Purification and Properties of Colicin E3 Immunity Protein*

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SUMMARY
A protein which inhibits the in vitro effects of colicin E3 (E3) was purified from cells colicinogenic for E3 by chromatography on DEAE-Sephadex and Sephadex G-75. This "immunity protein" could be isolated both from uninduced and mitomycin C-induced colicinogenic cells, but not from cells lacking the Col E3 factor. It is a highly acidic protein of molecular weight about 10,000. At concentrations approximately equimolar to that of the colicin, it completely prevents colicin E3 from inhibiting in vitro protein synthesis. Even in vast molar excess, however, it has no effect on the in vivo killing of sensitive cells by the colicin.

Experiments to investigate the effect of immunity protein on inhibition of in vitro protein synthesis by colicin E3 are consistent with a model in which immunity protein interacts with the colicin to prevent its destruction of ribosomes. Preliminary direct binding experiments have failed to reveal interaction of immunity protein either with colicin or with ribosomes. However, highly purified colicin contains a component which co-migrates with immunity protein on sodium dodecyl sulfate-acrylamide-urea gels.

Colicin E3 is a protein antibiotic produced by certain strains of coliform bacteria (1). It inhibits protein synthesis in sensitive bacteria by causing cleavage of a small fragment from the 3' end of the 16 S RNA of the 30 S ribosomal subunit (2, 3). This cleavage occurs both in vivo and in vitro (4, 5). The in vitro cleavage requires the presence of the 50 S ribosomal subunit (6, 7). The action of the colicin in vivo or in vitro can be detected either by the appearance of the 3'-terminal ribosomal RNA fragment on polyacrylamide gels (2, 3) or sucrose gradients (3, 4) or by the loss of in vitro protein-synthesizing activity by E3-treated ribosomes (4, 5). Neither of these effects is seen in vitro, however, unless purified colicin preparations are used (9). Extracts prepared from E3 colicinogenic cells inhibit the in vitro activity of purified colicin, although ribosomes isolated from those extracts are susceptible to killing by those from non-colicinogenic bacteria (4, 5). The colicinogenic cells, however, are not susceptible to homologous colicin in vivo (10). These results have led to the postulation of an immunity substance which is specified by colicinogenic cells and confers upon them immunity to their colicin. It is this immunity substance, present in crude colicin preparations and in extracts from colicinogenic cells, which is believed to be responsible for the inhibition of in vitro colicin activity (4, 5).

We describe here the purification of the colicin E3 immunity protein from E3 colicinogenic cells. Its activity was studied primarily by means of its effect on the inhibition of in vitro protein synthesis by colicin E3.

MATERIALS AND METHODS

Bacterial Strains—Colicin E3 and E3 immunity protein were prepared from the colicinogenic strain W3110(E3), obtained from D. Helinski (University of California, San Diego). The E3-sensitive strain used as an indicator for in vitro E3 activity was W3110. The strain colicinogenic for E2 was E2TR2 from P. Fredericq (Université de Liège). S-30 extracts for protein synthesis were made from K56, a derivative of S26 from A. Garen (Yale University).

Colicins—Colicins E3 and E2 were purified as described by Herschman and Helinski (11) or by the modification described below for the preparation of immunity protein. Purification was sometimes carried only through the DEAE-Sephadex step.

In Vitro Colicin Assay Colicin activity was routinely assayed in vitro by spot testing dilutions on 10^6 sensitive bacteria spread in 2.5 ml of soft agar, as described by Herschman and Helinski (11). The inverse of the last dilution at which there is inhibition of indicator growth is defined as colicin activity, in units per ml.

