has been found to be present in corn ctDNA. The inability to localize the above three tRNA genes in ctDNA of corn and pea probably is a result of inadequate charging of the tRNAs rather than the lack of their genes in ctDNA.

A number of isoaccepting species of tRNAs have been reported in chloroplasts (Guillemaut & Weil, 1975). In experiments using three isoaccepting species of leucyl-tRNAs and two isoaccepting species of phenylalanyl-tRNA, the hybridization data obtained by Weil et al. (1977) have shown that the isoaccepting species of leucyl-tRNA are coded by the same gene(s). The same was found to be true for the isoaccepting species of phenylalanyl-tRNA. The multiplicity of tRNA genes in ctDNA must be tightly restricted because we have found that there are only ~40 tRNA genes in ctDNA. In addition, the data of Table II do not support the gross multiplicity of any particular aminoacyl-tRNA.

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Conformational Changes in Deoxyribonucleic Acid during Transcription†

William Wachsman and Donald D. Anthony*

ABSTRACT: Circular dichroism (CD) was used to examine changes in secondary structure of calf thymus DNA during in vitro transcription. Formation of a binary complex between DNA and RNA polymerase (nucleoside triphosphate:nucleotidyltransferase, EC 2.7.7.6) did not alter the CD spectrum of the DNA. Alterations in ellipticity in the spectral region between 245 and 300 nm occurred during synthesis of RNA. This change was consistent with a B- to A-like form transition in polynucleotide conformation. The increment of ellipticity consisted of two separate components; component I was insensitive to treatment with pancreatic ribonuclease whereas component II was a ribonuclease labile fraction. Cleavage by restriction endonucleases did not produce or significantly alter the ellipticity of transcription. In contrast, between 50% and 60% of the component I ellipticity was sensitive to pancreatic DNase I. The data indicate that component I is a property of DNA and suggest that the alteration in secondary conformation which affects this component extends cooperatively beyond the DNase I insensitive DNA–RNA polymerase complexes.

On the basis of X-ray diffraction data for DNA, RNA, and DNA/RNA hybrids, it has been suggested that the ability of DNA to be transcribed may be regulated by its secondary structure. DNA can be identified in a number of conformations (for review, cf. Arnott, 1970). These are dependent upon the degree of hydration and the type and concentration of counterions present. In contrast, RNA or DNA/RNA hybrids have more limited conformational flexibility and have been found to exist only in an A-like conformational form (Arnott et al., 1968, 1973). From such data it has been suggested that a B- to A-form transition in the DNA would favor its transcription (Arnott et al., 1968).

There has been little direct experimental work to support or contradict a major role for the structure of DNA in regulating transcription. Indirect biochemical evidence obtained by Travers' group (Travers et al., 1973; Travers, 1974) and by Nankanishi et al. (1974, 1975) indicates that variations in temperature and ionic strength, as well as the presence of

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Me_2SO and glycerol, can alter the quality of RNA synthesized in vitro. The data suggested that the target of these environmental variations or chemicals was DNA. In a more direct examination of structure–function interrelationships, Beabealashvily et al. (1972) showed alterations in circular dichroism (CD)[1] during transcription of DNAase I resistant binary complexes of DNA and RNA polymerase. It was suggested that these changes could be due to either formation of RNA product or alterations in DNA structure.

The work in the present communication demonstrates CD changes during transcription of native, double-stranded calf thymus DNA qualitatively similar to those reported by Beabealashvily et al. (1972). The data indicate that a B- to A-like form transition in the structure of DNA occurs which is greater than that predicted by accumulation of RNA product. These alterations in CD are dependent upon the addition of nucleoside triphosphates but do not have an absolute requirement for the polymerization of RNA. The changes in DNA base stacking were found to extend beyond the immediate environment of protection afforded DNA by formation of the binary complex with RNA polymerase.

