ences between the two molecules must be small, since no evidence of heterogeneity can be detected by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate.

References

Bautz, E. K. F., Bautz, F. A., and Dunn, J. J. (1969), *Nature (London) 223*, 1022.

Bautz, E. K. F., Dunn, J. J., Bautz, F. A., Schmidt, D. A., and Mazaitis, A. J. (1970), *in* Lepetit Colloquim on RNA Polymerase and Transcription, Silvester, L., Ed., Amsterdam, North-Holland, p 90.

Bolle, A., Epstein, R. H., Salser, W., and Geiduschek, E. P. (1968), *J. Mol. Biol. 31*, 325.

Bremer, H., Konrad, M. W., Gaines, K., and Stent, G. S. (1965), *J. Mol. Biol. 13*, 540.

Brody, E., Sederoff, R., Bolle, A., and Epstein, R. (1970), *Cold Spring Harbor Symp. Quant. Biol. 35*, 203.

Burgess, R. R., Travers, A. A., Dunn, J. J., and Bautz, E. K. F (1969), *Nature (London) 221*, 43.

Chamberlin, M., and Berg, P. (1962), *Proc. Nat. Acad. Sci. U. S. 48*, 81.

Downey, K. M., and So, A. G. (1970), *Biochemistry 9*, 2520.

Glynn, I. M., and Chappell, J. B. (1964), *Biochem. J. 90*, 147.

Goff, C. G., and Minkley, E. G. (1970), *in* Lepetit Colloquium on RNA Polymerase and Transcription, Silvester, L., Ed., Amsterdam, North-Holland, p 124.

Hall, B. D., Fields, K., and Hager, G. (1970), *in* Lepetit Colloquium on RNA Polymerase and Transcription, Silvester, L., Ed., Amsterdam, North-Holland, p 148.

Hinkle, D., and Chamberlin, M. J. (1970), *Cold Spring Harbor Symp. Quant. Biol. 35*, 65.

Losick, R.,and Sonenshein, A. L. (1969), *Nature (London) 224*, 35.

Maitra, U., and Hurwitz, J. (1965), *Proc. Nat. Acad. Sci. U. S. 54*, 815.

Maitra, U., Nakata, Y., and Hurwitz, J. (1967), *J. Biol. Chem. 242*, 4908.

Richardson, J. P. (1969), *Progr. Nucl. Acid Res. Mol. Biol. 9*, 65.

So, A. G., and Downey, K. M. (1970), *Biochemistry 9*, 4788.

So, A. G., Downey, K. M., and Jurmark, B. S. (1971), *in* Miami Winter Symposia, Vol. 2, Ribbons, D. W., Woessner, J. F., and Schultz, J., Ed., Amsterdam, North-Holland, p 221.

Sugiura, M., Okamoto, T., and Takanami, M. (1970), *Nature (London) 225*, 598.

Szybalski, W., Kubinski, H., and Sheldrick, P. (1966), *Cold Spring Harbor Symp. Quant. Biol. 31, 123.*

Travers, A. A. (1970), *Nature (London) 225*, 1009.

Travers, A. A., and Burgess, R. R. (1969), *Nature (London) 222*, 537.

Zillig, W., Palm, P., Sethi, V. S., Zechel, K., Rabussay, D., Heil, A., Seifart, W., and Schachner, M. (1970), *Cold Spring Harbor Symp. Quant. Biol., 35*, 47.

F_1 Sex Factor of *Escherichia coli*. Size and Purification in the Form of a Strand-Specific Relaxation Complex of Supercoiled Deoxyribonucleic Acid and Protein*

Bruce C. Kline† and Donald R. Helinski‡

ABSTRACT: Supercoiled F_1 sex factor DNA complexed with cellular material was isolated from logarithmically growing *Escherichia coli* cells. The sedimentation coefficient of the complex is approximately 84 S whereas noncomplexed, supercoiled F_1 DNA is 80 S. Treatment of a mixture of complexed and noncomplexed supercoiled DNA with proteases, alkali, or sodium dodecyl sulfate results in a 50% conversion of the complexed DNA to the open-circular form of DNA with no significant effect on the noncomplexed DNA. The open-circular form resulting from this conversion possesses a nick or gap specifically in the denser of the two strands of F_1 DNA separated by equilibrium centrifugation in a CsCl gradient containing poly(U,G). The average contour length of the open-circular form of F_1 DNA was determined to be 31.7 ± 2.1 μ corresponding to a molecular weight of 60.9 ± 4.1 × 10^6.

Several supercoiled extrachromosomal elements have been isolated from *Escherichia coli* as DNA–protein complexes, for example, colicinogenic factors *Col*E_1 (K30) (Clewell and Helinski, 1969), *Col*E_2 (P9) (Clewell and Helinski, 1970a; Blair *et al.*, 1971), *Col*E_3 (CA38) (Clewell and Helinski, 1970a), and the colicinogenic factor–sex factor, *Col*I_b (P9) (Clewell and Helinski, 1970b). A characteristic property of these supercoiled DNA–protein complexes, designated relaxation complexes, is their conversion to the open-circular DNA form possessing a nick or gap in one of the two DNA strands after

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in vitro treatment with proteases, detergents, or alkali. In each case the corresponding noncomplexed supercoiled DNA form of the extrachromosomal element is not nicked by these treatments. For the relaxation complexes of the relatively low molecular weight plasmids, *Col*E$_1$ and *Col*E$_2$, the nick or gap in the open-circular form resulting from treatment with a protease has been shown to be strand specific (Clewell and Helinski, 1970c; Blair *et al.*, 1971).

