EXISTENCE OF THE COLICINOGENIC FACTOR-SEX FACTOR ColI$_b$-P9 AS A SUPERCOILED CIRCULAR DNA-PROTEIN RELAXATION COMPLEX

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SUMMARY: The colicinogenic factor ColE$_1$ has previously been isolated as a supercoiled circular DNA-protein complex designated relaxation complex. When treated with proteases, strong ionic detergents, or alkali, the supercoiled DNA in the complex is converted to the relaxed, or open circular, form. In these studies the colicinogenic factor-sex factor ColI$_b$-P9 has been purified from Escherichia coli as a circular DNA molecule with sedimentation values of 73S and 45S for the supercoiled and open circular forms, respectively, and as a supercoiled DNA-protein relaxation complex with properties similar to those described for the ColE$_1$ relaxation complex.

INTRODUCTION: The colicinogenic factor E$_1$ (ColE$_1$) (m.w. 4.2 x 10^6) has been isolated from Escherichia coli as a unique supercoiled DNA-protein complex, designated relaxation complex (1,2). The relaxation complex is characterized by the conversion of the supercoiled DNA in the complex to an open circular DNA form with a strand specific nick or gap when the complex is treated with strong ionic detergents, proteases, or alkali (pH 12.5) (1,2).

The colicinogenic factors ColE$_2$ and ColE$_3$ (m.w. of approximately 5.0 x 10^6 daltons in each case (3)), have also been isolated as relaxation complexes (4). These three colicinogenic E factors do not exhibit sex factor properties; however, the conjugal transfer of these factors can be promoted by a sex factor (5-7). ColI$_b$-P9 is a colicinogenic factor that determines the production of colicin I$_b$ and in contrast to the ColE factors also exhibits sex factor properties (8,9). In the studies presented here ColI$_b$ was isolated both as a supercoiled DNA molecule of a considerably higher molecular weight than the ColE factors and also as a supercoiled DNA-protein complex with properties similar to those of the ColE$_1$ relaxation complex.

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METHODS: E. coli K12 strains AB1157 (10) and AB1157(ColI$_b$) were used in this study. The ColI$_b$ factor was transfered to the AB1157 strain from Salmonella typhimurium L2 cys D36 (ColI$_b$-P9) by a procedure previously described (9). The medium employed for bacterial growth and the procedure of labeling with ^{3}H-thymine, ^{14}C-thymine or $^3H^{32}PO_4$ have been described in detail elsewhere (1,2).

The procedure for lysing the cells has been described previously (1,2) and essentially consists of the treatment 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. The centrifugation step normally pellets approximately 95% of the chromosomal DNA in the supernatant. The supernatant is referred to as "cleared lysate". Cleared lysates were either sedimented on 15-50% sucrose gradients to purify the ColI$_b$ DNA-protein complex or subjected to dye-buoyant density centrifugation as described earlier (1,2) to purify the non-complexed DNA form of ColI$_b$. The ColE$_1$ DNA used as a reference was non-complexed material prepared by dye-buoyant density centrifugation (1,2).

Material pooled from the peak from the 15-50% sucrose gradient was treated with pronase (Calbiochem) or sodium dodecyl sulfate (SDS) (Fisher Scientific) under the conditions previously described for the ColE$_1$ relaxation complex (1).

Sucrose density gradient centrifugation, dropwise fractionation of the gradients, and the counting or radioisotopes were carried out as previously described (1,2). The 15-50% sucrose gradient 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: The supercoiled circular DNA form of the ColI$_b$ factor was purified from the AB1157(ColI$_b$) strain by dye-buoyant density centrifugation. Separate 15 ml cultures of AB1157(ColI$_b$) and non-colicinogenic AB1157 cells were grown to log phase in the presence of ^{3}H-thymine and ^{14}C-thymine, respectively. The cells were harvested, mixed together, and a cleared lysate was prepared (see methods). A portion of the cleared lysate was centrifuged to equilibrium in a

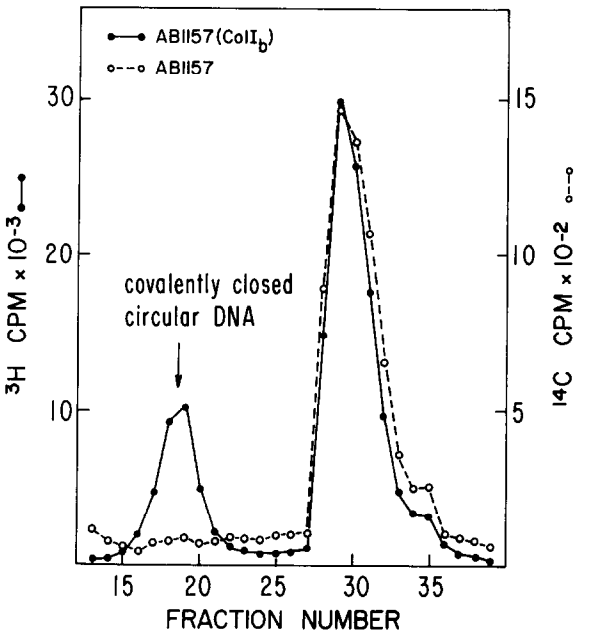

FIGURE 1: Dye-buoyant density gradient centrifugation of cleared lysate of a mixture of ^{3}H-labeled colicinogenic cells and ^{14}C-labeled non-colicinogenic cells.