Materials and Methods

Enzymes. RNA polymerase was isolated from strain MRE-600 *Escherichia coli* according to a modification of the procedures of Babinet (1967). Bacterial cells harvested in mid–late log phase were suspended in an equal volume of buffer A (50 mM Tris–HCl, pH 7.9, 0.1 mM EDTA, and 0.1 mM dithiothreitol). Acid-washed glass beads (250 μm in diameter) were added in a ratio of 300 g/100 g of bacteria. Cellular membranes were disrupted by high-speed homogenization in a Waring blender, and the soluble fraction was washed free of membrane by centrifugation at 16000 g for 10 min. The soluble fraction was then fractionated as described by Babinet except that buffer A was utilized for all extractions, elution II was done with 1.6 M NaCl instead of 2.0 M NaCl, and elution III employed poly(ethylene glycol) (PEG), 20000, in place of PEG, 6000. Further purification by DE-52 anion-exchange chromatography and subsequent high salt concentration elution from Bio-Gel A 1.5m Agarose gel was done as described by Burgess (1969). Polymerase was stored at 4 °C in buffer containing 0.01 M Tris, pH 7.9, 0.01 M $MgCl_2$, 0.1 M KCl, 0.1 mM dithiothreitol, 0.1 mM EDTA, and 50% glycerol. Specific activity with calf thymus DNA as the template was 900–1300 units/mg protein (a unit was defined as the incorporation of one nmol of [^{3}H]CTP into acid-precipitable RNA in 20 min at 37 °C). Between 85 and 95% of the protein of the final enzyme preparation migrated in bands which corresponded in molecular weight to RNA polymerase subunits when electrophoresed in 0.1% sodium dodecyl sulfate–2.5% polyacrylamide gel. On the basis of relative content of the band which corresponded to the $\beta\beta'$ subunit, the stoichiometry for the σ subunit was 0.35–0.5. The polymerase was free of ribonuclease and deoxyribonuclease as determined by incubation with radiolabeled ribosomal RNA and by conversion of SV40 form I DNA to form II DNA.

Bovine pancreatic ribonuclease was purchased from Calbiochem. It was dissolved in 50 mM Tris-HCl (pH 7.8) and subsequently heated at 100 °C for 20 min. Electrophoretically pure pancreatic deoxyribonuclease I was a product of Schwarz/Mann. *Eco*R1, *Hin*dIII, and *Bam*I restriction endonucleases were obtained from Miles Biochemicals.

[1] Abbreviations used: CD, circular dichroism; NTP, nucleoside triphosphate; PEG, poly(ethylene glycol).

DNA and RNA. Calf thymus DNA was isolated by the method of Kay et al. (1952). The concentration of DNA was determined by the modified diphenylamine method of Burton (1956). Whole-cell guinea pig RNA, a gift from A. Berkman, was isolated according to the procedure of Cooper & Kay (1969) as modified by Purtell & Anthony (1975). RNA complementary to calf thymus DNA was synthesized in vitro in a reaction mixture containing 1 mg of calf thymus DNA and 200 units of *E. coli* RNA polymerase per mL of 50 mM Tris-HCl (pH 7.8), 8 mM $MgCl_2$, 2 mM $MnCl_2$, 5.4 mM β-mercaptoethanol, and 0.4 mM each of ATP, GTP, UTP, and CTP. The final volume of the reaction mixture was 10 mL. Following incubation at 37 °C for 45 min, 300 μg of DNAase I was added, and the incubation was continued for an additional 30 min. The cRNA was purified by extraction with an equal volume of phenol saturated with 10 mM Tris-HCl, pH 7.2. The aqueous phase was precipitated in 2.5 volumes of 95% ethanol at −20 °C, and cRNA was pelleted and resuspended in 10 mM Tris-HCl, pH 7.2. The phenol extraction was repeated. Traces of phenol and small molecular weight substrates were removed by three additional ethanol precipitations. RNA prepared in this manner had an $OD_{260/280}$ ratio of 1.96 and an $OD_{260/235}$ ratio of 2.12. The orcinol reaction (Lin & Schjeide, 1969) was utilized to determine RNA concentration.