In Vitro Protein Synthesis, Immunity Protein Assay—S-30 extracts for protein synthesis were prepared as described by Webster et al. (12). Immunity activity was assayed by in vitro protein synthesis stimulated by f2 RNA. Aliquots, generally 1 to 20 μl, of fractions to be tested for immunity activity were added to tubes containing Buffer A (50 mM Tris HCl-30 mM ammonium chloride-10 mM magnesium acetate, pH 7.6), 50 mM KCl, 3 mM ATP, 0.2 mM GTP, 10 mM phosphoenolpyruvate, 5 mM glutathione, all amino acids except lysine at 0.1 mM, and S-30 extract. Three micrograms of purified colicin E3 were added per 100- to 120-μl reaction and a 20-min preincubation at 37°C was begun. At the end of 20 min, 25 μg of f2 RNA (12) and 0.5 μCi of L-[14C]lysine (270 to 300 Ci per mole) were added and
the tubes were incubated for another 20 min at 37°. The reaction was stopped by addition of 3 ml of 5% trichloroacetic acid. The tubes were heated at 90° for 10 min, filtered on glass-fiber filters, dried, and counted in toluene-based scintillator. Fractions containing immunity activity were those which prevented the inhibition of in vitro protein synthesis by purified colicin E3. Ribosomes for use in the in vitro protein synthesis assay for colicin or immunity activity were prepared by centrifuging S-30 extracts in 0.6-ml tubes for 4 hours at 35,000 rpm in the SW 50.1 rotor. The upper two-thirds of the supematant was removed and used as S-100 for protein synthesis. The pellet was resuspended in 0.1 ml of Buffer A, debris was removed by a low speed spin, and the concentration of the ribosomes was adjusted to 600 A260 per ml in Buffer A.

Under the conditions of these assays, the amount of added mRNA was such that the amount of protein synthesis was limited by the ribosomes and not by the message concentration. The amount of protein synthesis is directly proportional to the number of active ribosomes. When colicin-treated and untreated ribosomes were mixed in varying proportions and then used for in vitro protein synthesis, the amount of synthesis was equivalent to the proportion of untreated ribosomes in the reaction. Thus, colicin-inactivated ribosomes do not interfere with synthesis by viable ribosomes.

Growth and Induction of E3 Immunity Protein—Immunity protein was purified from colicinogenic cells by a modification of the method of Herschman and Helinski for the purification of colicin E3 (11). Forty liters of W3110(E3) were grown in an American Sterilizer Biogen to a density of 5 × 10^8 per ml and then induced for 50 min with 0.4 mg per liter of mitomycin C (Sigma). Cells were harvested and colicin and immunity protein were immediately extracted from the cell surface by washing the cells twice, for 30 min per wash, in a Lourdes blender, in 250 ml of ice-cold buffer, 10 mM potassium phosphate-1 mM sodium chloride, pH 7.0. The two washes were pooled and precipitated with ammonium sulfate in the cold as described by Herschman and Helinski (11). The 20 to 40% ammonium sulfate fraction was resuspended in 40 ml of Buffer A and the 40% ammonium sulfate supernatant were dialyzed extensively against Buffer A. The ammonium sulfate pellet and supernatant were then processed by chromatography separately but in exactly the same way, as described under "Results."

Polyacrylamide Gel Electrophoresis—Ten per cent acrylamide-0.1% SDS-8 M urea cylindrical gels were made and run approximately as described by Henry and Pratt (13). Running buffer was 0.1 M sodium phosphate-0.1% SDS, pH 7.2. Fifteen per cent acrylamide-0.1% SDS-8 M urea slab gels were run on the apparatus described by Studier (14) using the modifications developed by P. Model.1 The buffers used were described by O’Farrell et al. (15).

RESULTS

Immunity Activity of Uninduced and Induced Colicinogenic Cultures—A material which inhibited the in vitro activity of colicin E3 was first detected and purified from a 100,000 × g supernatant from 40 liters of uninduced W3110(E3) grown to 5 × 10^8 per ml, harvested, and prepared as described by Webster et al. (12). This activity was purified from the 100,000 × g supernatant by DEAE-Sephadex A-50 and Sephadex G-75 chromatography as described in detail below for an induced culture. No such activity could be found in extracts from non-colicinogenic cells (results not shown). The amount of material isolated from 40 liters of uninduced cells colicinogenic for E3 was not sufficient for detailed characterization.

Forty liters of W3110(E3) were induced with mitomycin C and the cells were extracted with 1 M NaCl in phosphate buffer, pH 7.0. The cell washes were pooled and precipitated with ammonium sulfate as described under "Materials and Methods." An activity which masked or inhibited the in vitro effects of colicin E3 was detected both in the 20 to 40% ammonium sulfate cut and in the 40% supernatant. This immunity activity was then purified by chromatography.

