initiators in vivo, especially if V_{max} is not achieved due to nucleotide inhibitors or submaximal nucleotide concentrations. This may be especially true of the *E. coli* ribosomal cistrons, all of which contain a promoter with CTP as the initiating nucleotide. A very high $K_{i,A}$ for this species could make the promoter subject to initiation control. The level of transcription in vitro does indeed depend on the concentration of CTP (Gilbert et al., 1979). Although initiation rate control of transcription is probably not typical, it may be important for certain promoters, especially under physiological conditions involving suboptimal activity of nucleoside triphosphates.

Acknowledgments

We are grateful to Mary Ann Kaestner for her excellent technical assistance.

References

Carpousis, A. J., & Gralla, J. D. (1980) *Biochemistry 19*, 3245.
Chamberlin, M. J. (1976) in *RNA Polymerase* (Losick, R., & Chamberlin, M., Eds.) p 17, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Court, D., & Rosenberg, M. (1979) *Annu. Rev. Genet. 13*, 319.
Gilbert, S. F., de Boer, H. A., & Nomura, M. (1979) *Cell (Cambridge, Mass.) 17*, 211.
Gonzalez, N., Wiggs, J., & Chamberlin, M. J. (1977) *Arch. Biochem. Biophys. 182*, 404.
Johnsrud, L. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 5314.
Johnston, D. E., & McClure, W. R. (1976) in *RNA Polymerase* (Losick, R., & Chamberlin, M., Eds.) p 413, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Kennel, D., & Riezman, H. (1977) *J. Mol. Biol. 114*, 1.
Krakow, J. S., Rhodes, G., & Jovin, T. M. (1976) in *RNA Polymerase* (Losick, R., & Chamberlin, M., Eds.) p 127, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Maizels, N. M. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 3585.
Mangel, W. F., & Chamberlin, M. J. (1974) *J. Biol. Chem. 249*, 2995.
McClure, W. R., & Cech, C. L. (1978) *J. Biol. Chem. 253*, 8949.
McClure, W. R., Cech, C. L., & Johnston, D. E. (1978) *J. Biol. Chem. 253*, 8941.
Nierman, W. C., & Chamberlin, M. J. (1979) *J. Biol. Chem. 254*, 7921.
Nierman, W. C., & Chamberlin, M. J. (1980) *J. Biol. Chem. 255*, 1819.
Reeve, A. E., & Huang, R.-C. C. (1979) *Nucleic Acids Res. 6*, 81.
Rhodes, G., & Chamberlin, M. J. (1975) *J. Biol. Chem. 250*, 9112.
Schmitz, A., & Galas, D. J. (1979) *Nucleic Acids Res. 6*, 111.
Silverstone, A. E., Arditti, R. R., & Magasanik, B. (1970) *Proc. Natl. Acad. Sci. U.S.A. 66*, 773.
Simpson, R. B. (1979) *Cell (Cambridge, Mass.) 18*, 277.
Stefano, J. E., & Gralla, J. (1979) *Biochemistry 18*, 1063.
Stefano, J. E., Ackerson, J., & Gralla, J. (1980) *Nucleic Acids Res. 8*, 2709.

Single-Stranded Poly(deoxyguanylic acid) Associates into Double- and Triple-Stranded Structures†

Achilles Dugaiczyk,* Donald L. Robberson, and Axel Ullrich

ABSTRACT: Circular plasmid deoxyribonucleic acid (DNA), pBR322, was digested with the restriction endonuclease *PstI* to give full-length double-stranded DNA molecules, terminated by two self-complementary single-stranded sequences:

pG–C-T-G-C-A
A-C-G-T-C–Gp

The protruding 3′ termini were extended with dG by using calf thymus terminal deoxynucleotidyl transferase and dGTP, to form single-stranded tails of oligo(dG). At a length of about dG_{15}, such tails become resistant to single strand specific endonuclease S_1, and also cease to function as substrate (initiator) for the terminal deoxynucleotidyl transferase. This altered reactivity arises from association of the oligo(dG) tails into double- and triple-stranded structures, resulting in linear, circular, and branched polymers of the monomeric linear plasmid DNA. All these polymeric structures of the plasmid DNA are stable at room temperature, can be observed in the electron microscope, and can be separated from each other by agarose gel electrophoresis. At 60 °C or in 50% formamide, most of the oligo(dG) self-association can be reversed (melted), and the plasmid DNA is again found as the original linear monomer.