CsCl-ethidium bromide buoyant density gradient. The result, shown in Fig. 1, indicates the presence of a ^{3}H-labeled satellite band of DNA in a position characteristic of covalently closed circular DNA. No equivalent peak is observed for the lysate of the ^{14}C-labeled non-colicinogenic AB1157 cells. Material in the satellite peak of DNA was pooled, dialyzed and sedimented on a neutral sucrose

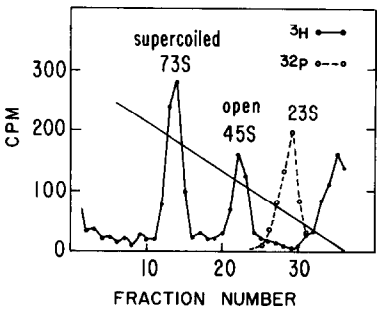

FIGURE 2: Sedimentation analysis of ^{3}H-labeled ColI$_b$ DNA obtained from dye-buoyant density centrifugation. Sedimentation was (from right to left) through 5-20% sucrose gradients and carried out in the Spinco SW65 rotor (15°C) at 50,000 rpm for 45 minutes. The ^{32}P-labeled 23S marker was supercoiled ColE$_1$ DNA.

density gradient in the presence of ^{32}P-labeled 23S ColE$_1$ supercoiled DNA. The result, shown in Fig. 2, indicates 2 peaks sedimenting at approximately 73S and 45S relative to the 23S ColE$_1$ DNA marker. Storage, in solution at 4°C, of the DNA pooled from the satellite peak results in a decrease of the 73S materials and a corresponding increase of the 45S material. Pronase or SDS treatment of the satellite DNA does not affect the relative amounts of the 2 peaks observed on sucrose gradient centrifugation. The 73S and 45S peaks are interpreted as representing the supercoiled and open circular DNA forms, respectively, of the ColI$_b$ factor. This interpretation is consistent with the observation by electron microscopy of supercoiled and open circular DNA forms in these preparations of ColI$_b$ DNA. The sedimentation values of the supercoiled or open circular DNA forms correspond to a molecular weight of 61.5 x 10^6 daltons for the ColI$_b$ factor (11).

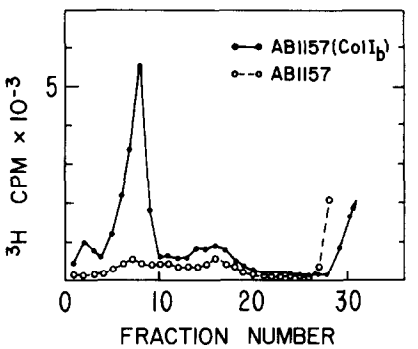

FIGURE 3: Sedimentation of cleared lysates on 15-50% sucrose density gradients in the Spinco SW25.1 rotor (2°C) at 22,500 rpm for 16 hrs. The profiles for both the colicinogenic and non-colicinogenic cells are superimposed. Sedimentation was from right to left.

The ColI$_b$ factor was also isolated as a complexed supercoiled DNA form exhibiting properties of the previously described relaxation complex of ColE$_1$ DNA. A cleared lysate of cells was sedimented on a 15-50% sucrose density gradient. As seen in Fig. 3 sedimentation of a portion of cleared lysate of AB1157(ColI$_b$) on a 15-50% sucrose gradient yields a very pronounced peak in the lower half of the gradient. This peak represents 1.44% of the total DNA in the crude lysate and is absent in a similarly prepared lysate of non-colicinogenic

cells. Material within this peak was pooled, diluted with TES (0.05 M NaCl, 0.005 M Na_2 EDTA, 0.03 M Tris pH 8.0) and treated with 0.25% SDS. To a sample serving as a parallel control TES was added in place of the detergents. The samples were then sedimented on neutral sucrose density gradients with $\underline{ColI}_b$ DNA isolated from a CsCl-ethidium bromide equilibrium gradient serving as an external marker. As shown in Fig. 4 the untreated sample sediments to a position

