Characterization of Colicin Ia and Colicin Ib

PURIFICATION AND SOME PHYSICAL PROPERTIES*

(Received for publication, January 21, 1970)

JORDAN KONISKY† AND FREDERIC M. RICHARDS

From the Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06520

SUMMARY

Colicins Ia and Ib have been purified from mitomycin C-induced *Escherichia coli* strains W3110 str-r (col Ia) and W3110 str-r (col Ib) by ammonium sulfate precipitation, treatment with diethylaminoethylcellulose, and chromatography on DEAE-Sephadex, phosphocellulose, and hydroxylapatite. Sedimentation velocity and equilibrium centrifugation as well as polyacrylamide gel electrophoresis at several pH values indicated that both colicin preparations were homogeneous. The molecular weight of colicin Ia was determined from its $s_{20,w}$ (3.57 S) and $D_{20,w}$ (4.12 $\times$ 10$^{-7}$ cm$^2$ sec$^{-1}$) as well as from sedimentation equilibrium centrifugation and dodecyl sulfate polyacrylamide gel electrophoresis to be approximately 77,600. The molecular weight of colicin Ib was approximately 79,000 ($s_{20,w}$ = 3.60 S; $D_{20,w}$ = 4.32 $\times$ 10$^{-7}$ cm$^2$ sec$^{-1}$). Colicins Ia and Ib had $f/f_0$ values of 1.82 and 1.76, respectively.

Colicins are bactericidal substances produced by various *Escherichia coli* strains and active against other related strains. The capacity to produce colicins resides in an extrachromosomal genetic element called the colicinogenic factor which can be induced to initiate colicin synthesis by such agents as mitomycin C and ultraviolet light. Some isolated colicins have been shown to be simple proteins with molecular weights ranging from 55,000 to 75,000 (1–3).

Three distinct specificities are found in colicin molecules: mode of action, receptor specificity, and immunity specificity. Treatment of sensitive cells with a colicin leads to specific metabolic changes in the challenged cell which may be different depending on the particular colicin (4). Colicins adsorb to specific receptors on the surface of *E. coli* and can be grouped according to receptor specificity (5). Cells which have the capacity to produce a particular colicin are immune to the lethal effect of the colicin of the same type that they produce (6) but sensitive to heterologous types. Thus, colicin molecules have immunity specificity in that the challenged colicinogenic cell is able to distinguish between homologous and heterologous colicins. Immunity is not caused by failure of adsorption; immune cells retain receptors for adsorption (7) and, in fact, adsorb the homologous colicin (8, 9).

Colicins of the I group consist of two classes, Ia and Ib. Both of these colicins not only adsorb to the same specific cell receptor, but superficially at least have a common mode of action, inhibition of all macromolecular synthesis (9). The two differ, however, in immunity specificity; cells able to produce colicin Ia are immune to low concentrations of Ia but not to Ib and vice versa (10). Thus these two colicins afford us an ideal system in which to correlate the structure of colicin molecules with their biological specificities. It is reasonable to suppose that colicins Ia and Ib have common structural regions with regard to mode of action and receptor specificity, yet different structural regions involved in immunity specificity. Thus comparative studies of the physical and chemical properties of these molecules may lead to information concerning the structural features involved in the immunity mechanism. The present paper describes the purification of colicins Ia and Ib as well as some of their physical properties.

EXPERIMENTAL PROCEDURE

Bacterial Strains and Media—*Escherichia coli* K-12 strains W3110 str-r, W3110 str-r (col Ia-CA53), W3110 str-r (col Ib-P9), and W3110 str-r (col Ib-P9) 1 r were obtained from M. Nomura. The col Ia-CA53 and col Ib-P9 factors were introduced into W3110 str-r from *E. coli* K-12 AB1193 (col Ia-CA53) and *Salmonella typhimurium* cys D36 (col Ib-P9), respectively. TB (tryptone, Difco) medium and Tris medium were prepared as described previously (8) with the exception that Tris medium contained 0.15% Casamino acids and 0.15% glucose. Nutrient soft agar and nutrient agar plates contained TB medium plus 0.7 and 1.3% agar (Difco), respectively. Buffer PBM, a 0.05 M potassium phosphate buffer containing 0.01 M $\beta$-mercaptoethanol and adjusted to the indicated pH at room temperature with KOH.