Among the principal nucleic acid components, it is notorously difficult to obtain experimental data on guanylic acid because of its low solubility and aggregation into viscous gels (Shapiro, 1968). An exact structure of its homopolymer remains uncertain. X-ray diffraction patterns for poly(rG) (Zimmerman et al., 1975) have been found to be virtually identical with those for poly(rI) (Rich, 1958; Arnott et al., 1974) and have been interpreted to represent four-stranded structures. Guanylic acid continues to reveal new and unpredicted properties, adding to our understanding of nucleic acids. For example, the structure of the self-complementary hexanucleotide d(CpGpCpGpCpG) has been unexpectantly established (Wang et al., 1979) to be an antiparallel left-

† From the Department of Cell Biology, Baylor College of Medicine, Texas Medical Center, Houston, Texas 77030 (A.D.), the Department of Biology, The University of Texas System Cancer Center, Houston, Texas 77030 (D.L.R.), and the Division of Molecular Biology, Genentech Inc., South San Francisco, California 94080 (A.U.). *Received May 14, 1980.* Supported in part by the Baylor Center for Population Research and Reproductive Biology (A.D.) and by Grant No. CA-16527 awarded by the National Cancer Institute, Department of Health, Education, and Welfare (D.L.R).

0006-2960/80/0419-5869$01.00/0

handed double helix, with the dG residues having the syn conformation rather than the usual anti as in the right-handed helix for the B deoxyribonucleic acid (DNA) model of Watson & Crick (1953).

Our interest in the properties of oligo(dG) arose from work on recombinant DNA, when we observed that (dG)- and only dG-tailed plasmids aggregate. This observation presented us with a new opportunity to investigate some properties of dG polymers, because any self-association of the dG tails would be reflected in the plasmid DNA molecules and thus readily observed by a variety of experimental methods not applicable to short dG stretches. It is demonstrated here that such single-stranded dG_{15}[1] oligomers readily associated into double- and triple-stranded structures, giving rise to linear, circular, and branched polymers of the monomeric plasmid DNA.

Materials and Methods

Enzymatic Reactions. Reactions involving the *Escherichia coli* DNA ligase (Dugaiczyk et al., 1975), calf thymus terminal deoxynucleotidyl transferase (Bollum, 1974), and single strand specific endonuclease S_1 (Vogt, 1973) were carried out as described in the literature.

Tailed Plasmid Derivatives. Plasmid pBR322 DNA was digested with the restriction endonuclease *Pst*I (BRL) according to the supplier's recommendations. The DNA was phenol extracted and precipitated with ethanol at −20 °C. The incorporation of labeled dGTP into the linear plasmid DNA was followed by determination of Cl_3AcOH–precipitable material. The reaction was stopped when the incorporation leveled off at 20–30 min. The relatively short reaction time should have prevented the formation of short oligomers, generated in an uninitiated reaction of the terminal transferase and dGTP, which has been demonstrated to take place in the absence of an initiator after a prolonged lag period (Bollum, 1974). The tailed plasmid DNA was further purified from inorganic pyrophosphate, dGTP, or other short oligomers by Sephadex G-100 column chromatography. The purified tailed plasmid derivative was analyzed by agarose gel electrophoresis (Helling et al., 1974). No radioactivity was found other than in regions corresponding to the mobility of plasmid DNA.