This report presents data on the size of F_1 DNA and evidence for the existence in *E. coli* of a supercoiled DNA relaxation complex of the F_1 sex factor. As in the cases of the *Col*E$_1$ and *Col*E$_2$ relaxation complexes, the nick or gap in the open-circular form produced by the induced relaxation of the supercoiled F_1 DNA complex is in a unique strand of the DNA.

Materials and Methods

Materials. Sources of reagents used in this work have been given previously (Clewell and Helinski, 1969) except for α-chymotrypsin, C. F. Boehringer and Soehne, and the copolymer, polyuridylic acid–guanylic acid (poly(U,G), U–G, 1:1.0), Miles Laboratory (lot 14-343-381). ^{32}P-Labeled MS2 phage was generously provided by Dr. P. Bonner. Plkc prophage DNA was purified from *E. coli* W1485 *TrpC*$_1$ *ThyA* carrying Plkc by the procedure described below for the purification of noncomplexed F_1 DNA. This strain was provided by Dr. I. Crawford. ϕX174 RFII DNA employed in the electron microscopy studies was purified by sucrose density gradient centrifugation and provided by G. Clinton and M. Hayashi.

Bacterial Strains. The thymine-requiring *E. coli* CR34 (F_1) strain and the isogenic F$^-$ strain have been described previously (Bazaral and Helinski, 1970). A thymine-requiring *E. coli* JC411 strain (Clewell and Helinski, 1970c), selected by David Kingsbury, also was used in this study. Both of these strains were converted to maleness by conjugation with W1485 (F_1) and shown to be sensitive to the male specific phages MS2 and μ.

Isolation of Noncomplexed and Complexed F_1 DNA. Bacteria were grown aerobically at 37° in a Tris minimal medium containing Casamino acids, glucose (for noncomplexed F_1 DNA) or glycerol (for complexed F_1 DNA), and radioactive thymine; harvested by centrifugation midway in the logarithmic period (cell concentration of 5×10^8 cells/ml); and subsequently converted to spheroplasts by treatment with lysozyme. The spheroplasts were then lysed (crude lysate) by treatment with a mixture of Brij 58 and deoxycholate. A detailed description of the growth medium, reagents, labeling conditions, and lysis procedure has been given previously (Clewell and Helinski, 1969). The crude lysate was repeatedly (five times) frozen in an ethanol–Dry Ice bath and thawed in a 37° water bath to increase the final yield of F_1 DNA. The lysate was then passed five times through a 1-ml pipet to shear the chromosomal DNA. Finally, the crude lysate was centrifuged 5 min at 48,000g to remove the bulk of chromosomal DNA and cellular debris. The resulting supernatant, containing F_1 DNA, is designated a cleared lysate.

Noncomplexed, supercoiled F_1 DNA was purified from cleared lysates by the dye–buoyant density procedure of Radloff *et al.* (1967). The denser band was isolated, pooled, mixed with 1–2 μg of nonradioactive *E. coli* chromosomal DNA in 5 ml of TES (50 mM Tris-HCl–5 mM EDTA–50 mM NaCl), and pelleted in a nitrocellulose centrifuge tube at 200,000g for 180 min. The pellet was dissolved by storage overnight in 0.5 ml of TES at 4°.

Complexed, supercoiled F_1 DNA was partially purified from the cleared lysates by sedimentation in a 20–31%, or 15–50%, neutral sucrose gradient as shown in Figure 2. The partially purified F_1 DNA, designated once-purified F_1 DNA, was pooled, pelleted, and resuspended, as described for noncomplexed F_1 DNA, except that carrier DNA was omitted, and the resuspending TES buffer contained 5% sucrose, 1 mg/ml of bovine serum albumin, and 6 mM β-mercaptoethanol (ME).[1] Although similar results were obtained if carrier DNA was added, or ME was omitted, the addition of ME appeared to increase the stability of the complex. In those cases where it was necessary to purify the F_1 DNA complex further, the material was additionally centrifuged through a 5-ml 20–31% sucrose density gradient lacking bovine serum albumin but containing 6 mM ME. Fractions (170 μl) were collected and a 10-μl portion of each fraction was spotted on filter paper, trichloroacetic acid washed, and counted. The supercoiled F_1 DNA peak pooled from this gradient is referred to as twice-purified F_1 complex. Trichloroacetic acid washing of filter papers and counting of radioisotope were carried out as described previously (Clewell and Helinski, 1969).