Nucleotides. Unlabeled and ^{3}H-labeled ribonucleoside triphosphates were obtained from Schwarz/Mann. Purity of the compounds was determined by thin-layer chromatography on PEI-cellulose by using a 1.0 M LiCl solvent system (pH 7.0).

Biochemical Assays. RNA polymerase activity was ascertained by in vitro incorporation of ribonucleoside triphosphates into acid-precipitable material. Unless otherwise noted, the standard reaction contained 50 mM Tris-HCl (pH 7.8), 8 mM $MgCl_2$, 2 mM $MnCl_2$, 5.4 mM β-mercaptoethanol, 0.4 mM each of ATP, GTP, UTP, and [^{3}H]CTP ($1–2 \times 10^3$ cpm/nmol), 50 μg/mL calf thymus DNA, and RNA polymerase. Following incubation at 37 °C, aliquots of 0.1 mL were removed and precipitated in 5 mL of 5% trichloroacetic acid at 4 °C. The precipitates were filtered on Millipore nitrocellulose filters (0.45 μm). Membranes were then washed and counted in a scintillation system as previously described (Anthony et al., 1969).

Circular Dichroism. CD spectra were recorded with a Jasco J-20 CD spectropolarimeter. All measurements were thermostatically controlled at 37.0 ± 0.2 °C in a water-jacketed cuvette (Optacel Co.). CD data are presented in terms of molar ellipticity based on total nucleoside content of DNA for each reaction unless otherwise specified.

For CD spectra, the standard biochemical reaction contained 0.1 mM each ATP, GTP, UTP and CTP, 100 μg/mL calf thymus DNA, and 60 units of RNA polymerase. Additions of ribonuclease and/or deoxyribonuclease were as described in legends to figures. Reaction components were mixed and equilibrated at 4 °C prior to addition of RNA polymerase. The sample was then placed into a preheated (37 °C) cuvette or vessel, and CD spectral determinations were begun within a 20-s delay time. Biochemical assays done in parallel to corresponding CD analysis contained identical concentrations of reaction components except that [^{3}H]CTP was used in place of CTP. Synthesis of RNA was monitored as described.

Results

Presented in Figure 1 are the CD spectra for calf thymus DNA, *E. coli* RNA polymerase, and the association complex of these two molecules. The DNA exhibits a conservative

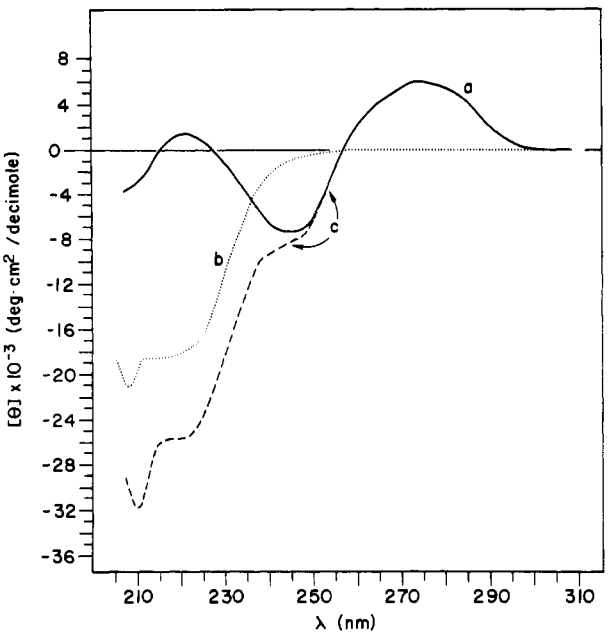

FIGURE 1: CD spectra of calf thymus DNA and RNA polymerase. Spectra were obtained for RNA polymerase, DNA, and the binary complex of DNA and RNA polymerase. Components were preincubated prior to spectral analysis for 30 min at 37 °C to equilibrate binding between DNA and the enzyme. Reactions contained 50 mM Tris-HCl, pH 7.8, 5.4 mM β-mercaptoethanol, 8 mM $MgCl_2$, and 2 mM $MnCl_2$ in a final volume of 1.0 mL. In addition, reaction a contained 100 mg of calf thymus DNA, reaction b, 60 units of RNA polymerase, and reaction c, 100 μg of DNA plus 60 units of RNA polymerase. Spectra were determined at 37 °C as described under Materials and Methods.