Electron Microscopy. Samples were prepared for electron microscopy by the basic protein Kleinschmidt technique essentially as described previously, with or without inclusion of formamide (Davis et al., 1971). Minor modifications of these procedures have been described earlier (Robberson et al., 1971, 1972). Partial thermal denaturation of one DNA sample, at a concentration of 1 μg/mL in 10 mM 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris) (pH 7.8), was achieved by heating at 60 °C for 90 s, followed by quenching in ice water for 2 min, after which the sample was prepared for electron microscopy by the aqueous Kleinschmidt technique (Davis et al., 1971) as modified (Robberson et al., 1971). Grids were rotary-shadowed with Pt–Pd (80:20) and examined with a Philips 300 electron microscope. Enlarged images of molecules, photographed on 35-mm film, were projected and traced. The length of individual molecules was measured with either a map measure or a Numonics digitizer, relative to an internal standard of PM2 DNA. The length of PM2 DNA was measured in other experiments relative to an internal standard of φX174 RF DNA, itself containing 5386 nucleotide pairs (Sanger et al., 1978). The length of PM2 DNA was thus determined to be 10 260 ± 200 nucleotide pairs.

[1] dG_{15} represents a mean value of 15 dG residues per DNA terminus. The same applies for dG_4 and dG_{65}.

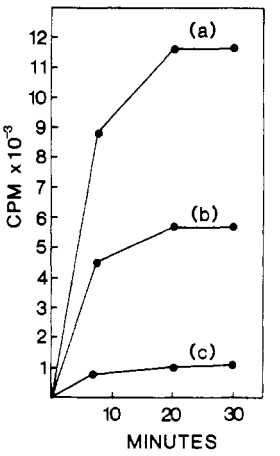

FIGURE 1: Incorporation of [^{3}H]dGTP into pBR322/*Pst*I DNA, catalyzed by the calf thymus terminal deoxynucleotidyl transferase (Bollum, 1974). At a DNA concentration of (a) 1.17 mg/mL, 2.65% of the dGTP present in the reaction mixture was incorporated, resulting in 15.4 dG residues per DNA terminus as the limit of incorporation. At a DNA concentration of (b) 0.585 mg/mL, 1.3% of total dGTP was incorporated, giving a limit incorporation of 14.8 dG residues per DNA terminus. At a DNA concentration of (c) 0.117 mg/mL, 0.24% of total dGTP was incorporated, resulting in 13.9 dG residues per DNA terminus.

Results

Length of Oligo(dG) Tails. The reaction catalyzed by the calf thymus terminal deoxynucleotidyl transferase (Bollum, 1974) was carried out at three different concentrations of the *Pst*I-linearized pBR322 DNA: 1.17, 0.585, and 0.117 mg/mL. Such conditions favor the formation of linear *n*-mers over circularization of this DNA (Dugaiczyk et al., 1975). We found that the reaction always stopped after the addition of 15 dG residues per DNA terminus, regardless of the concentration of the DNA termini (Figure 1). This limit incorporation was specific for the addition of dG residues. Long dT or dA tails could be polymerized not only at the *Pst*I-generated termini (–CTGCA), but also at these termini after prior extension by three to four dG residues (–CTGCAGGG). This indicates that a 3′-terminal dG nucleotide serves as an equally good initiator substrate for the enzyme.

Although the length of 15 dG residues per DNA terminus should be considered as a mean value, dG_{15}, the distribution of this value is rather narrow. This was verified on individual, cloned molecules. We have determined the DNA sequences across the dG–dC linkers in recombinant plasmids derived from the pBR322/*Pst*I-dG_{15} vector. In three instances, the linkers were found to be composed of 15 and once of 16 dG–dC residues. It is true that the length of such linkers in recombinant plasmids will depend both on the length of the original dG tails and on that of the dC tails; nevertheless, the experimental findings of 15 and 16 such linker residues corroborates the present data of dG_{15} residues per pBR322/*Pst*I terminus.