Strand Specificity of Induced Relaxation. A solution of predominantly supercoiled, noncomplexed, ^{14}C-labeled F_1 DNA in 0.1 N NaOH was heated for 1 min in a boiling-water bath resulting in the random nicking of 40–50% of the supercoiled molecules. The complementary single strands of F_1 DNA were then separated from covalently closed, double-stranded F_1 DNA by sedimentation through a 5-ml preparative, 20–31% alkaline sucrose gradient (pH 12.5) using the centrifugation conditions described in Figure 2. This material served as a source of reference heavy and light strands in subsequent poly(U,G)–CsCl centrifugations. A solution of twice-purified, ^{3}H-labeled, complexed, supercoiled F_1 DNA was layered directly onto preparative 5-ml alkaline sucrose gradients both to induce relaxation and to denature the open-circular, double-strand product. Subsequently, the single-strand circular and linear DNA of the relaxed product were purified by prolonged centrifugation as described in Figure 5. Portions of these purified circular or linear strands were then mixed with a purified mixture of circular and linear strands of randomly nicked, noncomplexed F_1, neutralized, and subjected to poly(U,G) CsCl buoyant density analysis according to the procedure of Opara-Kubinska *et al.* (1964) as described by Vapnek and Rupp (1970).

Electron Microscopy. Sample preparation and determination of contour lengths were performed essentially as previously described (Roth and Helinski, 1967). Open-circular F_1 DNA was obtained from covalently closed-circular DNA (purified by the dye–CsCl procedure) by exposure of the DNA–dye complex to light from a 100-W light bulb for 6 hr at 4°. The DNA was then used directly or dialyzed overnight against several changes of 0.15 M ammonium acetate (pH 8.5). A significant difference in contour length of F_1 DNA was not observed between dialyzed or nondialyzed preparations. ϕX174 RFII DNA, dialyzed against the same buffer, was added to solutions of F_1 DNA just prior to examination. For visualization, 45 μl of DNA solution was mixed with 5 μl of 0.1% cytochrome *c* (dissolved in 5 M ammonium acetate, pH 8.5), held 15 min at room temperature, and then spread over a 0.25 M ammonium acetate, pH 8.5, buffer. The DNA–cytochrome films were picked up on carbonized and parlodion-coated grids and uranium shadowed.

[1] Abbreviations used are: ME, β-mercaptoethanol; SDS, sodium dodecyl sulfate.

TABLE I: Contour Lengths of Open-Circular F_1 and ϕX174 DNA.

F_1 DNA			RFII DNA of ϕX174			$X(F_1)/X(\phi X174)$
n^a	X (μ)	σ (μ)	n	X (μ)	σ (μ)	
13[b]	33.8	1.43	8	1.83	0.09	18.37
8	32.4	1.10	14	1.79	0.08	18.10
7	30.4	0.94	26	1.67	0.05	18.20
12	29.8	1.25	15	1.60	0.04	18.63

[a] n, X, and σ refer to the sample size, average length of molecule, and standard deviation, respectively. [b] This preparation of F_1 DNA was not dialyzed to remove CsCl and ethidium bromide prior to mixing with ϕX174 DNA and cytochrome *c* and spreading. The other preparations of F_1 DNA were dialyzed as described in Materials and Methods.

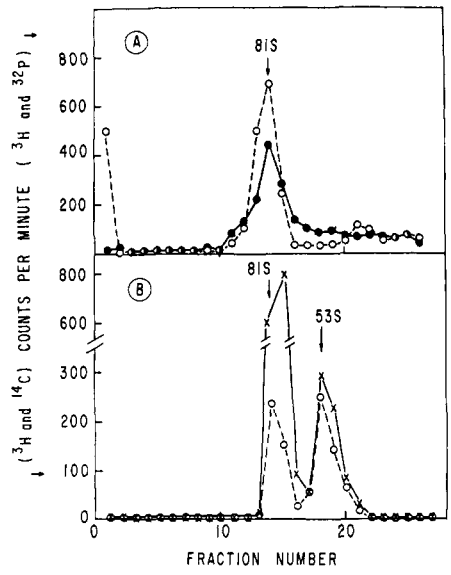

FIGURE 1: Sucrose gradient analysis of F_1 DNA purified by dye–CsCl equilibrium centrifugation. ^{3}H-Labeled noncomplexed F_1 DNA, purified by centrifugation in a CsCl gradient containing ethidium bromide was mixed with ^{32}P-labeled MS2 phage (A) or ^{14}C-labeled Plkc prophage DNA (B) and layered onto a sucrose density gradient. In the case of part A a 5-ml 5–20% sucrose gradient in TES was employed and the centrifugation was carried out at 50,000 rpm for 30 min in a Spinco SW65 rotor at 15°. A 5-ml 20–31% sucrose gradient in TES and 0.5 M NaCl was used for part B and the centrifugation conditions were 45,000 rpm for 55 min in a Spinco SW50.1 rotor at 15°. The direction of sedimentation is from right to left. Fractions (12 drop) were collected directly onto paper, trichloroacetic acid washed, and counted. Recovery of each radioisotope is greater than 90%. (●------●) ^{3}H-labeled F_1 DNA; (●——●) ^{32}P-labeled MS2 phage; (×——×) ^{14}C-labeled Plkc DNA.