DEAE-Sephadex and Sephadex G-75 Chromatography—Colicin and immunity activity separate clearly upon DEAE-Sephadex chromatography of ammonium sulfate precipitates. While colicin elutes from DEAE-Sephadex A-50 in the early part of a 0 to 0.5 M KCl in Buffer A gradient, immunity activity binds tightly to the column, with the bulk of the high specific activity material eluting in a 1 M salt wash. A typical elution profile is shown for the ammonium sulfate precipitate in Fig. 1. The material in the 20 to 40% ammonium sulfate cut was dialyzed into Buffer A and applied to a column (4.5 × 15 cm) of DEAE-Sephadex A-50 which had been equilibrated with Buffer A. Proteins were then eluted with a 500-ml linear gradient from Buffer A to 0.5 M KCl-Buffer A. Arrow indicates end of gradient and beginning of 1 M KCl-Buffer A step elution. O—O, A obscene; O—O, immunity activity assayed by protection of in vitro protein synthesis from inhibition by E3. Fractions under the bracket contained peak in vitro E3 activity.

![Graph](http://www.jbc.org/)

**Fig. 1.** DEAE-Sephadex chromatography of ammonium sulfate-precipitated E3 and immunity protein. Bed volume was 230 ml and 10-ml fractions were collected. Elution was with 250-ml linear gradient from Buffer A to 0.5 M KCl-Buffer A. Arrow indicates end of gradient and beginning of 1 M KCl-Buffer A step elution. O—O, A obscene; O—O, immunity activity assayed by protection of in vitro protein synthesis from inhibition by E3. Fractions under the bracket contained peak in vitro E3 activity.
were collected at a flow rate of 12 ml per hour. The immunity equilibrated and eluted with Buffer A. Two-milliliter fractions were collected. o- - o, AzsO; O- - O, immunity activity. Solid arrow indicates blue dextran marker for excluded volume; open arrow indicates [14C]lysine included marker.

the percentage of surviving protein synthesis. Three microliters of each column fraction assayed were added to a standard [14C]-lysine-f2 RNA incorporation reaction containing 5 µg of DEAE-Sephadex-purified colicin E3. A control incorporation, to which colicin but no immunity protein was added, gave 10% of the incorporation of a reaction without colicin; 100% synthesis was determined from an incorporation with no added colicin.

Whereas Fig. 1 shows an elution profile for the ammonium sulfate pellet, DEAE-Sephadex chromatography of the 40% supernatant results in elution of immunity activity under the same salt conditions.

The amount of the DEAE-Sephadex material necessary to completely block the in vitro activity of 5 µg of E3 in a 100-µl f2-directed incorporation can be extrapolated to determine the total amount of immunity activity in the pooled activity peaks. The pooled DEAE-Sephadex peak from the ammonium sulfate pellet was calculated to be sufficient to inhibit the action of 75 mg of colicin E3 in the in vitro protein synthesis reaction. The yield from the 40% ammonium sulfate supernatant at this stage of purification is sufficient to "neutralize" the effects of 150 mg of E3 when calculated in this way. By this criterion, the cell wash of the mitomycin C-induced culture yielded 50 to 100 times more immunity activity than the supernatant from an uninduced culture.

The pooled immunity activity peak (90 A260) was dialyzed into Buffer A and concentrated by reapplying it to a column (0.9 x 8 cm) of DEAE-Sephadex A-50 equilibrated with Buffer A. As soon as the whole volume of the pooled peak had been applied to the column, it was eluted in a single step with 1 M KCl-Buffer A. Virtually all of the A260 came off the column with the salt front. One- to two-milliliter fractions were collected beginning with the high salt step. The peak fractions containing the A260 were pooled, yielding 27 A260 in a total of 4 to 5 ml.

This concentrated immunity activity was further purified by Sephadex G-70 gel filtration. Fig. 2 shows an elution pattern of this material from a Sephadex G-75 column (2 x 40 cm) equilibrated and eluted with Buffer A. Two-milliliter fractions were collected at a flow rate of 12 ml per hour. The immunity activity of 5-µl aliquots of various fractions was assayed in 100-µl incorporations containing 5 µg of colicin E3. Those fractions in the A260 peak which gave complete protection against killing, by 5 µg of colicin, of in vitro protein synthesis were used as purified immunity protein for all subsequent experiments. Fractions containing maximum activity generally had an A260 of 0.2 to 1.6 and were used without further concentration.