Reagents—Mitomycin C was obtained from Calbiochem. Reagents used for preparation of polyacrylamide gels were purchased from Eastman Kodak. Amido black and bromphenol blue were obtained from Hartman-Leddon, Philadelphia, Pennsylvania. Coomassie brilliant blue and sodium deoxycholate were obtained from Sigma. Sodium dodecyl sulfate, $\beta$-mercaptoethanol, and EDTA were from Matheson Coleman and Bell. Propylene glycol monolaurate was obtained from K and E Rare and Fine Chemicals. Ammonium sulfate (special...
enzyme grade) and guanidine hydrochloride (ultra pure) were purchased from Mann.

DEAE-Sephadex A50 was obtained from Pharmacia. DEAE (DE52) and phosphocellulose (P11) were obtained from Whatman. Hydroxylapatite was obtained from Bio-Rad, Richmond, California.

Bovine serum albumin (Fraction V) was purchased from Nutritional Biochemicals. Lysosyme, DNase I, myoglobin, pepsin, and ovalbumin were obtained from Worthington.

Colicin Assay and Protein Determination—During purification colicin activity was determined by serially diluting the colicin solution with PBM (pH 7) and spotting a drop of each dilution on a nutrient agar plate spread with 2.5 ml of nutrient soft agar containing 0.2 ml of a chilled overnight culture of strain W3110 str-r grown in Tris medium. The plates were incubated for 5 hours at 37°C. The number of colicin units per strain W3110 str-r grown in Tris medium. The plates were incubated for 5 hours at 37°C. The number of colicin units per ml was defined as the highest dilution giving a clear zone of inhibition of growth of the overnight culture. This assay is semiquantitative.

Protein concentration was determined in the purification steps by the method of Lowry et al. (11), with bovine serum albumin as the standard. The protein concentrations of solutions of purified colicin were determined from their extinction coefficients, which were obtained by determining the total weight of amino acids present in a protein solution of known optical density. The content of the various amino acids was determined by amino acid analysis.1 By this method aqueous solutions of colicin Ia and Ib containing 1 mg per ml of protein have an optimal density of 1.03 and 1.09 at 260 μm in a 1-cm cell.

Electrophoresis—Polyacrylamide gel electrophoresis at pH 4.5 was performed with the buffer system and gel compositions described by Reisfeld, Lewis, and Williams (12). Disc gel electrophoresis at pH 6.6 was carried out exactly as described in the manual supplied by Canal Industrial Corporation, Bethesda, Maryland. The gels (0.5 × 8 cm) were run at room temperature under the conditions described in the legend to Fig. 3, stained for 1 hour with a 1% solution of Amido black in methanol-water-acetic acid (6:5:1), and destained at room temperature by diffusion into 7% acetic acid.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis was carried out as described by Shapiro, Vineria, and Maizel (13). Lyophilized samples (100 μg) were dissolved in 160 μl of 0.01 M sodium phosphate buffer (pH 7.2) containing 1% SDS and 1% β-mercaptoethanol. After incubation at 37°C for 3 hours and overnight dialysis against 0.1 M sodium phosphate (pH 7.2) containing 0.1% SDS, 0.1% β-mercaptoethanol, 160 μl of a 20% sucrose solution and 4 μl of a 0.1 M solution of bromophenol blue, all in the dialysis buffer were added. This mixture, 200 μl, was layered over an SDS acrylamide gel (0.5 × 10 cm) prepared as described by Shapiro et al. (13). Electrophoresis was carried out at room temperature in 0.1 M sodium phosphate buffer (pH 7.2) containing 0.1% SDS. Other conditions of electrophoresis are described in the legend to Fig. 3. When the bromophenol blue had migrated to the bottom of the gel, the gels were removed, fixed in 20% sulfosalicyclic acid for 16 hours, stained with 0.25% Coomassie brilliant blue in water for 4 hours, and destained by diffusion into 7% acetic acid. For molecular weight determinations the distance of the stained zone from the origin was measured.

1 J. Konisky and F. M. Richards, in preparation.

2 The abbreviation used is: SDS, sodium dodecyl sulfate.

Ultracentrifugal Analysis—Analytical sedimentations were performed in a Spinco model E ultracentrifuge with schlieren optics and Eastman Kodak metallographic plates to photograph the patterns, which were measured with a Nikon 6C profile projector.