Results

Determination of Size of F_1 DNA. DNA extracted from *E. coli* bacteria carrying the F_1 sex factor can be separated into a dense minor component and a light major component in a dye–CsCl buoyant density gradient (Bazaral and Helinski, 1970). The minor component, found in the characteristic position for supercoiled DNA in the dye–CsCl gradient, is not observed in extracts of otherwise isogenic F⁻ bacteria. Examination of this F_1 DNA by electron microscopy has shown that the majority of these DNA molecules are supercoiled. To determine the size of the supercoiled F_1 DNA, the DNA was purified by equilibrium centrifugation in an ethidium bromide–CsCl density gradient and subjected to sedimentation and electron microscopy analyses.

Sedimentation values of 80 and 48 S for the supercoiled, and open-circular DNA forms, respectively, of F_1 DNA previously have been obtained by sucrose gradient velocity analyses with the considerably smaller supercoiled *Col*E$_1$ DNA ($s_{20,w}$ = 23 S) as reference DNA (Bazaral and Helinski, 1970). These studies were repeated with the more closely sedimenting ^{32}P-labeled MS2 phage ($s_{20,w}$ = 81.5 S) (Moller, 1964) and the prophage DNA of the bacteriophage Plkc (molecular weight of 61 × 10^6) (Ikeda and Tomizawa, 1968) as reference markers. As shown in Figure 1A,B the fast-sedimenting supercoiled form of F_1 DNA (labeled with [^{3}H]thymine) sedimented to the same position as MS2 phage and slightly faster than Plkc prophage DNA. The slower sedimenting peak of DNA at the 53S position in Figure 1B probably corresponds to the spontaneously generated open-circular DNA form of F_1 and Plkc DNA. The neutral $s_{20,w}$ values of the supercoiled and open-circular DNA forms of F_1 DNA were also determined by band sedimentation analysis according to the procedure of Vinograd *et al.* (1963). The average values obtained from three determinations were 80.5 and 53.1 S, respectively, for the two DNA forms.

The contour length of the open-circular DNA form of F_1 DNA was determined by electron microscopy. As shown in Table I the average length of four different preparations of F_1 DNA varied from 29.8 to 33.8 μ. The average for the total number of molecules was 31.7 μ with a standard deviation of 2.1 μ. Assuming a linear density of 192/Å (MacHattie *et al.*, 1965), this average contour length corresponds to a molecular weight of 60.9 ± 4.1 × 10^6. This value is in good agreement with the molecular weight of 62.4 ± 0.8 × 10^6 calculated from the average length ratio of 18.36 ± 0.24 of F_1 and ϕX174 RFII DNA (Table I) and the reported molecular weight of 3.4 × 10^6 for ϕX174 RF DNA (Sinsheimer, 1959).

Comparison of F_1 DNA Obtained by the Dye–Buoyant Density or Sedimentation Velocity Procedures. Extracts of F_1 containing CR34 or JC411 strains of *E. coli* contain a rapidly sedimenting DNA component that is missing from extracts of F⁻ bacteria also when examined by sedimentation velocity analysis (Figure 2). Examination by electron microscopy of the rapidly sedimenting F_1 DNA isolated by this procedure has shown that the majority of these DNA molecules are supercoiled. Supercoiled F_1 DNA isolated by the dye–CsCl procedure was mixed with a portion of F_1 DNA isolated by the sedimentation velocity procedure and subsequently reexamined by sedimentation velocity and dye–buoyant density centrifugation. The results of Figure 3A show that supercoiled F_1 DNA purified by sucrose gradient centrifugation sediments faster (84–87 S) than the 80S supercoiled F_1 DNA purified by the dye–CsCl procedure. Upon storage, the 84S to 87S F_1 DNA spontaneously "relaxes" to 57S to 59S material while the spontaneous relaxation of 80S F_1 DNA yields 53S DNA. These and other observations reported below indicate a significant amount of material is complexed to the 80S F_1 DNA when isolated by the sucrose gradient procedure but not when isolated by the dye–CsCl procedure. As in the case of the *Col*E$_1$ relaxation complex (Clewell and Helinski, 1970c), reconstruction experiments with the 84S to 87S F_1 DNA have indicated that this material is not recovered from the supercoiled or satellite band of the dye–CsCl gradient but is preferentially relaxed by the centrifugation procedure leaving only