S_1 Resistance of Oligo(dG). By use of the terminal deoxynucleotidyl transferase (Bollum, 1974) and labeled deoxyribonucleotide triphosphates, two tailed derivatives were prepared from the *Pst*I-linearized pBR322 DNA. One of these derivatives had ^{32}P-labeled dG_{15} per terminus, the other had ^{32}P-labeled dG_4 further extended by ^{3}H-labeled dT_{65} per DNA terminus. Samples containing 1.8 μg of one of these tailed plasmids were digested for 1 h at 37 °C with such an amount of single strand specific endonuclease S_1 (Vogt, 1973) that would digest 10 μg of single-stranded DNA under these conditions. All samples were subsequently precipitated with 5% trichloroacetic acid in the presence of carrier DNA, and the radioactivity was counted in a scintillation counter. Table

Table I: Endonuclease S_1 Resistance of Single-Stranded dG and dT Hompolymers, Protruding from 3′ Termini of pBR322/*Pst*I DNA[a]

	^{32}P (cpm)	% S_1 resistance	^{3}H (cpm)	% S_1 resistance
$-[^{32}P](dG)_{15}$				
$-S_1$	1536	100		
$+S_1$	1398	90.4		
$-[^{32}P](dG)_4[^{3}H](dT)_{65}$				
$-S_1$	548	100	38696	100
$+S_1$	95	17.3	1957	5.1

[a] Samples of pBR322/*Pst*I-$[^{32}P]dG_{15}$ or pBR322/*Pst*I-$[^{32}P]-(dG)_4[^{3}H](dT)_{65}$ were digested with an excess of single strand specific endonuclease S_1. The results present limits of digestion.

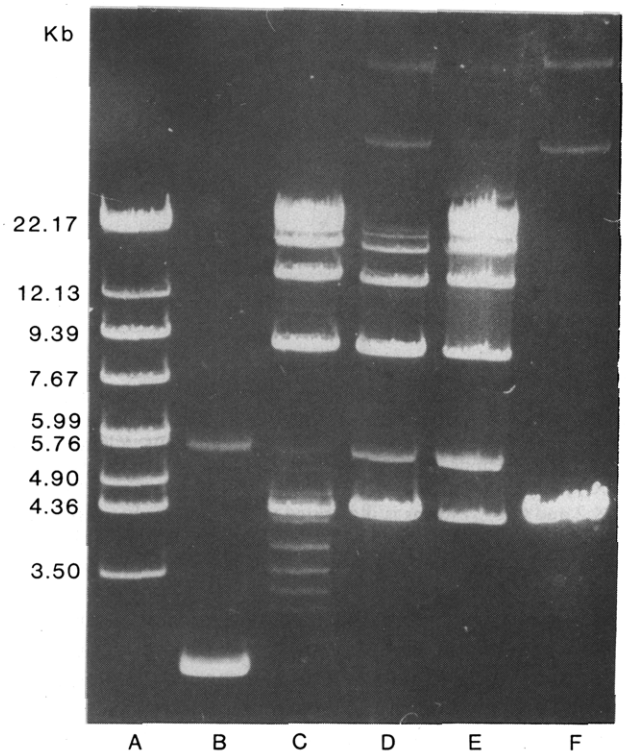

FIGURE 2: Agarose gel electrophoretic separation (Helling et al., 1974) of the products of ligation of pBR322/*Pst*I DNA (C) and of the products of association of the oligo(dG)-extended plasmid pBR322/*Pst*I-dG_{15} DNA (D-F). The same amount of DNA was loaded into slots C–F. The sample loaded into slot D had been previously stored at a concentration of 50 μg/mL in 10 mM Tris and 1 mM ethylenediaminetetraacetic acid (EDTA) (pH 7.4), and that in slot E had been stored at 1.84 mg/mL in the same buffer. Prior to loading, the sample in slot F was heated at 60 °C for 5 min and then quenched in ice water. Linear molecular DNA markers are separated in lane A; their molecular weights are as indicated. The fastest moving species in lane B is the super-coiled form of pBR322 DNA; the minor slower species is the relaxed circular form of the same plasmid DNA. The faint bands seen in lane C represent circular forms of pBR322 DNA differing in the number of supercoils.