RESULTS

Purification of Colicin Ia and Ib

Growth and Induction of Colicinogenic Strains—A 2-liter overnight culture of either strain W3110 str-r (col Ia-CA53) or W3110 str-r (col Ib-P9) grown in TB medium at 37°C was inoculated into 100 liters of sterile TB medium in a New Brunswick model F-130 100-liter Ferracel. Two milliliters of propylene glycol monolaurate were added as antifoam. The culture was grown at 37°C with vigorous aeration with the fermentor agitation control set at 200 rpm. When the culture density reached 3 to 4 × 10^8 cells per ml, 0.2 μg per ml of mitomycin C was added, and incubation was continued for another 4 hours after which time the Ferracel temperature control was set to 10°C. The Ferracel exit port was next attached to two refrigerated Sharples models AS-16 centrifuges, and the cells harvested by forcing the culture into the centrifuge bowls by positive pressure. The cell pellet was stored overnight at 4°C in the centrifuge bowls.

Extraction of Colicin from Cell Pellet—Preliminary experiments (see below) showed that the crude extract prepared from the cell pellet (see below) from each of the induced strains contained greater than 95% of the total measurable colicin activity (crude extract plus growth medium). The growth medium of strain W3110 str-r (col Ib-P9) 1-r which lacks the colicin I receptor contained about 10 to 20% of the total measurable colicin activity. Conversion of induced cells to spheroplasts by lysozyme-EDTA treatment failed to release significant amounts of colicin into the medium.

Neither I-type colicin could be extracted quantitatively from the cell pellet by repeated washing with PBM (pH 7) solutions containing either 1 M NaCl, 0.5 M guanidine-HCl, 0.5% SDS, 0.75% sodium deoxycholate, or when saturated with chloroform. To extract colicin I, the cell pellet from the 100-liter culture was suspended in 200 ml of PBM (pH 7), containing 0.025 M MgCl₂, and the cells were disrupted by passage through a French pressure cell at 5000 p.s.i. DNase (5 μg per ml) was next added, and the extract was stirred at 37°C for 45 min to reduce the viscosity. The extract was next centrifuged at 18,000 rpm in a Spino No. 20 rotor, and the supernatant, containing 85 to 95% of the total colicin activity, was saved.

Ammonium Sulfate Fractionation—All subsequent steps were carried out at 4°C. To the supernatant from the last step was added solid ammonium sulfate (114 g per liter of solution). The solution was stirred for 1 hour and then centrifuged at 18,000 rpm for 20 min. Ammonium sulfate (193 g per liter of solution) was added to the supernatant and after stirring and centrifugation as above the precipitate was dissolved in 200 ml of PBM (pH 7) and dialyzed overnight against 20 liters of the same buffer. This fraction contained 80 to 100% of the total colicin activity found in the cell extract.

DEAE Treatment—The ammonium sulfate fractions (300 to 1000 ml) were stirred into suspensions (100 g per liter) of diethylaminoethyl cellulose previously equilibrated with PBM (pH 7). Ten grams of DEAE were used per g of protein. After stirring for 90 min the mixtures were filtered (grade 617 paper,
Table I