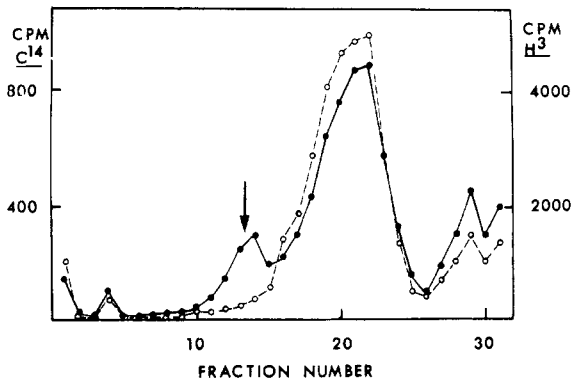

FIGURE 2: Sucrose gradient analysis of cleared lysates prepared from a mixture of JC411 (F_1) and JC411 spheroplasts. Preparation of cleared lysates is described in Materials and Methods. Specific activities of ^{14}C-labeled (●———●) JC411 (F_1) and ^{3}H-labeled (O-----O) JC411 DNA were 2.5×10^4 and 1×10^5 cpm per μg, respectively. The gradient is 20–31% neutral sucrose containing 1 mg/ml of bovine serum albumin and 6 mM ME. Sedimentation is from right to left in a SW25.1 rotor at 18,000 rpm for 14 hr at 6°. Each fraction (0.2 ml) was spotted on paper, trichloroacetic acid washed, dried, and counted. Recovery of each label is greater than 90%. The F_1 DNA peak (designated by the arrow) is calculated to be 0.5% of the total DNA contained in the crude lysate.

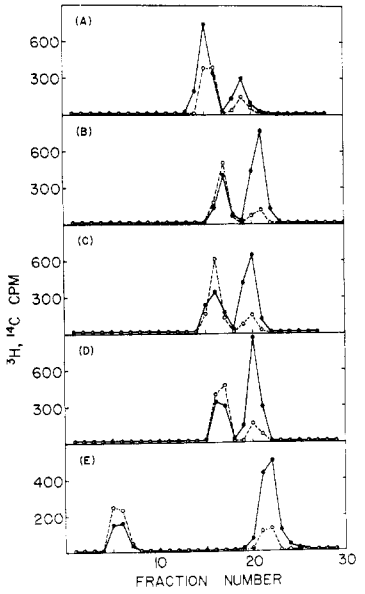

FIGURE 3: Sucrose gradient analysis of untreated and treated 84S F_1 DNA complex. Samples of once purified and tenfold concentrated ^{3}H-labeled 84S F_1 DNA complex (●———●) were mixed with ^{14}C-labeled noncomplexed F_1 DNA (O----O) in TES (total volume of 0.4 ml) and incubated for 30 min at 37° in the presence of the agents listed below. A 0.3-ml portion of each mixture was layered onto a 20–31% neutral sucrose density gradient in TES and containing 0.5 M NaCl. (A) No addition to the incubation mixture; (B) 0.25% SDS; (C) 1.25 mg/ml of autodigested Pronase; (D) 1.25 mg/ml of α-chymotrypsin; (E) a 0.3-ml portion without prior treatment was layered onto a 20–31% alkaline (pH 12.5) sucrose gradient. The gradients were then centrifuged in a six place, SW50.1 Spinco rotor at 15° for 55 min at 45,000 rpm (sedimentation is from right to left). In neutral gradients A–D the peaks at fractions 15–17 and 20–22 represent the supercoiled and open-circular F_1 DNA forms, respectively. In the alkaline gradient (E) the rapidly sedimenting material at fraction 6 represents the collapsed supercoiled DNA form whereas the slow peak at fraction 22 represents a mixture of the denatured single-strand linear and circular DNA forms. 12-drop fractions were collected directly onto paper, trichloroacetic acid washed, and counted. Recovery of each label is greater than 95% in all cases except (E) in which case the recovery is approximately 80% for the [^{14}C]DNA.

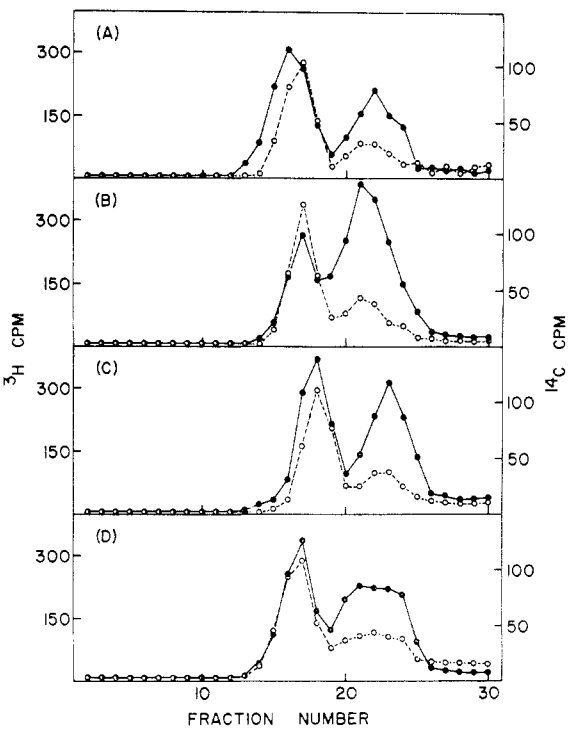

FIGURE 4: Heat inactivation of 84S F_1 complex. Two mixtures of ^{3}H-labeled 84S F_1 DNA (●———●) and ^{14}C-labeled noncomplexed F_1 DNA (O-----O) in TES containing 0.55 M NaCl (total volume 0.30 ml) were incubated at 25° in one case and 60° in the other case for 30 min. Each mixture was then divided into two equal portions and SDS (0.25% final concentration) added to one portion and equal volume of TES to the second portion. The four reaction mixtures were then incubated for 30 min at 37° and subjected to sedimentation velocity analysis according to the conditions described in Figure 2. (A) 25°, untreated; (B) 25°, SDS treated; (C) 60°, untreated; (D) 60°, SDS treated. The calculated per cent of ^{3}H counts in the supercoiled peak is 58, 25, 48, and 45 for A, B, C, and D, respectively. The amount of ^{14}C counts in the supercoiled peak is approximately 85% of the total in each case. The recovery of total counts is greater than 95% in each case.