I summarizes the results which are limits of digestion of the single-stranded tails. It can be seen that whereas poly(dT) is readily digested by the endonuclease S_1, even relatively short dG_{15} homopolymers are quite resistant to this enzyme under the same conditions.

Separation of Polymers of pBR322/PstI–dG$_{15}$ DNA. When *Pst*I-linearized pBR322 DNA was tailed with $\overline{15}$ dG residues and then subjected to agarose gel electrophoresis, a separation into molecular species was observed which closely resembled the separation of DNA species obtained in a reaction of ligation of the same plasmid DNA except terminated by complementary termini (Figure 2). The dG_{15}-tailed

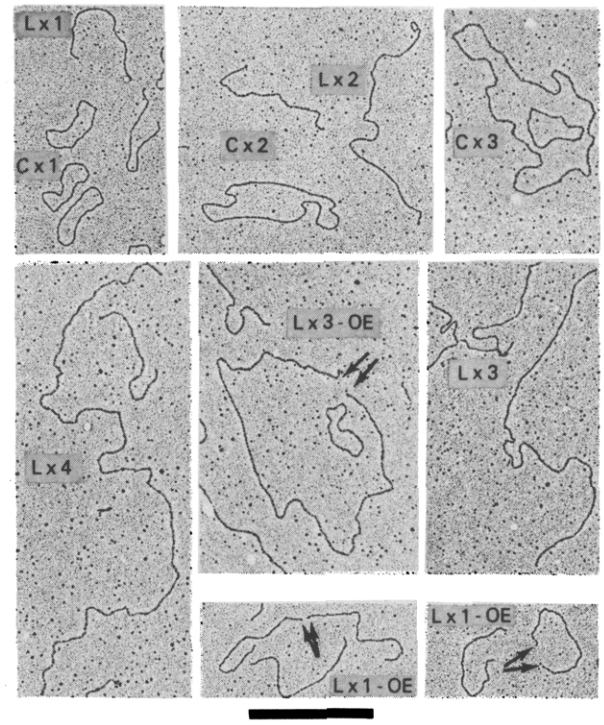

FIGURE 3: Electron micrographs of linear, circular, monomeric, and oligomeric unbranched forms of pBR322/*Pst*I–dG_{15} DNA. Samples were prepared for electron microscopy by the aqueous Kleinschmidt technique. The appearance of monomeric linear (L × 1) and circular (C × 1) as well as oligomeric linear (L × 2, L × 3, and L × 4) and circular (C × 2 and C × 3) forms is depicted. Linear forms with opposed ends (OE, indicated by arrows) are also shown. Magnification is the same for all micrographs and is indicated by a bar length of 1 μm.

plasmid DNA gives rise to apparently the same linear *n*-mers and a monomeric circular form as can be obtained by ligating *Pst*I-linearized pBR322 DNA. The only molecular species that are absent from the dG_{15}-held polymers are the circular forms with different numbers of supercoils, which are seen as faint bands among the products of ligation of *Pst*I-terminated pBR322 DNA (Figure 2C). Upon a brief exposure to 60 °C prior to electrophoresis, the dG_{15}-held polymers can be melted, giving rise to the original linear monomeric species (Figure 2F). We think that the faint bands of lowest electrophoretic mobility (Figure 2D–F) represent branched polymers of the plasmid DNA that are held together by triple-stranded dG_{15} structures (see electron microscopy). They exhibit an exceptionally low electrophoretic mobility in gels and a higher thermal stability in comparison with all the remaining linear and circular *n*-mers. We have never observed any DNA, even of the highest molecular weight, to exhibit such low mobility in agarose electrophoresis as do those few species that we believe represent branched structures of DNA.