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Protein (mg)</th>
<th>Colicin (units x 10^-6)</th>
<th>Colicin (specific activity)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colicin Ia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I. Crude extract</td>
<td>6.4 x 10^4</td>
<td>5.0</td>
<td>79</td>
<td>100</td>
</tr>
<tr>
<td>II. Ammonium sulfate</td>
<td>4.9 x 10^4</td>
<td>6.3</td>
<td>127</td>
<td>126</td>
</tr>
<tr>
<td>III. DEAE (batch)</td>
<td>945</td>
<td>1.5</td>
<td>2,807</td>
<td>30</td>
</tr>
<tr>
<td>IV. DEAE-Sephadex</td>
<td>217</td>
<td>1.6</td>
<td>7,350</td>
<td>32</td>
</tr>
<tr>
<td>V. Phosphocellulose</td>
<td>96</td>
<td>1.2</td>
<td>12,200</td>
<td>24</td>
</tr>
<tr>
<td>VI. Hydroxylapatite</td>
<td>79</td>
<td>0.7</td>
<td>10,000</td>
<td>14</td>
</tr>
<tr>
<td>Colicin Ib</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I. Crude extract</td>
<td>6.8 x 10^4</td>
<td>4.8</td>
<td>71</td>
<td>100</td>
</tr>
<tr>
<td>II. Ammonium sulfate</td>
<td>4.2 x 10^4</td>
<td>4.0</td>
<td>95</td>
<td>85</td>
</tr>
<tr>
<td>III. DEAE (batch)</td>
<td>1.2 x 10^4</td>
<td>5.9</td>
<td>490</td>
<td>122</td>
</tr>
<tr>
<td>IV. DEAE-Sephadex</td>
<td>204</td>
<td>1.6</td>
<td>7,800</td>
<td>33</td>
</tr>
<tr>
<td>V. Phosphocellulose</td>
<td>66</td>
<td>0.9</td>
<td>13,600</td>
<td>19</td>
</tr>
<tr>
<td>VI. Hydroxylapatite</td>
<td>60</td>
<td>0.7</td>
<td>12,000</td>
<td>15</td>
</tr>
</tbody>
</table>

Fig. 1. DEAE-Sephadex chromatography of colicin Ib (Fraction II).

Eaton-Dikemann Company) on a Buchner funnel under negative pressure. Ammonium sulfate (472 g per liter) was added to the filtrates, and the precipitates were collected by centrifugation, dissolved in 40 to 50 ml of PBM (pH 7), and dialyzed against 10 liters of the same buffer.

DEAE-Sephadex Chromatography—The samples from the previous step were applied to individual DEAE-Sephadex A50 columns (5 x 115 cm) previously equilibrated with PBM (pH 7). The flow rates were maintained at 40 ml per hour by means of a Beckman model 746 solution metering pump. The columns were washed with PBM (pH 7), and fractions were assayed for absorbance at 280 m\(\mu\) and colicin activity. A fraction (30 to 60%) of the total amount of colicin applied did not adsorb to the column (see below). Tubes containing unadsorbed colicin of highest activity were pooled.

Phosphocellulose Chromatography—The pooled samples from the previous step were applied directly to individual DEAE-Sephadex A50 columns (5 x 115 cm) previously equilibrated with PBM (pH 7). The flow rates were maintained at 25 ml per hour. The columns were washed with the above buffer until the effluent was free of unadsorbed protein. The adsorbed protein was then eluted by a continuous phosphate gradient (total volume, 1.5 liters) between 0.05 M phosphate PBM (pH 6.8) and 0.5 M phosphate buffer (pH 6.8) containing 0.01 M mercaptoethanol. Both colicins eluted at between 0.2 and 0.25 M phosphate (Fig. 2). The peak fractions were pooled, dialyzed extensively against distilled water and lyophilized.

Comments on Purification

The results of the purification are shown in Table I. We routinely obtained a final specific activity which was 80- to 150-fold higher than that found in the original cell extract. When assayed for carbohydrate content by the indole-sulfuric acid method of Dische (14) both colicins showed less than 5 \(\mu\)g of carbohydrate per mg of protein. Both colicins contain less than 3 \(\mu\)g of hexosamine per mg of protein as assayed on the amino acid analyzer. The ultraviolet absorption spectra of both colicins in PBM (pH 7.0) had the characteristics found for proteins containing tryptophane and tyrosine and both colicins had an \(A_{max}\) at 278 m\(\mu\). \(A_{max}/A_{280}\) for colicins Ia and Ib are 1.74 and 1.79, respectively.
FIG. 3. Polyacrylamide disc (A, B, C, and D) and SDS polyacrylamide (E, F) gel electrophoresis of colicins Ia and Ib. A, and B, electrophoresis in pH 4.5 gels, (current 5 ma per gel, 34 hours, origin at top, cathode at bottom). A, colicin Ia, 75 µg of protein. B, colicin Ib, 80 µg of protein. C and D, electrophoresis at pH 6.6 (current 5 ma per gel, 4 hours, origin at top, cathode at bottom). C, colicin Ia, 50 µg of protein. D, Colicin Ib, 55 µg of protein. E and F, electrophoresis at pH 7.0 (current 6 ma per gel, 3 hours, origin at top, anode at bottom). Colicin Ia, 35 µg of protein. Colicin Ib, 45 µg of protein.