noncomplexed (80S) F_1 DNA in the satellite band. In subsequent experiments, the F_1 DNA purified by sucrose gradient and dye–buoyant density centrifugation are designated complexed and noncomplexed F_1 DNA, respectively.

Induced Relaxation of F_1 DNA Complex to a Slower Sedimenting Form. ^{3}H-Labeled F_1 DNA complex was mixed with ^{14}C-labeled, 80S noncomplexed F_1 DNA and the mixtures were subjected to separate treatments by Pronase, chymotrypsin, SDS, and alkali as described under Figure 3. As shown in Figure 3B,C,D, respectively, SDS, Pronase, and chymotrypsin induced approximately a 50% conversion of F_1 DNA complex to 53S sedimenting molecule. Under these conditions the noncomplexed 80S F_1 DNA in the mixture did not undergo a significant conversion to the 53S form. The percentage of specific conversion of the F_1 DNA complex to a slower sedimenting form in the alkaline sucrose gradient (Figure 3E) was also approximately 50% after correcting for the nonspecific conversion (14%) of complexed F_1 DNA. The degree of nonspecific conversion is calculated from the amount of relaxation exhibited by the noncomplexed F_1 DNA contained in the same gradient.

Heat treatment (60°, 30 min) induced 17% conversion of the supercoiled, complexed F_1 DNA to the 53S DNA form without significantly affecting the admixed 80S DNA (Figure 4A,C). After this heat treatment the unconverted supercoiled

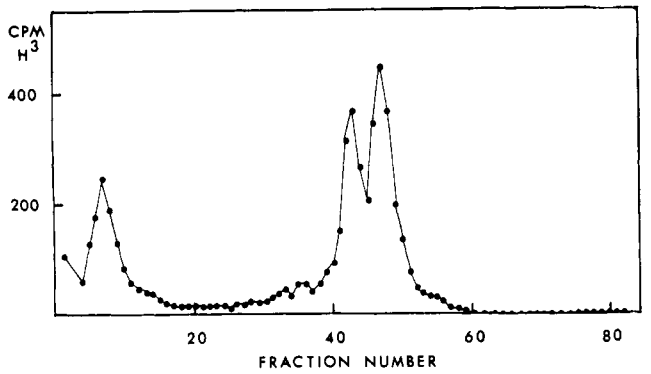

FIGURE 5: Sucrose gradient analysis of the product of alkaline-induced relaxation. Twice-purified F_1 DNA complex (approximately 90% 84 S) was layered directly onto a 20–31% alkaline sucrose density gradient maintained at 4° (0.3 M NaOH–1.0 M NaCl–5.0 mM EDTA). The material was then centrifuged at 4° in an SW65 titanium rotor at 50,000 rpm for 180 min. 4-drop fractions were collected directly onto papers, TCA washed, and counted. The peaks at fractions 44 and 48, respectively, represent single-strand circular and linear DNA. The peak at fraction 5 represents collapsed supercoiled DNA.

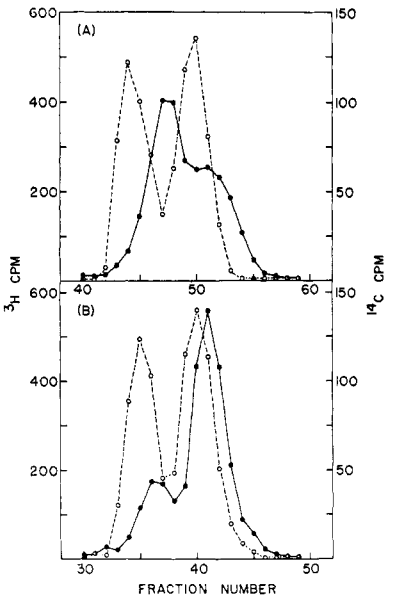

FIGURE 6: Test of strand specificity of nick in relaxed F_1 DNA. Twice-purified ^{3}H-labeled (●———●) complexed F_1 DNA was subjected to prolonged centrifugation on 20–31% alkaline sucrose gradients to induce relaxation and fractionate the single-stranded DNA as in Figure 5. Fractions (55 μl) were collected into polypropylene tubes and 5-μl portions of each fraction were counted. The leading half of the fast peak (circular single strands) and the trailing half of the slow peak (linear single strands) of the ^{3}H-labeled DNA were pooled separately, neutralized with TES, mixed with 50 μg of poly(U,G) and a small portion of denatured, randomly nicked, noncomplexed ^{14}C-labeled F_1 DNA (O-----O), and centrifuged to equilibrium (40 hr at 44,000 rpm in a Ti 50 fixed-angle rotor) in a neutral CsCl solutions, ρ = 1.750 g/cm^3 (A) linear single strands; (B) circular single strands. Fractions (6 drop) were collected directly onto paper, trichloroacetic acid washed, and counted. Recoveries were greater than 90% in all cases. Density increases from right to left.