Sephadex G-75-purified immunity protein runs as a single band on cylindrical 10% acrylamide-0.1% SDS-8 M urea phosphate gels and on 15% acrylamide-0.1% SDS-8 M urea Laemmli-type slab gels (Fig. 6).

The amino acid analysis, for which we thank Dr. Stanford Moore and Dr. Paul Fletcher (The Rockefeller University), is shown in Table I. It also indicates that the G-75 purified immunity protein is homogeneous, since integral values were obtained for individual amino acids. The amino acid analysis is consistent with a minimum molecular weight near 10,000 for immunity protein. On the slab gel shown in Fig. 6, immunity protein has a mobility very close to that of commercial pancreatic ribonuclease, molecular weight 13,700. The molecular weight indicated from the amino acid analyses is probably more correct; there are reported anomalies in molecular weight determinations for a Tris-SDS gel system similar to the one we used (17).

The molecule is usually acidic; almost one-third of its residues are aspartate or glutamate, accounting for its very strong affinity for DEAE-Sephadex.

Absence of E3-specific Immunity Protein in Cells Colicinogenic

TABLE I

| Amino acid        | Residues |
|-------------------|----------|
| Aspartic acid     | 18       |
| Threonine         | 9        |
| Serine            | 16       |
| Glutamic acid     | 10       |
| Proline           | 3        |
| Glycine           | 8        |
| Alanine           | 2        |
| Half-cystine (or cysteine) | 1 (or more) |
| Valine            | 6        |
| Methionine        | 1        |
| Isoleucine        | 3        |
| Leucine           | 6        |
| Tyrosine          | 4        |
| Phenylalanine     | 8        |
| Histidine         | 1        |
| Lysine            | 5        |
| Arginine          | 1        |

* Calculated on the basis of methionine, histidine, and arginine = 1.

* Corrected for 5% threonine, 10% serine destruction.

* The acid hydrolysates showed half-cysteine (or cysteine) to be present, but a precise determination as cysteic acid was not performed.

* On an isoelectric focusing gel run by Dr. M. Rifkind (The Rockefeller University), immunity protein had a pI of approximately 4.3.
for E2—The specificity of E3 immunity protein for cells colicinogenic for E2 was demonstrated by carrying out a parallel purification on cells colicinogenic for E2. Colicin E2 shares its cell surface receptor with E3 (1) and has very similar physical properties (11). It has no effect when added to an in vitro protein synthesis reaction (results not shown), nor does it interfere with the killing of that reaction by E3 or with the protection from E3 afforded by E3 immunity protein. E2-producing E2TR2 cells were induced with mitomycin C and the 1 M NaCl cell wash was purified by exactly the same procedure used for the purification of E3 immunity protein. At each stage of the purification, column fractions were assayed in vitro for E3 immunity activity. At no point was there any such activity detected. A homogeneous protein was isolated from E2-producing cells which has similar ionic properties and a similar molecular weight to E3 immunity protein. However, it has a slightly different amino acid composition and mobility on 15% acrylamide-SDS-urea slab gels than E3 immunity protein. This protein is thus not the same molecule as E3 immunity protein, nor does it have any effect on the inhibition of in vitro protein synthesis by colicin E3. Thus cells not carrying the Col E3 factor do not make an immunity protein in approximately stoichiometric amounts with respect to colicin is sufficient to block colicin activity in vitro.

If a titration of immunity protein like the one shown in Fig. 3 is done at two different colicin concentrations, the resulting curves are parallel, except at their upper and lower limits (not shown). A 2.5-fold increase in the amount of colicin added to the reaction requires about a 2.5-fold increase in immunity protein to maintain the same level of protein synthesis. This suggests that immunity protein is interacting in some way with the colicin to reduce its effective concentration in the in vitro reaction.