FIG. 4. Determination of the diffusion coefficient of colicin Ia. The conditions of centrifugation are described in the text. The height (H) and area (A) of the single boundary represented in the schlieren photographs were determined as a function of time.

Early attempts at purification were hampered by the instability of the colicins during the early steps in the procedure which were carried out in dilute buffer (0.01 M phosphate, pH 7.0). Although addition of mercaptoethanol (0.01 M) afforded some protection against inactivation, increasing the buffer concentration to 0.05 M prevented any significant loss in activity. After lyophilization the salt-free purified preparations showed no significant loss in activity when stored at -30° for several months.

Confirming earlier studies (9) we have found that crude preparations of colicins Ia and Ib (Fraction II of the preparation) exhibit two peaks of activity on DEAE-Sephadex chromatography (Fig. 1). Whereas about 50% of the total activity found in the crude extract does not adsorb to the column, a second peak of activity can be eluted by 0.3 M NaCl. When the colicin activity from each peak was pooled and rerun under the same conditions, each activity peak of colicin chromatographed in a single peak in a manner identical with the parent peak. Since previous studies on the biological properties of colicins Ia and Ib were limited to the unadsorbed colicins (9), we have confined our studies to purification and characterization of these unadsorbed activities.

Homogeneity of Preparations

Fig. 2 shows the chromatography of colicins Ia and Ib from Step V of the purification procedure on hydroxylapatite columns, the last step of the purification procedure. In both cases the single peak of colicin activity coincides with the single protein peak. The recovery of activity from this step varies from 80 to 100% and results in no significant change in specific activity (see Table I).

Acrylamide gel electrophoresis at pH 4.5 and 6.6 (Fig. 3, A, B, C, and D) shows a single staining zone which migrated toward the cathode. When both preparations were initially incubated with 1% SDS and 1% β-mercaptoethanol and electrophoresis carried out in acrylamide gels containing 0.1% SDS at pH 7.0, both migrated as a single zone toward the anode (Fig. 3, E, and F).

The schlieren pattern obtained during the centrifugation of Fraction VI under conditions described below for the determination of the sedimentation coefficient by analytical ultracentrifugation showed a single symmetrical peak indicating the existence of only a single sedimenting component in the purified preparation.
The sedimentation coefficient of purified colicin Ia (Fraction VI) in 3.7 M KC1 was calculated for both colicins. In the second method the peak of colicin was determined by killing activity. An osm value of 0.2 ml/g for colicin Ia concentration (15), through a linear sucrose gradient and calculation of the osm value for the particular colicin from the relative peak positions after 24 hours of centrifugation at 36,000 rpm in a Spinco SW39 rotor according to the method of Martin and Ames (16). The position of the single obtained for colicin Ib showed no significant concentration dependence at protein concentrations ranging from 1 to 7 mg per ml.

Sedimentation and Diffusion Coefficients

The sedimentation coefficient of both colicins was determined by two methods. The first involved cosedimentation of colicin Ia or Ib (Fraction IV of the purification procedure) with rabbit hemoglobin, osm value 4.31 (15), through a linear sucrose gradient and calculation of the osm value for the particular colicin from the relative peak positions after 24 hours of centrifugation at 36,000 rpm in a Spinco SW39 rotor according to the method of Martin and Ames (16). The position of the single peak of colicin was determined by killing activity. An osm value of 3.7 was calculated for both colicins. In the second method the sedimentation coefficient of purified colicin Ia (Fraction VI) in 0.25 M PBM (pH 6.8) and Ib in 0.05 M PBM (pH 6.8) containing 0.1 M KCl was determined by sedimentation at 59,100 rpm at 20° in a 4°, 12-mm Kel-F cell in the Spinco model E analytical ultracentrifuge. Corrected osm values of 3.57 and 3.67 S were obtained for colicin Ia and Ib, respectively. Although there was a dependence of osm on colicin Ia concentration (8) was calculated to be 0.2 dl per g, where  = (1 - kc), osm obtained for colicin Ib showed no significant dependence at protein concentrations ranging from 1 to 7 mg per ml.