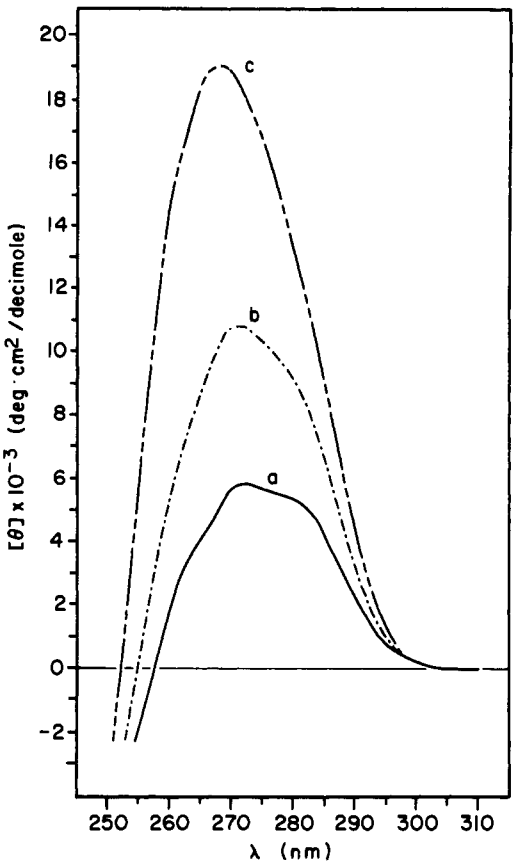

FIGURE 2: Comparison of the CD spectra of DNA, DNA plus AGUC, and the complete transcription system. Reaction conditions were as described in the legend to Figure 1. Reaction a contained 100 μg of calf thymus DNA, reaction b, 100 μg of DNA plus 0.1 mM each of ATP, GTP, UTP and CTP, and reaction c, 100 μg of DNA, 0.1 mM AGUC, and 60 units of RNA polymerase. Transcription (reaction c) was initiated by addition of enzyme. Following incubation for 45 min at 37 °C, spectra were determined at 37 °C as described under Materials and Methods.

spectrum in which the positive ellipticity between 257 and 300 nm is approximately equal to the negative ellipticity between 225 and 257 nm (spectrum a). Such conservation of positive and negative rotatory strength is representative of B form DNA as described by Tunis-Schneider & Maestre (1970). At the concentration of RNA polymerase utilized in this and subsequent experiments in this paper, it contributed no resolvable ellipticity above 255 nm (spectrum b).

Under the conditions used, RNA polymerase is known to bind to DNA (Anthony et al., 1969). Except for an additive contribution from the CD spectrum of RNA polymerase, the formation of this enzyme–DNA complex did not alter either the qualitative or quantitative CD spectral characteristics of DNA between 235 and 310 nm (spectrum c). This identity of peak, crossover, and trough indicated that the base-stacking parameters of DNA, as defined by CD, were not altered by the association between DNA and polymerase. In contrast, negative ellipticity below 235 nm was greater than the additive contributions of enzyme and DNA. It is likely that the interaction between the protein and DNA results in structural change within the protein. Such an interpretation would be consistent with the data of Hinkle & Chamberlin (1972).

Changes in CD during the Process of Transcription. Addition of the four nucleoside triphosphate substrates to DNA caused the spectral shift denoted by the area between curves a and b of Figure 2. This change in spectra was due to additive contributions between the CD of DNA and of the four nucleoside triphosphates (data not shown). In contrast, when transcription was initiated by addition of the polymerase, a further spectral shift denoted by the area between curves b and c occurred. This change was associated with a blue shift both in the peak of ellipticity and in the crossover point. A relative decrease in trough also occurred as positive ellipticity above 255 nm increased. The transition to these spectral features is consistent with the development of a nonconservative spectrum and is indicative of alterations in the base stacking of polynucleotides. Such alterations occur during formation of an RNA polymer or by a B- to A-form transition in the structure of DNA.