Electron Microscopy of Linear and Circular Oligomers. The frequency distribution of lengths of linear and circular molecules of pBR322/*Pst*I–dG_{15} DNA was determined by electron microscopy. In one sampling, 212 linear molecules were detected having an average length of 4390 ± 260 base pairs. In the same sampling, 56 monomeric circles gave an average valaue of 4410 ± 280 base pairs. These numbers agree well with those predicted from electrophoretic mobility of the monomer (Figure 2) and with the value of 4361 base pairs for pBR322, determined by sequencing of the plasmid DNA (Sutcliffe, 1978).

The various other molecules that were seen in the electron microscope were always integral *n*-mers of the monomeric

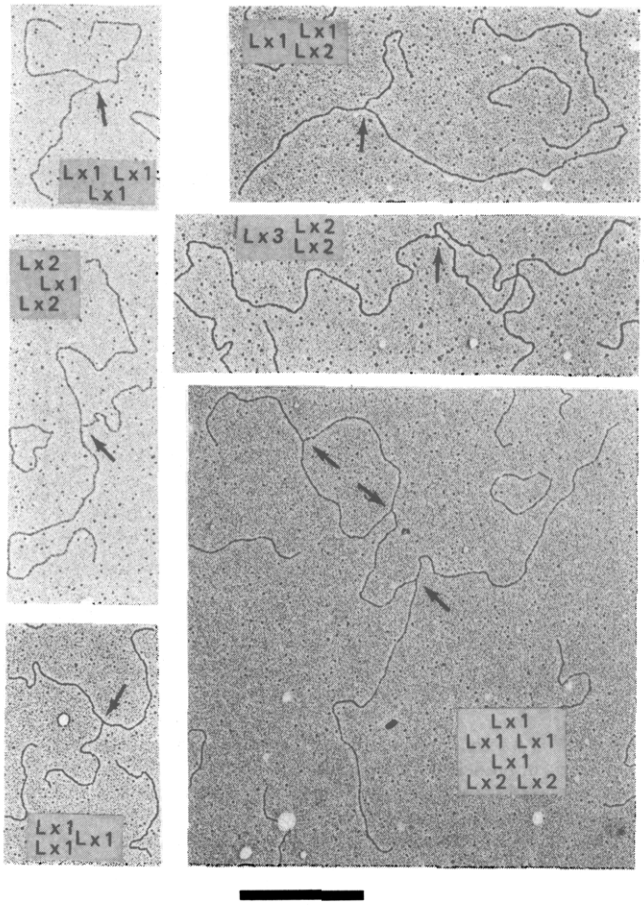

FIGURE 4: Electron micrographs of linear branched oligomeric forms of pBR322/*Pst*I–dG_{15} DNA. Samples were prepared for electron microscopy by the aqueous Kleinschmidt technique. The appearance of linear monomers (L × 1) and oligomers (L × 2 and L × 3) is illustrated with branch points indicated by arrows. Magnification is the same for all micrographs and is indicated by a bar length of 1 μm.

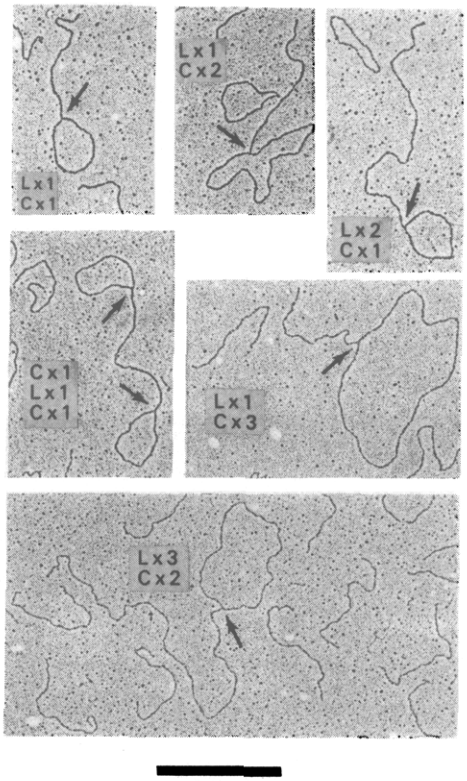

FIGURE 5: Electron micrographs of branched oligomers of pBR322/*Pst*I–dG_{15} DNA involving circular forms. Samples were prepared for electron microscopy by the aqueous Kleinschmidt technique. The appearance of monomeric linear (L × 1) and circular (C × 1) as well as oligomeric linear (L × 2 and L × 3) and circular (C × 2 and C × 3) forms is illustrated with junctions between linear and circular forms indicated by arrows. Magnification is the same in all micrographs and is indicated by a bar length of 1 μm.

