules in apparently pure preparations is low (7).

Acknowledgements—We would like to thank E. Bjes for skilled technical assistance, S. W. Provencher for providing access to his CD analysis programs, P. Argos, L. J. Bishop and V. L. Davidson for important discussions, and Lucy Winchester for preparation of this manuscript.

REFERENCES