Contribution of RNA Product to CD of Transcription. Under reaction conditions identical with those of the CD experiments, the quantity of acid-precipitable RNA was measured as described under Materials and Methods. When the reaction had reached a plateau, 69 nmol of product had accumulated. The experimentally derived molar ellipticity at 270 nm for RNA complementary to calf thymus DNA in 0.05 M Tris-HCl (pH 7.8), 8 mM $MgCl_2$, 2 mM $MnCl_2$, and 5.4 mM β-mercaptoethanol was 11 665. On the basis of this and the quantity of product synthesized, the maximal contribution from RNA was 35% of the CD alteration induced during transcription.

For a more direct assessment of the contribution of product, pancreatic RNase was included in the transcription system (Figure 3). Spectrum a represents the complete transcription system minus RNA polymerase and spectrum c the complete system including the enzyme. The addition of RNase to the complete system (spectrum b) produced a decrease in the CD of transcription (spectrum b vs. spectrum c). The stipled region in Figure 3 designates the increment of transcription-related CD which persisted after treatment with the RNase. In a comparable experiment in which RNA synthesis was measured, the addition of the same amount of RNase reduced

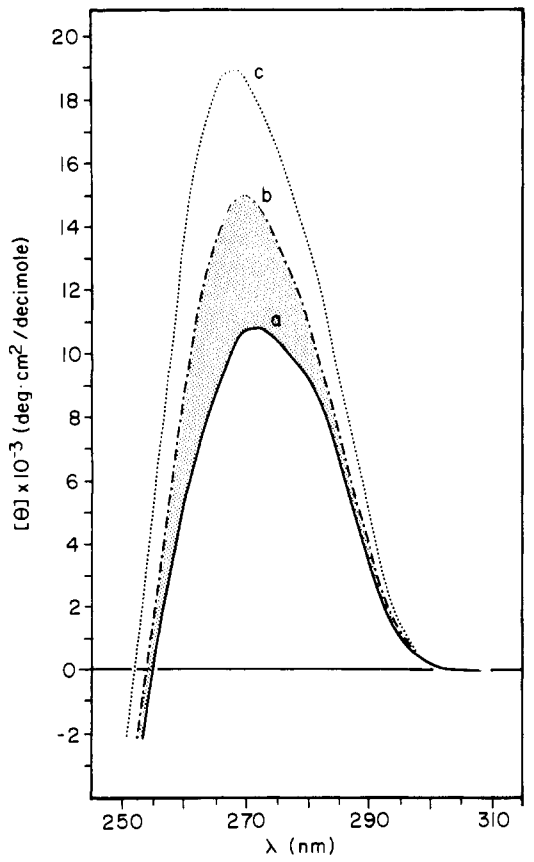

FIGURE 3: Effect of RNase on the CD of transcription. Reaction conditions were as described in the legends to Figures 1 and 2. Reaction a contained DNA plus NTP, and reactions b and c contained the complete transcription system. Reaction b contained, in addition, 20 μg/mL pancreatic ribonuclease. Following addition of RNA polymerase, reactions b and c were incubated 45 min at 37 °C. CD was determined at 37 °C as described under Materials and Methods.