The diffusion coefficient of both colicins (Fraction VI) in 0.25 M PBM (pH 6.8) was calculated from the spreading of the schlieren boundary at 8000 rpm in a 2°, 12-mm filled Epon double sector synthetic boundary cell as a function of time (17). The small dependence of osm on concentration of the colicins, the low speed of centrifugation, and the short time of the run (40 min) made corrections for the sharpening of the boundary caused by dependence of osm on concentration unnecessary (18). The method and the data of a typical experiment are shown in Fig. 4. The corrected osm values of 4.19 X 10^-7 and 4.32 X 10^-7 cm^2 sec^-1 were calculated for colicins Ia and Ib, respectively.

Molecular Weight Determinations

From a combination of sedimentation and diffusion coefficients the molecular weights of colicins Ia and Ib were calculated from the Svedberg equation (19) to be 78,500 and 78,100, respectively. The partial specific volume  (computed (20) from the amino acid composition of the colicins) were calculated to be 0.733 and 0.736 ml per g for colicins Ia and Ib, respectively.

Centrifugation of colicin Ia at 24,000 rpm and Ib at 22,000 rpm in 0.25 M PBM (pH 6.8) at 20° to sedimentation equilibrium in a six-channel cell yields distributions (Fig. 5) in which the protein concentration at the meniscus is zero. From a determination of d(ln c)/dc(+) where r (in centimeters) is the distance from the axis of rotation and c is the protein concentration at r, we have calculated (10) molecular weights of 75,000 ± 600 and 80,500 ± 500 for colicin Ia and Ib, respectively. The linearity of the plots in the case of colicin Ia attests to the homogeneity of the preparation. The slight upward curvature seen toward the bottom of the cell in the case of colicin Ib may indicate either aggregation of the colicin at high protein concentration or may be caused by the presence of a high molecular weight minor contaminant.

We also determined the molecular weight of both colicins by the method of SDS polyacrylamide gel electrophoresis in which the desired molecular weights can be obtained from determination of the distance of migration of the colicins and comparison to a plot of the relative migration of proteins of known molecular weight against the log of their respective molecular weights (13, 21). The data for an experiment in which the colicins were run in gels together with the standard proteins is summarized in Fig. 6. By this method we obtained a molecular weight of 79,000 ± 1400 for colicin Ia and 80,000 ± 1400 for colicin Ib.

Frictional Coefficient

Table II summarizes the results of the physical studies which were carried out with the purified colicins. The average fri-
tional coefficient, $f_\text{s}$ calculated from sedimentation and diffusion data is $9.50 \times 10^{-8} \pm 0.20$ g sec$^{-2}$ for colicin Ia and $9.55 \times 10^{-8} \pm 0.17$ g sec$^{-2}$ for colicin Ib. These values correspond to an $f/f_\text{s}$ of 1.82 for colicin Ia and 1.76 for colicin Ib, where $f_\text{s}$ is the frictional coefficient of an unhydrated sphere of molecular weight equal to that of the particular colicin, based on equilibrium centrifugation, and of their respective partial specific volumes. Assuming ellipsoidal shapes and either one of two extreme degrees of hydration for the colicins, they can be considered as either unhydrated prolate ellipsoids of axial ratio 16 and 15, oblate ellipsoids of axial ratio 21 and 20 (25), or hydrated spheres binding 3.7 and 3.3 g of water per g of colicin Ia and Ib, respectively (26).

**DISCUSSION**

The common biological properties of colicins Ia and Ib suggest that both colicins have structural similarities. This has been verified by several criteria. Both colicins have been purified in comparable yields by a common purification scheme. Both colicins exhibit identical chromatographic behavior on DEAE-Sephadex, phosphocellulose, and hydroxylapatite as well as identical electrophoretic behavior in acrylamide gels at pH 4.5 and 6.6. Physical data obtained for both colicins indicate that both molecules are of similar size and shape (see below). Preliminary amino acid analysis suggests that both colicins have similar yet nonidentical primary structures. Comparative chemical studies of colicins Ia and Ib are in progress to elucidate the chemical basis for the immunity specificities found in these molecules. Isolation of mutant colicinogenic strains producing colicins with altered immunity specificities should prove to be a useful approach.

