EVIDENCE FOR THE EXISTENCE OF THE COLICINOGENIC FACTORS E_2 AND E_3 AS SUPERCOILED CIRCULAR DNA-PROTEIN RELAXATION COMPLEXES

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SUMMARY: Colicinogenic factor E_1 previously has been isolated as a supercoiled circular DNA-protein complex that undergoes a conversion to the relaxed, or open circular, state when the complex is treated with certain proteases, strong ionic detergents, or alkali. In this study, data is presented demonstrating the existence of similar relaxation complexes for the bacterial colicinogenic factors E_2 and E_3 of Escherichia coli.

INTRODUCTION: The colicinogenic factor E_1, (ColE_1), is a bacterial plasmid (m.w. 4.2×10^6 daltons) that determines the production of the extracellular antibiotic protein colicin E_1. ColE_1 DNA has recently been isolated from Escherichia coli as a supercoiled circular DNA-protein complex (designated relaxation complex) which when treated with agents or conditions affecting protein structure uniquely undergoes a conversion to a state where the DNA is in the relaxed or open circular form (1,2). The relaxed DNA has been found to contain a single nick or gap that is strand specific.

The colicinogenic factors E_2 (ColE_2) and E_3 (ColE_3), bacterial plasmids that determine the production of colicins E_2 and E_3, respectively, have been previously isolated from Escherichia coli as supercoiled DNA molecules each with a molecular weight of approximately 5.0×10^6 daltons (3). In this communication we present evidence for the existence of these plasmids as relaxation complexes.

METHODS: E. coli K12 strains W3110(ColE_2) and W3110(ColE_3) carrying the ColE_2 and ColE_3 factors, respectively, were made colicinogenic by conjugal transfer of the ColE_2 factor from Shigella P9 (4) and the ColE_3 factor from E. coli CA38 (5). The W3110(ColE_3) strain also carries the ColI factor from CA38 but this

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relatively large molecular weight plasmid is in an inactive state in this strain with respect to colicin I production. A non-colicinogenic strain of W3110 served as a control strain in these studies. The cells were grown in the presence of ^{3}H-thymine in a Tris-casamino acids-glycerol medium that has been described in detail elsewhere (1). The lysing prodedure has been described previously (1,2) and essentially consists of the lysing of EDTA-lysozyme spheroplasts with a detergent mixture of Brij 58 and sodium deoxycholate followed by a low speed centrifugation (clearing spin) of the lysate. This centrifugation step pellets about 95% of the chromosomal DNA while most of the plasmid DNA remains in the supernatant. The supernatant is referred to as a cleared lysate.

Plasmid DNA pooled from peaks in the 15-50% sucrose gradient was treated with pronase (Calbiochem) or sodium dodecyl sulfate (SDS) (Fischer Scientific) in the manner described previously for the ColE$_1$ complex (1,2).

Non-complexed supercoiled circular DNA (^{32}P-labeled) was obtained as a satellite peak after subjecting cleared lysate to dye-buoyant density centrifugation in ethidium bromide-CsCl gradients (2).

Sucrose density gradient centrifugation, dropwise fractionation of the gradients, and the counting of radioisotope were carried out as previously described (2). The 15-50% sucrose gradients contained 0.01 M Na_2 EDTA, 0.06 M KCl and 0.02 M Tris pH 7.3. The 5-20% sucrose gradients contained 0.05 M NaCl, 0.005 M Na_2 EDTA and 0.03 M Tris pH 8.0.