Measurement of protein synthesis at two different immunity concentrations in the presence of increasing amounts of colicin and a constant amount of ribosomes also suggests a quantitative relationship between the amount of colicin in the reaction and the amount of immunity protein required to inhibit the in vitro activity of that colicin. In Fig. 4, a 3-fold increase in immunity protein requires a corresponding 3-fold increase in colicin to reach the same extent of ribosome inactivation. Plotted on the same graph is the inactivation caused by 3 pg of colicin, with the amount of immunity protein required to inhibit the in vitro activity of that colicin. In Fig. 4, a 3-fold increase in immunity protein requires about a corresponding 3-fold increase in colicin to reach the same extent of ribosome inactivation. Plotted on the same graph is the inactivation caused by 3 µg of colicin, with the amount of immunity protein required to inhibit the in vitro activity of that colicin. In Fig. 4, a 3-fold increase in immunity protein requires a corresponding 3-fold increase in colicin to reach the same extent of ribosome inactivation. Plotted on the same graph is the inactivation caused by 3 µg of colicin, with the amount of immunity protein required to inhibit the in vitro activity of that colicin. In Fig. 4, a 3-fold increase in immunity protein requires about a corresponding 3-fold increase in colicin to reach the same extent of ribosome inactivation. Plotted on the same graph is the inactivation caused by 3 µg of colicin, with the amount of immunity protein required to inhibit the in vitro activity of that colicin.

**Stoichiometry of Immunity Protein and Colicin in in Vitro Protein Synthesis**—The potency of purified immunity preparations can be titrated to determine the minimum amount required to neutralize a given amount of colicin in vitro. Such a titration is shown in Fig. 3. Dilutions of a purified preparation of immunity protein at 800 µl per ml were added to standard 100-µl incubations containing all components except f2 RNA and [14C]lysine. Three micrograms of colicin E3 were added to each tube, the reaction was preincubated 20 min at 37°C, and then label and message were added and synthesis was allowed to proceed at 37°C for 20 min. Synthesis for each sample is shown as the percentage of a control reaction containing no added E3.
TABLE II

Effect of immunity protein on in vivo killing by colicin E3

Exponentially growing colicin-sensitive cells, 2 x 10^9 in 100 μl, were added to tubes containing dilutions of colicin E3 and purified immunity protein, where appropriate, in 50 μl of broth. The tubes were incubated for 25 min at 37°C. Dilutions were spread on agar plates and scored for surviving colonies.

| Colicin E3 | Immunity protein | Surviving cells per ml | Surviving cells % |
|-----------|-----------------|------------------------|------------------|
| 0         | 0               | 1.65 x 10^6            | 100              |
| 2.5 x 10^-3 | 0           | 2.5 x 10^6             | 1.5              |
| 2.5 x 10^-3 | 1           | 2.2 x 10^6             | 1.3              |

![Graph](https://via.placeholder.com/150)

**Fig. 5. A, in vitro inhibition of protein synthesis by E3 in presence and absence of immunity protein.** Amounts of ribosomes shown were preincubated with 3 μg of E3 for 20 min in Buffer A. O—O, no immunity protein during preincubation; ⋆—*, 0.4 μg of immunity protein during preincubation. At the end of the preincubation, excess immunity protein was added to stop colicin. S-100, f2 RNA, L-[14C]lysine, and other components for protein synthesis were added after the preincubation. Results are expressed as the percentage of control synthesis with no colicin at every ribosome concentration shown. B, the percentage of synthesis at each point in A was converted to the A_{660} per ml ribosomes inactivated by multiplying the percentage inactivated by the concentration at each point. ⋆—*, no immunity protein during preincubation; O—O, 0.4 μg of immunity protein during preincubation.

...and the colicin-immunity mixture is then incubated with colicin-sensitive cells in vitro, the immunity protein does not inhibit the colicin at all (Table II).

**Relationship of Ribosome Concentration to in Vitro Colicin Killing in Presence of Immunity Protein—**Increasing amounts of ribosomes were preincubated with 3 μg of colicin in the presence and absence of a constant amount of purified immunity protein. The amount of immunity protein in the preincubation, 0.4 μg, was set so as to give less than full protection against the colicin under the conditions of this experiment. At the end of the 20-min preincubation, S-100 extract and the other components necessary for protein synthesis were added to the ribosome mixtures, and protein synthesis was allowed to proceed for 20 min. Immunity protein (2.4 μg) sufficient to completely block further colicin action, was added with the components for protein synthesis. The results of this experiment also support a model for immunity action in which the immunity protein interacts with some form of the colicin, rather than with the ribosomes. At every ribosome concentration tested, over a 10-fold range, the ratio of synthesis in the presence of immunity protein to synthesis in its absence remained constant (Fig. 5A). If a certain number of the ribosomes were being protected by the binding of a constant amount of immunity protein, then the proportion of protected ribosomes in the reaction could be expected to decrease with increasing ribosome concentration. The upper curve in Fig. 5A would have a decreasing slope in such a case. If on the other hand, a constant amount of colicin were inactivated by the immunity protein, the immunity protein should reduce killing by colicin by a constant amount at any ribosome concentration and the two curves should be parallel. Since the curves are parallel, this experiment is further evidence of an interaction between colicin and immunity protein.