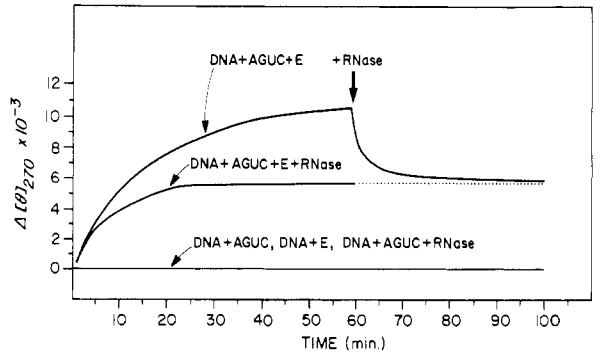

FIGURE 4: Effect of RNase on the kinetics of the transcription CD. The reaction for complete transcription (DNA + AGUC + E) was as described in the legend to Figure 2. Pancreatic RNase (20 μg/mL) was added to aliquots of this reaction at time zero and at 60 min as designated. Circular dichroism with enzyme or AGUC absent from the system was adjusted to the same base-line value as designated. Incubations and determination of CD at 270 nm were at 37 °C as previously described.

acid-precipitable RNA product to less than 8% of the total amount synthesized. In subsequent kinetic experiments, CD was measured at 270 nm.

Figure 4 illustrates the time course of the CD change during the process of transcription in the presence and absence of pancreatic ribonuclease. When ribonuclease was added to the reaction mixture at zero time, the initial rate of change in CD was similar to that of a complete reaction mixture minus RNase. The total change in CD at the plateau level was 54% of that obtained in the absence of RNase. When RNase was added at 60 min after the reaction was started, a decrease in ellipticity occurred which plateaued at a level comparable to that obtained when RNase was present from time zero. Thus, the reaction can be separated into two components, component I, which is not influenced by RNase, and component II, which is RNase labile.

Table I: Effect of Reaction Products and Restriction Endonucleases on the CD of Transcription[a]

expt no.	reaction components (additions or delections)	−RNase $\Delta[\theta]_{270}$ (deg)	−RNase % of control	+RNase $\Delta[\theta]_{270}$ (deg)	+RNase % of control
1		9630	100	5545	100
2	$-Mn^{2+}$, Mg^{2+}	0	0		
3	−AGUC	0	0		
4	−polymerase	0	0		
5	+PPi	9535	99.9		
6	−AGUC + PPi	0	0		
7	+*Eco*R1			5467	99
8	−AGUC + *Eco*R1	0	0		
9	+*Hin*dIII			5711	103
10	−AGUC + *Hin*dIII	0	0		
11	+*Bam*I			4686	85
12	−AGUC + *Bam*I	0	0		

[a] Reaction mixtures for the complete transcription system contained 100 μg of calf thymus DNA, 60 units of RNA polymerase, 2 mM $MnCl_2$, 8 mM $MgCl_2$, 5.4 mM β-mercaptoethanol, 50 mM Tris-HCl, pH 7.8, and 0.1 mm each of ATP, GTP, UTP, and CTP (AGUC) in a final volume of 1.0 mL. Additions or deletions were as designated in column 2. Pyrophosphate was added to experiments 5 and 6 in a final concentration of 0.4 mM. Restriction endonucleases *Eco*R1, *Hin*dIII, and *Bam*I were added in final concentrations of 24, 24, and 20 units per mL, respectively (experiments 7–12). Reactions were initiated by addition of RNA polymerase at 37 °C. Circular dichroism was measured as described under Materials and Methods. Percent change in ellipticity at 270 nm was determined after reactions had reached plateau (60 min).

Effect of Reaction Products and Endonucleolytic Cleavage of DNA on the CD of Transcription. Table I outlines the requirements of the complete transcription system. These experiments were done either in the presence of ribonuclease, which measured component I CD, or in its absence, which measured components I plus II CD. The ellipticity increase requires metal cofactors in addition to DNA, ribonucleoside triphosphate substrates, and enzyme. Since pyrophosphate is released during synthesis of RNA, the contribution of this molecule was examined. Addition of 0.4 mM pyrophosphate did not cause any increment of change in the CD of the complete transcription system (experiment 5) or when ribonucleoside triphosphate substrates were absent from the system (experiment 6).