RESULTS AND DISCUSSION: Cleared lysates were prepared (see Methods) from separate log phase cultures of W3110, W3110(ColE$_2$) and W3110(ColE$_3$) cells and fractionated on 15-50% sucrose gradients. The result is shown in Fig. 1. In the cases of the W3110(ColE$_2$) and W3110(ColE$_3$) strains a well-defined peak is observed in the middle of the gradient and in approximately the same position described for the ColE$_1$ factor prepared under the same conditions (1). This peak is absent in the W3110 non-colicinogenic strain. The smaller peak observed near the bottom of the gradient in the case of the W3110(ColE$_3$) cells represents the relatively large ColI plasmid DNA that was transfered together with the

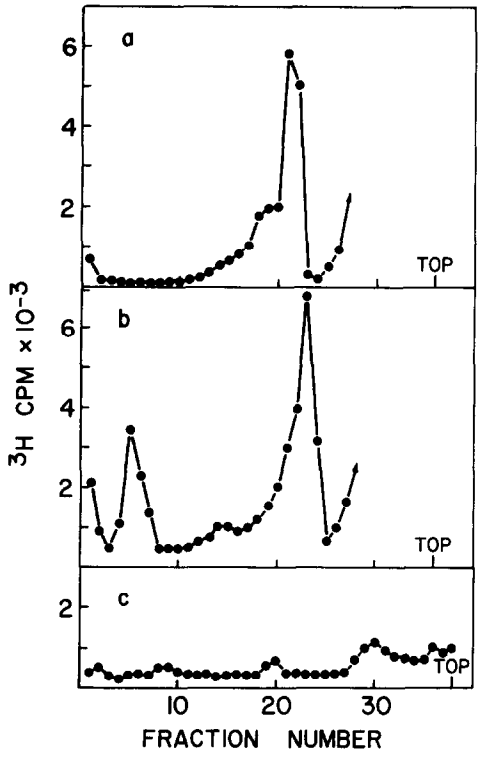

FIGURE 1: Sedimentation of cleared lysates on 15-50% sucrose density gradients in the Beckman SW25.1 rotor (2°C) at 25,000 rpm for 16 hrs. a) W3110 (ColE$_2$). b) W3110(ColE$_3$). c) W3110. In (a) and (b) the counts near the top of the gradients increase off scale and represent TCA soluble material. In (c) the TCA soluble counts were removed by washing the filter papers in TCA.

ColE$_3$ from the CR38 strain. In each case fractions within the ColE$_2$ and ColE$_3$ peaks were pooled, diluted with TES (0.05 M NaCl, 0.005 M Na$_2$ EDTA, 0.03 M Tris pH 8.0), mixed with the appropriate ^{32}P-labeled non-complexed ColE$_2$ or ColE$_3$ DNA, and treated with either 0.25% SDS or 250 μg/ml pronase for 10 min at 25°C. Samples serving as controls had TES added in place of SDS or pronase. The control and treated samples were then analyzed on 5-20% sucrose density gradients As shown in Fig. 2, in the untreated control sample, the ^{3}H-labeled ColE$_2$ and ColE$_3$ DNA sediments to varying degrees slightly faster than the ^{32}P-labeled ColE$_2$ and ColE$_3$ non-complexed supercoiled DNA's. In the case of ColE$_2$, the leading edge of the ^{3}H peak is rather broad. This occurs reproducibly, and presumably represents plasmid DNA molecules with varying amounts of cellular