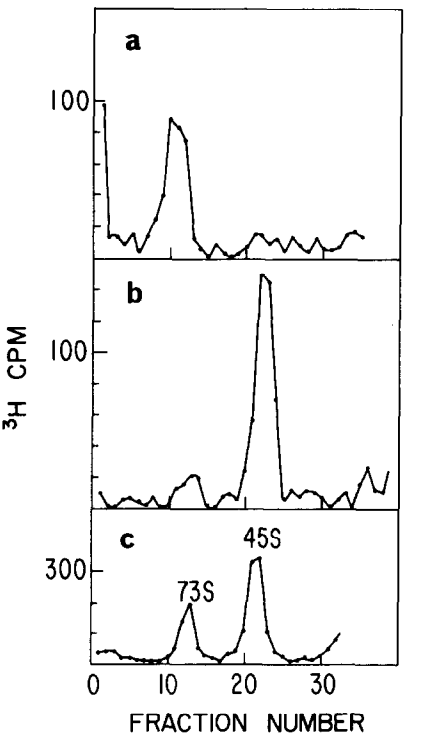

FIGURE 4: Sedimentation analysis of $\underline{ColI}_b$ material obtained from the 15-50% sucrose gradient. Sedimentation was (from right to left) through 5-20% sucrose gradients and carried out in the SW65 rotor (15°C) at 50,000 rpm for 45 minutes. a) untreated control sample (recovery of ^{3}H counts was 77%). b) sample treated with SDS prior to centrifugation (recovery of ^{3}H counts was 80%). c) external marker representing the supercoiled and open circular forms of $\underline{ColI}_b$ DNA obtained as the satellite peak resulting from dye-buoyant density centrifugation of a cleared lysate.

very close to that of the 73S supercoiled form of the external marker. Treatment with SDS resulted in a very pronounced conversion to a position closely corresponding to that of the relaxed or open circular form of the external marker. Fig. 5 shows the results of a similar experiment where ^{32}P-labeled $\underline{ColI}_b$ non-

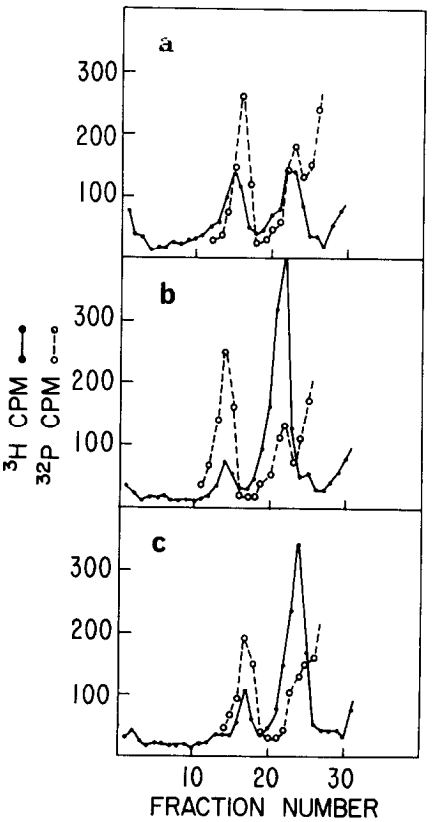

FIGURE 5: Sedimentation analysis of ^{3}H-labeled ColI$_b$ DNA obtained from the 15-50% sucrose gradient. Sedimentation conditions were as described in Fig. 4 a) untreated control sample (recovery of ^{3}H counts was 64%). b) sample treated with SDS prior to centrifugation (recovery of ^{3}H counts was 72%). c) sample treated with pronase prior to centrifugation (recovery of ^{3}H counts was 70%). The ^{32}P-labeled non-complexed ColI$_b$ DNA was present during treatment of the ^{3}H-labeled material.

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complexed DNA was present during treatment with SDS or pronase. In this preparation of ColI$_b$ DNA approximately one-half of the ^{3}H-labeled DNA was in the slower sedimenting DNA form prior to treatment. It is observed that the faster sedimenting ^{3}H-labeled peak sediments slightly ahead of the 73S ^{32}P-labeled non-complexed ColI$_b$ DNA at approximately 76S. Both SDS and pronase treatment induced a pronounced conversion of the 76S sedimenting form to the slower sedimenting form, while the ^{32}P-labeled non-complexed supercoiled ColI$_b$ DNA remained unchanged. The ColI$_b$ DNA remaining as the faster sedimenting form sedimented coincident with the 73S marker.

These data resemble the results obtained for the ColE$_1$ relaxation complex in the following respects. 1) Supercoiled ColI$_b$ DNA, purified from a Brij-DOC lysate by sucrose gradient centriguation sediments slightly faster than the supercoiled from of ColI$_b$ DNA isolated by the dye-buoyant density gradient centrifugation technique. 2) Treatment of ColI$_b$ DNA obtained by the gentle lysis procedure with SDS or pronase results in a conversion of the DNA to a form that possesses the sedimentation properties of the open circular form of ColI$_b$ DNA while the supercoiled ColI$_b$ DNA obtained by dye buoyant density centrifugation is unaffected by this treatment. On the basis of these observations it is concluded that ColI$_b$ DNA can exist as a relaxation complex similar to that of the ColE$_1$ plasmid.

The ColI$_b$ factor differs functionally from the ColE$_1$ factor in that unlike the ColE$_1$ factor, the ColI$_b$ factor possesses genetic determinants of sexuality (8,9). In addition, whereas there are 10 to 15 copies of the ColE$_1$ factor per chromosome in E. coli cells (2), the recovery data on the ColI$_b$ factor indicate the presence of one or at most a few copies of ColI$_b$ DNA molecules per chromosome. In these respects the ColI$_b$ factor resembles the F factor of E. coli, an episomal element that also exists in the form of a relaxation complex (12).

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