The ordinate in Fig. 5B represents the number of ribosomes inactivated in the colicin reaction, as measured by protein synthesis. The *upper line* shows ribosome inactivation by colicin in the absence of immunity protein. In the presence of immunity protein (*lower curve*), the amount of ribosomes inactivated is reduced, but not by a constant amount over the whole range of ribosome concentrations, since there is a sharp decrease in the slope of the curve at higher ribosome concentrations. If immunity protein were interacting with the ribosomes, the slope of the lower curve should increase, rather than decrease. At a high enough ribosome concentration, there would not be enough immunity protein to protect the ribosomes, so the proportion inactivated would increase. Since instead, the number of ribosomes inactivated decreases, immunity protein seems to be lowering the effective colicin concentration. At the break in the curve, colicin has probably become limiting in the ribosome inactivation reaction, and the reaction has reached its maximum velocity.

**Interaction of Immunity Protein with Colicin—**Since the data from in vitro protein synthesis experiments with colicin and immunity protein suggest that immunity protein interacts with the colicin, we have attempted to demonstrate this interaction directly. Preliminary experiments using sedimentation in sucrose gradients, gel filtration on Sephadex G-75, affinity chromatography, and antigen-antibody precipitation have failed to demonstrate any measurable interaction of immunity protein with either colicin or with ribosomes (results not shown).

There is, however, some evidence from gel electrophoresis which indicates that immunity protein may interact specifically with colicin E3. Fig. 6 shows a slab gel on which several preparations of colicin at various stages of purity were run next to a sample of purified immunity protein. In every sample of colicin, there is a band which runs at the same position as purified immunity protein. Since the purification procedure for colicin favors the separation of molecules with as widely different ionic properties as colicin and immunity protein, it is unlikely that the two would copurify to this extent if they were not interacting. Even in colicin purified through the CM-Sephadex chromatography described by Herschman and Helenius (11), there is a relatively prominent band where immunity protein runs. It is the only band on the gel besides that of colicin, and this slab gel system is extremely sensitive. Experiments are currently under way to investigate the nature of the “immunity” band found in these colicin preparations.

It is also interesting to note that purified colicin E2 also contains a minor gel band with a mobility corresponding to a molecular weight around 10,000 (not shown). This band co-electrophoreses with the protein isolated from the “immunity purification” from cells colicinogenic for E2 described in an earlier
synthesize protein. In the most complicated model, the immunity protein would interact with some form of the colicin-ribosome complex, allowing the ribosome to function normally while preventing its cleavage by colicin. Any of these models can be reconciled with the fact that immunity protein is effective against colicin when added to an *in vitro* reaction with colicin, but not when mixed with colicin that is then used to kill sensitive bacteria, *in vivo*. Immunity protein bound to colicin could be removed from the colicin as the colicin binds to the receptor on the cell surface or as it passes through the membrane into the cell. The colicin, with immunity protein thus removed, would be free to act on its target within the sensitive cell. Likewise, if immunity protein binds to the colicin’s substrate, the ribosome, immunity protein incubated with colicin outside the cell would have no means of reaching its own target without specific cell surface receptors and a means of passing through the membrane.

Our titration experiments with immunity protein and colicin in *in vitro* protein synthesis indicate that immunity protein is probably interacting with the colicin and not with the ribosomes. The presence in purified colicin of a band that co-electrophoreses with immunity protein on SDS acrylamide gels also strongly suggests that the two interact. The presence of a comparable band on gels of colicin E2 seems to bear this fact out. The low molecular weight marker that we purified from E2-producing cells may well be the “E2 immunity substance,” although we have no assay for that material. Glick et al. (18) have also reported seeing a low molecular weight component on SDS gels of some of their purified E3 preparations, although they do not further describe that band.