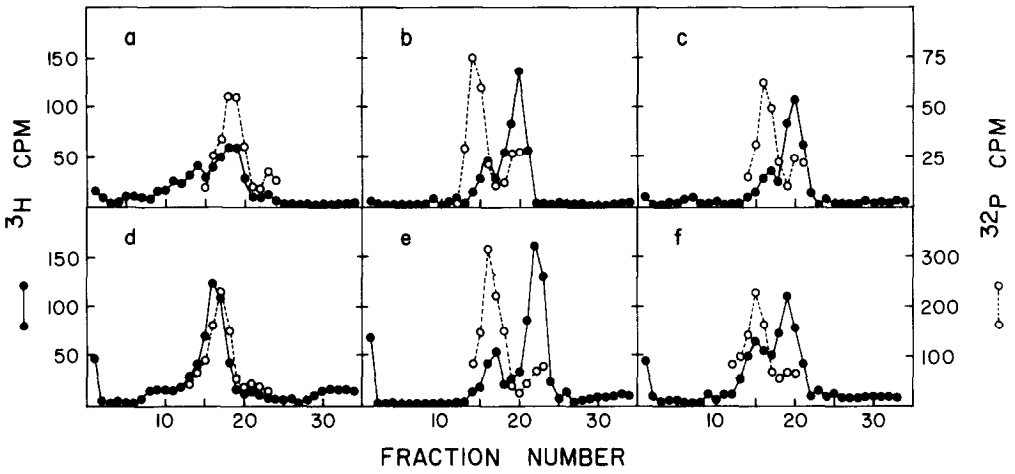

FIGURE 2: Sedimentation analysis of plasmid material on 5-20% sucrose density gradients. Samples were treated with TES (controls), SDS, or pronase prior to centrifugation in the Beckman SW65 rotor (15°C) at 50,000 rpm for 135 minutes. a) ColE$_2$ control. b) ColE$_2$, treated with SDS. c) ColE$_2$, treated with pronase. d) ColE$_3$, control. e) ColE$_3$, treated with SDS. f) ColE$_3$, treated with pronase. —●—●—, ^{3}H-labeled ColE$_2$ (a,b,c) or ColE$_3$ (d,e,f) being tested for the presence of complex. --o----o--, ^{32}P-labeled ColE$_2$ (a,b,c) or ColE$_3$ (d,e,f) non-complexed DNA. Sedimentation was from right to left. Recovery of ^{3}H counts was greater than 90% in each case.

material associated with it. Following treatment with either SDS or pronase, the majority (70-80%) of the ^{3}H-labeled plasmid DNA appears in a position corresponding to the open circular DNA form, while the ^{32}P-labeled (non-complexed) plasmid DNA remains unchanged. As in the case of the ColE$_1$ system, approximately 20 to 30% of the amount of plasmid ColE$_2$ and ColE$_3$ DNA is evidently not in the complexed state in that it is resistant to relaxation. Also as with the ColE$_1$ relaxation complex a conversion to the open circular DNA form is brought about by exposure of the ColE$_2$ or ColE$_3$ complexes to alkali in that when these materials are centrifuged on alkaline sucrose gradients (pH 12.5), approximately 70-80% of the ^{3}H-labeled DNA sediments as expected for the single stranded circular and linear products of open circular DNA that has been denatured (6,7). The remaining (approximately 20-30%) sediments with the characteristics of covalenlty-closed double stranded circular DNA. Extended alkaline sucrose gradient centri-

fugation results in a separation of the single stranded circular and linear pieces into two peaks of approximately equal size indicating that the relaxed molecules of ColE$_1$ DNA possess a single nick or gap (6,7).

These data indicate that the relaxation complex is not unique to the ColE$_1$ system but can be found in the two other plasmid systems ColE$_2$ and ColE$_3$. The similarity of the three relaxation complexes brings up the question as to whether or not the associated protein is in fact identical in each case. Further studies (6,7) on these three relaxation complexes purified from the isogenic E. coli strains have indicated that this may not be the case. Unlike the ColE$_1$ complex, the ColE$_2$ and ColE$_3$ complexes can be inactivated in vitro by heat treatment. Heat treatment induces relaxation in the ColE$_1$ complex while the ColE$_2$ and ColE$_3$ complexes become resistant to relaxation by SDS, pronase, or alkali upon prior exposure to heat. The ColE$_2$ and ColE$_3$ DNA molecules that have become resistant to relaxation appear as rapidly sedimenting material (i.e. characteristic of covalently closed circular molecules) when sedimented on alkaline sucrose gradients.

The above report has dealt with relaxation complexes of relatively low molecular weight plasmid DNA's. A relaxation complex involving the much higher moleculer weight ColI$_b$-P9 factor which, in addition to being a colicinogenic factor, is also a sex factor, has also been purified from E. coli (8,9). Similarly, the supercoiled DNA of the F$_1$ sex factor of E. coli can be isolated in the form of a relaxation complex (10).

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