Further experiments were done to determine if endonucleolytic cleavage of DNA is a causal factor for the CD change. Addition of *Eco*R1 or *Hin*dIII endonuclease had no effect on the component I CD of the complete transcription system (experiments 7 and 9) or on the CD of DNA in the presence of RNA polymerase (experiments 8 and 10), *Bam*I also had no effect on the CD of DNA plus RNA polymerase (experiment 12). *Bam*I in the presence of the complete transcription system, however, caused an ellipticity decrease of 15% at the plateau level of CD (experiment 11). Thus endonucleolytic cleavage as produced by these restriction enzymes was not the cause of the component I increase in CD.

For demonstration that component I was due to change in DNA structure, experiments were done with both pancreatic RNase and DNase present (Figure 5). All the experiments were done with RNase added at time zero. When DNase I was added to the transcribing system from time zero (curve

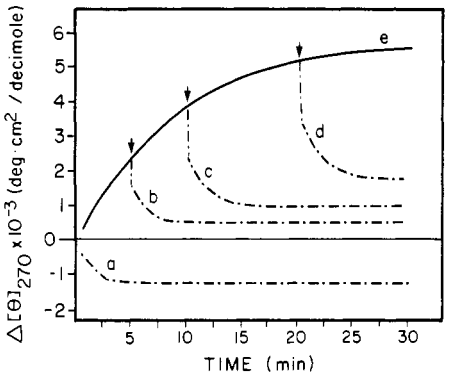

FIGURE 5: Effect of DNase on component I CD. The complete transcription reaction was as described in the legend to Figure 2. Pancreatic RNase (20 μg/mL) was added to all reactions at time zero. Pancreatic DNase I (20 μg/mL) was added at times designated. Incubations and determination of CD at 270 nm were at 37 °C as previously described. (a) Addition of DNase at time zero; (b) addition of DNase at 5 min; (c) addition at 10 min; (d) addition at 20 min; (e) the complete system minus DNase.

a), a decrease in molar ellipticity at 270 nm of 565 was observed. This same decrease was obtained when DNase was added to DNA alone (data not indicated). This diminution in rotatory strength represents a loss of order which occurs during degradation of the DNA polymer. Addition of DNase I at 5, 10, and 20 min after transcription was initiated caused, respectively, a 51%, 56%, and 52% decrease in ellipticity relative to that present at the time of DNase addition. This percent decrease in ellipticity was relative to the base line which was obtained when DNase I was added at zero time. The data indicate that component I CD is partially sensitive to degradation of DNA.

Discussion

Tunis-Schneider & Maestre (1970) were the first to report the use of CD to determine secondary structure of DNA. Their observation on thin-film preparations of DNA was extended to polynucleotides in aqueous solution by Ivanov et al. (1973). An important contribution by these authors was that conservative and nonconservative CD spectra were correlated to B- and A-like forms of DNA. The basis for this qualitative relationship between spectral characteristics and secondary conformation is assumed to be alterations in base stacking within the polynucleotide structure.

As a solution technique, CD provides a direct measure of polynucleotide conformation during transcription. Using DNase I digested fragments of DNA bound by RNA polymerase (kegs), Beabealashvily et al. (1972) observed alterations in CD during synthesis of RNA. The contribution of RNA product to this change was not assessed in these early experiments. The present communication reports similar spectral changes for a system that utilizes nondigested calf thymus DNA. Contribution of RNA product to the total CD change was separated from that caused by alteration in DNA structure in several ways. First, the contribution to ellipticity at 270 nm calculated from the amount of RNA synthesized under identical conditions was less than 35% of the total increase in ellipticity observed. Second, the experiments with pancreatic ribonuclease (Figures 3 and 4) confirmed that the contribution from RNA product was no more than 47% of the ellipticity increase when the CD reaction had reached a plateau. The data with ribonuclease illustrate further that the spectral change during transcription consists of at least two components. One is resistant to ribonuclease and follows first-order kinetics (component I), and the other is eliminated by ribonuclease (component II). In other experiments in which initiation of synthesis but not polymerization occurred (only ATP plus GTP present as substrate), only component I was observed (W. Wachsman and D. D. Anthony, unpublished results). Thus formation of RNA polymer is not necessary for this component of the spectral reaction.