The molecular weight of both colicins obtained from sedimentation equilibrium centrifugation, diffusion and sedimentation velocity data, and SDS acrylamide gel electrophoresis are in good agreement. Average values of 77,600 and 79,600 were obtained for colicins Ia and Ib, respectively. It should be noted that these values are somewhat higher than the previous estimate of 50,000 for partially purified colicins Ia and Ib (9), which assumed a roughly spherical shape for the two colicins. Since a large number of subunit proteins are dissociated in the presence of SDS, the fact that nearly identical molecular weights were obtained by analytical centrifugal methods and SDS acrylamide gel electrophoresis argues against a subunit structure for the two colicins. Since previous treatment with β-mercaptoethanol did not affect the mobility of either colicin on SDS acrylamide gel electrophoresis both seem to be composed of a single polypeptide chain.

The frictional coefficients of both colicins as calculated from either sedimentation or diffusion data are unusually high for proteins of this molecular weight (20). The average $f/f_\text{s}$ values of 1.82 for colicin Ia and 1.76 for colicin Ib correspond to axial ratios of 16 and 15 for unhydrated prolate ellipsoid-shaped molecules and 21 and 20 for oblate ellipsoids. Although the experiments yield no information concerning the degree of hydration of the purified colicins, it is unlikely that the high frictional coefficients found for these molecules can be explained entirely on the basis of bound water. Further physical and electron microscopic studies would be helpful in this regard. The $f/f_\text{s}$ values determined for colicins Ia and Ib can be compared to those found for other well characterized colicins. From the published physical data (1, 3) we have calculated an $f/f_\text{s}$ of 1.41 for colicins E2 and E3, and 1.75 for colicin E1 (9 assumed to be 0.725 ml per g). These results together with our own suggest that a high axial ratio may be a general feature of colicin molecules.

An elongated shape for colicin molecules leads to interesting speculations concerning the interaction of colicins with the bacterial surface. A prolate- or oblate-shaped molecule with a molecular weight of 80,000 and $\theta$ of 0.733 ml per g with an $f/f_\text{s}$ of 1.80 lying with its long axis parallel to the bacterial surface could be in contact with surface structures as much as 150 to 350 A apart. Studies on colicins have suggested that the bacterial surface can be considered as a structural mosaic consisting of several specific functional units (27) each of which can play a role in the functioning of various metabolic processes. It is possible to imagine that a single colicin could influence several metabolic systems, for example DNA, RNA, protein synthesis, oxidative phosphorylation, etc., by interacting with one or several of these functional units along its longitudinal axis. In this regard it is interesting that of those colicins which have been physically well characterized, colicins E1, Ia, and Ib which inhibit all macromolecular synthesis may have axial ratios as great as 15 to 20, while colicins E2 and E3 which specifically affect DNA and protein synthesis, respectively, have axial ratios which may be as high as 7 to 9. However, physical studies of other bacteriocins are necessary before any generalization can be drawn concerning correlations between the shapes and modes of action of bacteriocins.

Alternatively, the simultaneous shutoff of several metabolic systems by some colicins could derive from the direct interaction of the colicins with a single functional unit, the state of which could indirectly influence many metabolic systems by affecting a common component of each. Such a unitary hypothesis has been invoked to explain the action of several colicins. Present knowledge concerning the mechanism of colicin action has been recently reviewed (27).

The fact that trypsin reverses the biochemical effects induced by certain colicins (9, 28) together with evidence that radioactive colicin remains adsorbed to the cell envelope during the killing process (8) suggest that in their killing action colicins remain adsorbed to the cell surface. With pure colicin in hand we are at a stage where we can directly study the interaction of colicins with the surface receptor. The isolation and characterization of these receptors and a study of their interaction with colicin may prove to be necessary before the mechanism of colicin action can be fully understood.

**Acknowledgments**—We wish to thank Dr. M. Nomura, University of Wisconsin, for the bacterial strains. We are grateful to Mr. H. M. Steinman and Dr. R. C. Williams, Jr. for technical assistance and discussion concerning the ultracentrifugal studies. The expert technical assistance of Mr. Johnnie L. Moung is gratefully acknowledged.

**REFERENCES**

Characterization of Colicin Ia and Colicin Ib: PURIFICATION AND SOME PHYSICAL PROPERTIES
Jordan Konisky and Frederic M. Richards


Access the most updated version of this article at http://www.jbc.org/content/245/11/2972

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/245/11/2972.full.html#ref-list-1