form. Figures 3–5 illustrate examples of the various forms of molecules that were observed. Linear (L) and circular (C) monomeric and oligomeric forms of pBR322/*Pst*I–dG_{15} DNA can be seen in Figure 3. Molecules with opposed ends (double arrows in Figure 3) were seen quite frequently and are interpreted as coming from cyclized molecules which opened on drying in the electron microscope procedure. Figure 4 illustrates the appearance of various monomeric (L × 1) and oligomeric (L × 2, L × 3) linear branched forms of pBR322/*Pst*I–dG_{15} DNA. Figure 5 illustrates the appearance of branched oligomeric forms of pBR322/*Pst*I–dG_{15} DNA involving circular structures. Junctions between linear and circular forms are indicated by arrows. Table II summarizes the relative frequency of the various forms of the dG_{15}-tailed plasmid DNA before and after thermal and formamide denaturation. Although the relative amount of branched forms is rather small, branched forms are the most stable and resist denaturation (Table II). These branched DNA structures probably correspond to the forms that have the lowest electrophoretic mobility observed in agarose gels (Figure 2).

Discussion

In the present study we have analyzed certain of the associative properties of single-stranded dG homopolymers protruding from 3′ termini of plasmid DNA molecules. Their great tendency to self-associate results in the formation of very unusual structures of DNA. An analysis of this oligo(dG) self-association is nevertheless possible owing to a physical separation of interacting dG homopolymers by a stretch of plasmid DNA that does not participate in this interaction. Our electron microscopic observations of linear and circular as well as branched polymers of the monomeric linear plasmid DNA suggest that both double- and triple-stranded DNA structures, respectively, must have formed from the originally single-stranded dG homopolymers. The existence of double-stranded structures of the dG homopolymers is inferred from a substantial frequency of unbranched linear and circular oligomers of the plasmid DNA (Table II). The existence of triple-stranded structures is implied by the finding of branched polymers whose branch points contain three but never four arms. The lengths of such arms correspond to the length of pBR322 or an integral multiple thereof. In the association of three strands of oligo(dG), at least two of the strands have to assume a parallel orientation. The present results alone cannot, however, distinguish parallel vs. antiparallel orientation of strands in double-stranded structures of oligo(dG).

All of the oligomeric species of the dG-tailed plasmid DNA can also be observed by gel electrophoresis, indicating that the associations are quite stable at room temperature. The disappearance of the single-stranded character of the oligo(dG) is inferred from a resistance to digestion with the single strand specific endonuclease S_1 and also from the inability to function as substrate (initiator) for the terminal deoxynucleotidyl transferase, when a length of dG_{15} is reached. Although we can not determine from the present data whether hydrogen bonding or stacking interactions are the primary forces involved in the formation of the double- and triple-stranded dG structures, the results of this work should have practical implications in the field of recombinant DNA studies. Our

Table II: Frequencies of Monomeric and Oligomeric Forms of pBR322/PstI-dG_{15} DNA before and after Partial Denaturation Determined by Electron Microscopy