fraction becomes insensitive to further induced relaxation by SDS (Figure 4C,D). Similar results have been obtained with three different preparations of the supercoiled, complexed F_1 DNA.

The possibility that complex is generated *in vitro*, for example, by lysozyme-mediated binding of F_1 DNA to membrane, was eliminated by the results of a reconstruction experiment involving the addition of differentially labeled noncomplexed, supercoiled 80S F_1 DNA to spheroplasts of labeled CR34 (F_1) followed by lysis of the mixture by Brij–deoxycholate treatment. After lysis and purification of the F_1 DNA, it was observed that the added supercoiled F_1 DNA sedimented approximately 5% slower than the differentially labeled endogenous F_1 DNA. Moreover, the exogenous F_1 DNA was resistant to relaxation by SDS whereas the endogenous F_1 DNA characteristically was sensitive to the SDS treatment.

Characterization of the Induced Slower Sedimenting Form. When purified supercoiled F_1 DNA complex is layered on an alkaline sucrose gradient, alkaline-induced relaxation occurs (Figure 3E). The relaxation products can be resolved into two approximately equal peaks by prolonged sedimentation in alkaline sucrose (Figure 5). The peaks differ in their observed sedimentation coefficients by a factor of 1.10. This is the sedimentation behavior expected of denatured circular duplex molecules containing a break in one strand of the DNA duplex (Vinograd *et al.*, 1965). It has been shown by Vapnek and Rupp (1970) that the two complementary strands of F_1 DNA have a different buoyant density in poly(U,G)–CsCl gradients. Thus, if the alkali-induced relaxation is specific for a given strand, then the resultant linear and circular strands should represent one or the other density class in a poly(U,G)–CsCl gradient. The results of an experiment based on this prediction clearly demonstrate that the linear strand purified by alkaline sucrose gradient centrifugation is found predominantly in the more dense position (Figure 6A) and the circular strand is found predominantly in the less dense position (Figure 6B). The linear strand fraction shows less specificity presumably as a result of nonspecific nicking which occurs during the centrifugation procedure. Most curiously, both heavy and light strands derived from the F_1 DNA complex are less dense in the poly(U,G)–CsCl gradient than the corresponding strands isolated from randomly nicked noncomplexed F_1 DNA. A similar result with respect to strand-specific relaxation was obtained for F_1 DNA complex converted to the open-circular DNA form by chymotrypsin treatment.

Discussion

Supercoiled F_1 DNA has been extracted from logarithmically growing *E. coli* cells and purified both by preparative dye–CsCl buoyant density and sedimentation velocity centrifugation procedures. The physical–chemical and biochemical properties of F_1 DNA isolated by the two procedures have been compared and several striking differences were found. Supercoiled, F_1 DNA isolated by the sedimentation velocity procedure sediments 5–10% faster than 80 S or supercoiled F_1 DNA isolated by the dye–CsCl procedure. Treatment of this complexed F_1 DNA (84–87 S) with proteases or protein denaturing agents results in a conversion to approximately equal amounts of the 80S and 53S forms of F_1 DNA whereas noncomplexed 80S F_1 DNA is unchanged by these treatments. The product of the induced relaxation of supercoiled F_1 DNA complex is a circular molecule containing a single nick or gap since (a) it has the sedimentation coefficient of the open-circular form (53 S) and not that of the linear form (45 S); and, (b) it can be separated in alkaline sucrose gradients into approximately equal amounts of single-stranded DNA with the sedimentation characteristics of open-circular and linear DNA (Vinograd *et al.*, 1965). Moreover, the nick or gap appears to be strand

specific since the purified linear and circular single strands are found predominantly as the heavy and light DNA strands, respectively, in poly(U,G)–CsCl gradients. If the relaxation event were not strand specific, then the circular and linear single strands of F_1 should each have given rise to equal amounts of the heavy and light DNA. It is of interest that light and heavy DNA strands derived from complexed F_1 DNA always band lighter than their respective counterparts derived from noncomplexed F_1 DNA. The basis of the difference is presently under investigation.

It appears from the effect of the proteases on the complexed supercoiled F_1 DNA that protein is involved in the relaxation event. However, the data presented here do not distinguish between a direct or an indirect effect, nor do they eliminate or identify other macromolecular species as essential participants in the relaxation event. In view of the probable attachment of F_1 DNA to the bacterial membrane (Jacob *et al.*, 1963), it is reasonable to speculate that membrane material is complexed to the F_1 DNA and is responsible for the 5–10% increase in the sedimentation coefficient.