Requirements for generation of component I are DNA, RNA polymerase, nucleoside triphosphates, and magnesium and manganese (Table I). Binding of the enzyme to DNA in the absence of nucleotide triphosphates did not cause detectable alteration in the region of the spectrum characteristic of DNA conformation. This result contrasts with data generated by analysis of sedimentation properties (Saucier & Wang, 1972) and by the kinetic formaldehyde method (Zarudnaya et al., 1976). By these analytical techniques, binding of enzyme to DNA in the absence of nucleotide triphosphates produced changes that were interpreted as alteration in DNA structure. Addition of the nucleoside triphosphates did not substantially increase this change. There are several possible explanations for the difference between our results and these data. However, since DNA structural changes during the dynamic processes of transcription are not evaluated by the techniques used by these investigators, it is likely that the results are not comparable.

The elimination of greater than 50% of component I spectral change when DNase I was added to the reaction (Figure 5) demonstrated that at least this amount of component I is due to properties of DNA. Failure to eliminate more than 56% was very likely due to the DNase resistant cores of the enzyme–DNA complex. Consideration was given to the possibility that segments of the RNA product also contributed to this component and that these became sensitive to RNase as DNA digestion occurred. This was not likely for the following reasons: first, as noted earlier, the component I occurred without polymerization (unpublished experiments); second, after RNase digestion only 8% of the RNA synthesized remained; this amount of RNA was too small to account for the DNase sensitive change; and, finally, in experiments in which both DNase and RNase were used, the amount of RNA resistant to RNase was not changed from that which occurred with RNase alone.

From these data, it can be concluded that the component I reaction is caused by structural change in DNA. Prior to the time the reaction reaches plateau, the DNA must exist in at least four separate states: (a) RNA polymerase protected but structurally not altered, (b) RNA polymerase protected and structurally altered, (c) unprotected by RNA polymerase and structurally altered, and (d) unprotected by RNA polymerase and structurally not altered. The DNase resistant sites which are structurally not altered would correspond to DNA–enzyme association complexes (Figure 2, Table I). DNase resistant, structurally altered sites represent complexes on which transcription is occurring. Such sites would be equivalent to DNA "kegs" as originally described by Beabealashvily et al. (1972). Under the conditions of our experiments, between 40% and 50% of component I change (at plateau) was due to DNA in such protected environment. More significantly, 50–60% of the component I change was eliminated by treatment with DNase I (Figure 5). Such regions must represent structurally altered DNA outside of the immediately protected DNA–enzyme complexes. Assuming that the total regions of DNA altered (DNase resistant plus DNase sensitive) can be represented as a summation of wavelike functions, as theorized by Florentiev & Ivanov (1970), a minimum size can be estimated for the regions of

DNA cooperatively influenced (DNase I sensitive regions). On the basis of a Gaussian distribution and 18–40 protected DNA base pairs per DNA–enzyme complex (Heyden et al., 1972), the size for the spheres of DNA influenced but not protected would range between 60 and 180 base pairs.[2] When the plateau of component I occurs, it is not known whether protected or unprotected but structurally not altered regions of DNA would still exist.

The hypothesis that RNA synthesis should be thermodynamically favored by a B to A transition in DNA form was first advanced by Arnott et al. (1968). The data in this communication support the occurrence of such a transition in DNA form during the process of RNA synthesis. The cause–effect relationship between the change in DNA structure and transcription remains to be elucidated. The structural alteration could occur as a passive consequence of transcription, or it might serve as a mechanism for modulating either the initiation or the rate of transcription. Finally, the data which suggest that the change in DNA extends cooperatively beyond the immediate DNA–enzyme complex may have implications with respect to modulation by DNA–protein interaction at sites both contiguous and noncontiguous to specific genomes.

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[2] The formulation $Z = (\mu - \bar{x})/\sigma$ describes the normal frequency of distributions where Z = zeta score, μ is the limit of the curve in question, $\bar{x}$ is the mean standard distributions, and σ is standard deviation.