topological form	before partial denaturation[a]	after thermal partial denaturation[b]	after formamide partial denaturation[c]
linear unbranched			
monomer	47.9 (33.6)[d]	83.5 (71.8)	90.7 (82.6)
dimer	16.0 (22.4)	7.8 (13.5)	6.6 (12.0)
trimer	5.0 (10.7)	2.0 (5.2)	0.7 (1.3)
tetramer	1.5 (4:4)	0.0 (0.0)	0.2 (0.8)
higher oligomer	0.6 (2.5)	0.0 (0.0)	0.0 (0.0)
circular unbranched			
monomer	24.1 (17.0)	4.5 (3.9)	0.7 (0.6)
dimer	1.8 (2.5)	0.2 (0.3)	0.7 (0.0)
trimer	0.2 (0.4)	0.2 (0.4)	0.0 (0.0)
tetramer	0.2 (0.5)	0.0 (0.0)	0.0 (0.0)
higher oligomer	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
oligomeric branched			
with circle	0.8 (1.1)	0.7 (1.6)	0.2 (0.4)
without circle	1.9 (4.9)	1.1 (3.3)	0.9 (2.3)
no. of molecules classified	514	596	441
mass of molecules classified[e]	731	694	484

[a] Sample was prepared for electron microscopy by the basic protein Kleinschmidt technique. [b] Sample at a concentration of 1 μg/mL in 0.01M Tris (pH 7.8) was heated at 60 °C for 90 s then quenched in ice water. [c] Sample was partially denatured by dilution in the spreading solution used in the formamide modification of Kleinschmidt technique at 23 °C. This denaturing solution contained 50% formamide, 0.1M Tris (pH 8.5), and 0.01M EDTA. After addition of cytochrome *c* to a final concentration of 100 μg/mL the sample was layered onto a hypophase of 17.5% formamide, 0.01M Tris (pH 8.5), and 0.001M EDTA. [d] Numbers in parentheses are percentages of total mass of DNA. [e] Each oligomeric form is weighted by the number of monomer genomes it contained when the monomer is given unit value.

finding of a limit incorporation of dG_{15} per 3′ terminus of DNA should allow one to determine the molar concentration of 3′ termini in any sample of DNA, irrespective of size inhomogeneity of that DNA, by simply determining the limit molar incorporation of labeled dGTP and dividing the value by 15.

Acknowledgments

We thank Marion Gay for technical assistance, Elizabeth Sjoberg for help in measuring molecules, and James W. Hawkins for reading the manuscript.

References

Arnott, S., Chandrasekaran, R., & Martilla, C. M. (1974) *Biochem. J. 141*, 537–543.

Bollum, F. J. (1974) *Enzymes, 3rd Ed. 10*, 145–171.

Davis, R. W., Simon, M. E., & Davidson, N. (1971) *Methods Enzymol. 21*, 413–428.

Dugaiczyk, A., Boyer, H. W., & Goodman, H. M. (1975) *J. Mol. Biol. 96*, 171–184.

Helling, R. B., Goodman, H. M., and Boyer, H. W. (1974) *J. Virol. 14*, 1235–1244.

Rich, A. (1958) *Biochim. Biophys. Acta 29*, 502–509.

Robberson, D. L., Aloni, Y., & Attardi, G. (1971) *J. Mol. Biol. 55*, 267–270.

Robberson, D. L., Kasamatsu, H., & Vinograd, J. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 737–741.

Sanger, F., Coulson, A. R., Friedman, T., Air, G. M., Barrell, B. G., Brown, N. L., Fiddes, J. C., Hutchison, C. A., III, Slocombe, P. M., & Smith, M. (1978) *J. Mol. Biol. 125*, 225–246.

Shapiro, R. (1968) *Prog. Nucleic Acid Res. Mol. Biol. 8*, 73–112.

Sutcliffe, J. F. (1978) *Nucleic Acids Res. 5*, 7461–7467.

Vogt, V. M. (1973) *Eur. J. Biochem. 33*, 192–200.

Wang, A. H. J., Quingley, G. J., Kolpak, F. J., Crawford, J. L., Van Boom, J. J., Van der Marel, G., & Rich, A. (1979) *Nature (London) 282*, 680–686.

Watson, J. D., & Crick, F. H. C. (1953) *Nature (London) 171*, 737–738.

Zimmerman, S. B., Cohen, G. H., & Davies, D. R. (1975) *J. Mol. Biol. 92*, 181–192.