Various models have been proposed to explain the behavior of supercoiled DNA–protein relaxation complexes toward proteolytic and protein denaturing agents (Clewell and Helinski, 1970c). In one of the models these agents are postulated to activate a latent strand-specific endonuclease. Also, it has been proposed that these agents inactivate a binding protein that associates with the region of a preexisting single-strand nick or gap in the supercoiled DNA relaxation complex. The results of the heat experiment (Figure 4) where a significant portion of the complex is rendered insensitive to relaxation by SDS after heat treatment argues against the latter model.

A physiological role for relaxation complexes has not been demonstrated for the *Col*E_1, *Col*E_2, or F_1 DNA system. An obvious role suggested by the latent endonuclease model is that activation of the nuclease provides a nicked site for initiation of DNA replication (Clewell and Helinski, 1970c). It is possible that a strand-specific nick at the same site is involved in both the initiation of normal F_1 DNA replication and in the initiation of the conjugal transfer of a unique single strand of F_1 DNA (Rupp and Ihler, 1968; Ohki and Tomizawa, 1968). Thus, it is of interest to point out that the linear strand produced by the induced relaxation of F_1 DNA is the same as the strand shown by Vapnek and Rupp (1970) to be transferred during conjugation.

Finally, it should be noted that our estimate of 62×10^6 for the molecular weight of F_1 DNA is not in accord with the estimate of 45×10^6 for F_1 DNA deduced by Friefelder (1968) from a comparative study of the X-ray-induced nicking of the supercoiled form of F_1 and λ DNA. The uncertainty in contour length measurements and the value of mass per unit length for a DNA molecule has been discussed extensively by Lang (1970) and Friefelder (1970). Although it is conceivable that our estimate of the F_1 molecular weight is in error due to these uncertainties, it is unlikely that the potential error is great enough to account for the difference in molecular weight reported for F_1 DNA in this study and by Friefelder (1968). A contour length for F_1 DNA corresponding to a molecular weight of approximately 62×10^6 has also been observed by Sharp *et al.* (P. A. Sharp, M. T. Hsu, and N. Davidson, personal communication).

Current studies on the F_1 DNA complex involve a detailed characterization of the morphological and enzymatic properties of this material and an examination of the potential role of this complex in the maintenance of this sex factor in *E. coli*.

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References

Bazaral, M., and Helinski, D. R. (1970), *Biochemistry 9*, 399.

Blair, D. G., Clewell, D. B., Sherratt, D. J., and Helinski, D. R. (1971), *Proc. Nat. Acad. Sci. U. S. 68*, 210.

Clewell, D. B., and Helinski, D. R. (1969), *Proc. Nat. Acad. Sci. U. S. 62*, 1159.

Clewell, D. B., and Helinski, D. R. (1970a), *Biochem. Biophys. Res. Commun. 40*, 608.

Clewell, D. B., and Helinski, D. R. (1970b), *Biochem. Biophys. Res. Commun. 41*, 150.

Clewell, D. B., and Helinski, D. R. (1970c), *Biochemistry 9*, 4428.

Freifelder, D. (1968), *Cold Spring Harbor Symp. Quant. Biol. 33*, 425.

Friefelder, D. (1970), *J. Mol. Biol. 54*, 567.

Ikeda, H., and Tomizawa, J. (1968), *Cold Spring Harbor Symp. Quant. Biol. 33*, 791.

Jacob, F., Brenner, S., and Cuzin, F. (1963), *Cold Spring Harbor Symp. Quant. Biol. 28*, 329.

Lang, D. (1970), *J. Mol. Biol. 54*, 557.

MacHattie, L. A., Berns, K. I., and Thomas, C. A. (1965), *J. Mol. Biol. 11*, 648.

Moller, W. J. (1964), *Proc. Nat. Acad. Sci. U. S. 51*, 501.

Ohki, M., and Tomizawa, J. (1968), *Cold Spring Harbor Symp. Quant. Biol. 33*, 651.

Opara-Kubinska, A., and Kubinski, H., and Szybalski, W. (1964), *Proc. Nat. Acad. Sci. U. S. 54*, 923.

Radloff, R., Bauer, W., and Vinograd, J. (1967), *Proc. Nat. Acad. Sci. U. S. 57*, 1514.

Roth, T. F., and Helinski, D. R. (1967), *Proc. Nat. Acad. Sci. U. S. 58*, 650.

Rupp, W. D., and Ihler, G. (1968), *Cold Spring Harbor Symp. Quant. Biol. 33*, 647.

Sinsheimer, R. L. (1959), *J. Mol. Biol. 1*, 43.

Vapnek, D., and Rupp, W. D. (1970), *J. Mol. Biol. 53*, 287.

Vinograd, J., Bruner, R., Kent, R., and Weigle, J. (1963), *Proc. Nat. Acad. Sci. U. S. 49*, 902.

Vinograd, J., Radloff, R., Watson, R., and Laipis, P. (1965), *Proc. Nat. Acad. Sci. U. S. 53*, 1104.