We have, however, been unable to demonstrate directly the interaction of purified immunity protein with colicin or with ribosomes. Our purified colicin preparations generally have a specific activity of \(2 \times 10^6\) to \(1 \times 10^6\) units per mg *in vitro*, which is at least as high as previously described E3 preparations (11, 19). In these preparations, one killing unit corresponds to about 100 molecules, and *in vivo* killing by colicin follows a one-hit kinetics (20). This could mean either that every molecule has an equal, one in a hundred chance of providing the lethal event, or that only one out of a hundred molecules is viable and capable of killing a cell. If the latter is the case, then the majority of the colicin is indeed inactive, which is consistent with some of our *in vitro* data (Fig. 4). If only a few per cent of the colicin molecules are viable and capable of interacting with immunity protein, then it will be very difficult to see that interaction by any of the methods we have so far tried.

There is apparently a low constitutive level of immunity protein in colicinogenic cells, since the protein could be isolated in small amounts from uninduced cultures. Induction of colicinogenic cultures results in a corresponding 50- to 100-fold increase in the amount of immunity protein that can be isolated from the cells, and release of the immunity protein from the cells along with colicin.

Purified immunity protein should prove to be extremely useful in studying in detail the specific properties and requirements of the interaction between colicin and ribosomes, since it can be used to stop the colicin action at any given point during an *in vitro* reaction.

In the accompanying paper, Sidikaro and Nomura (21) have reached similar conclusions on the properties of the E3 immunity protein and the nature of its interaction with colicin E3.

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REFERENCES

1. Nomura, M. (1967) Annu. Rev. Microbiol. 21, 257–284
2. Bowman, C. M., Dahlberg, J. E., Ikemura, T., Konisky, J., and Nomura, M. (1971) Proc. Nat. Acad. Sci. U. S. A. 68, 964–968
3. Sexton, B. W., and Holland, J. B. (1971) Proc. Nat. Acad. Sci. U. S. A. 68, 959–963
4. Boon, T. (1971) Proc. Nat. Acad. Sci. U. S. A. 68, 2421–2425
5. Bowman, C. M., Sidikaro, J., and Nomura, M. (1971) Nature New Biol. 234, 133–137
6. Boon, T. (1972) Proc. Nat. Acad. Sci. U. S. A. 69, 549–552
7. Bowman, C. M. (1972) Fed. Eur. Biochem. Soc. Lett. 22, 73–75
8. Methack, R., Methack, I., and Aivazian, T. (1973) Proc. Nat. Acad. Sci. U. S. A. 70, 156–160
9. Konisky, J., and Nomura, M. (1967) J. Mol. Biol. 26, 181–195
10. Fredericoq, P. (1958) Symp. Soc. Exp. Biol. 12, 101–122
11. Herschman, H. R., and Helinski, D. R. (1967) J. Biol. Chem. 242, 5390–5398
12. Wadstrom, R. E., Engfeldt, D. L., Zinder, N. D., and Konigsberg, W. (1967) J. Mol. Biol. 29, 27–43
13. Henry, T. J., and Pratt, D. (1969) Proc. Nat. Acad. Sci. U. S. A. 62, 909–917
14. Studier, W. (1973) J. Mol. Biol., 79, 237–248
15. O’Farrell, P. Z., Huang, W., and Gold, L. M. (1973) J. Biol. Chem. 248, 5499–5501
16. Salnikow, J., Liao, T-H., Moore, S., and Stein, W. H. (1973) J. Biol. Chem. 248, 1480–1488
17. Laemmli, U. K. (1970) Nature 227, 680–685
18. Glick, M. J., Kerr, S. J., Gold, A. M., and Sheinin, D. (1972) Biochemistry 11, 1185–1198
19. Sabat, S. F., and Schnaitman, C. A. (1973) J. Biol. Chem. 248, 1797–1806
20. Nomura, M. (1964) Proc. Nat. Acad. Sci. U. S. A. 52, 1514–1521
21. Sidikaro, J., and Nomura, M. (1974) J. Biol. Chem. 249, 445–453
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