Conformational Mobility of Deoxyribonucleic Acid, Transfer Ribonucleic Acid, and Poly(adenylic acid) As Monitored by Carbon-13 Nuclear Magnetic Resonance Relaxation†

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ABSTRACT: The molecular motion of DNA, the native form of tRNA, and partially denatured poly(A) has been investigated by carbon-13 nuclear magnetic resonance (^{13}C NMR). The nuclear Overhauser effect of the RNA samples was measured at 25.1 and 50.3 MHz, and the spin–lattice relaxation time of all the samples was measured at 50.3 MHz. The NMR data indicate that the local motion of the ribose carbons is much less restricted than that of the bases for DNA and tRNA. The local motion correlation times of the ribose carbons are in the range of 1–7 ns for the samples investigated. The local motion correlation times of the different nucleic acids are quite similar with the exception that the 2′ carbon of DNA and poly(A) is apparently less restricted than that for tRNA. The local motion correlation times of the ribose carbons, except perhaps the 2′, do not appear to be strongly coupled to the conformation of the polynucleotide. The ^{13}C NMR results can be combined with those of other investigations to obtain a consistent picture of the internal and overall motions of polynucleotides which have a backbone that is much more flexible than that of the bases.

The role of conformational fluctuations in the biological processes of macromolecules has been gaining recognition during the past few years. The central cellular functions of transcription and translation quite plausibly involve distortion of the conformation of DNA. The packaging of polynucleotides into compact forms in chromatin, viruses, and ribosomes involves folding of the nucleic acids. These and other observations indicate that the deformation of polynucleotide structure is intimately related to the biological role of nucleic acids. Since the conformational mobility of nucleic acids is related to the energetics of deformation, it is important to have some information concerning the rates and types of molecular motion.

Several approaches have been used to monitor the conformational fluctuations of nucleic acids. The first information came from hydrodynamic studies which showed that long DNA double helixes are flexible (Eisenberg, 1974). On the average, the angle between base pairs separated by the persistence length, ~160 base pairs, is 90° (Eisenberg, 1974). Examination of the ^{31}P NMR relaxtion of DNA and double-stranded RNA has given support to the idea that double-stranded polynucleotides are not rigid rods and that the correlation time for the long-range bending motions of double-stranded RNA and DNA is on the order of a microsecond (Bolton & James, 1979, 1980).

Examination of the rate of decay of the fluorescence anisotropy of a drug molecule bound to polynucleotides has shown that there is significant local motion of the bases on the time scale of tens of nanoseconds (Wahl et al., 1970; Barkley & Zimm, 1979). ^{31}P NMR studies have indicated that the correlation time for the local motion of the phosphate group of RNA and DNA is on the order of 0.5 ns (Klevan et al., 1979; Bolton & James, 1979, 1980). Thus, there is local motion of polynucleotides which can occur ~1000 times faster than the bending motions which give rise to the persistence length.

Of special interest are the rate of conformational fluctuation of the ribose moiety of nucleic acids and the relationship between the mobility of the ribose and that of the bases. It is the conformation of the ribose which is indicative of the overall conformation of a polynucleotide. It is known that DNA can exhibit a wide variety of conformations, but RNA is generally found to exhibit only small deviations from a single conformation. This difference between RNA and DNA may be a manifestation of the conformational mobility of their respective riboses. Examination of the conformation of mononucleosides and mononucleotides, as well as a few small oligomers, leads to the concept of the "rigid nucleotide" (Davies, 1978). However, a theoretical study indicated that the ribose of polynucleotides is conformationally flexible (Levitt & Warshel, 1978), and a preliminary study of the ^{13}C NMR relaxation of DNA showed that the ribose is undergoing fluctuations on the time scale of nanoseconds (Bolton & James, 1979).

For the purpose of obtaining more information about the rates and types of conformational fluctuations of the ribose and bases of polynucleotides, the ^{13}C NMR relaxation of DNA, tRNA, and poly(A) has been examined. The polynucleotides were chosen to represent a variety of polynucleotide forms to allow investigation of the dependence, if any, of the rates and types of conformational fluctuations of the ribose and bases on the type of polynucleotide.

Materials and Methods

The poly(A) sample was prepared by dissolving 500 mg of the potassium salt from P-L Biochemicals in 14 mL of 0.1 M NaCl, 2 mM EDTA, and 10 mM H_2KPO_4 at pH 7. The buffer was made up of equal portions of H_2O and ^2H_2O. The tRNA sample was prepared by dissolving 500 mg of Sigma type X yeast tRNA in 14 mL of 0.1 M NaCl, 2 mM EDTA, 10 mM $MgCl_2$, and 10 mM H_2KPO_4 at pH 7. The buffer was made up of equal portions of H_2O and ^2H_2O. Calf thymus DNA from Sigma was briefly sonicated in high salt and dialyzed against 0.1 M NaCl and 10 mM H_2KPO_4 at the pH of the sample. The sample was then diluted with 20% of the

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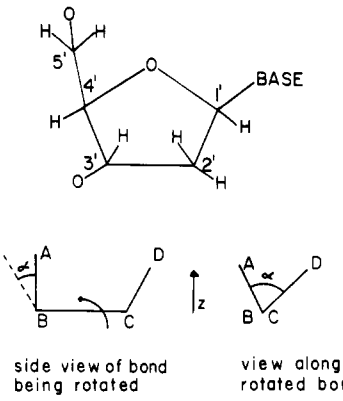

FIGURE 1: Puckering motion in the ribose ring. Rotation around the C–C bond, as depicted, reorients the carbon–hydrogen internuclear vector (thus affecting NMR relaxation) with angle ϕ for the internal rotation of 70.5° with respect to the axis of rotation for the tetrahedral carbons. Similar motions occur at the other ribose carbons.

same buffer made up with ^2H_2O. The DNA sample contained ~600 mg of DNA in 14 mL. The sample was made ~8 mM in EDTA by addition of a small volume of concentrated EDTA.

^{13}C NMR spectra at 50.3 MHz were obtained by using the Nicolet 200 spectrometer at the Magnetic Resonance Laboratory of the University of California at Davis. The samples were placed in 20-mm tubes for the experiments. Spectra at 25.1 MHz were obtained by using a Varian XL-100 equipped with a 20-mm probe built by Vladimir Basus. The DNA spectra were obtained at ~40 °C and the tRNA and poly(A) spectra at ~30 °C. Due to sample heating effects arising from the use of proton decoupling, the temperatures of the samples could not be accurately monitored. The same samples were used for the 25.1- and 50.3-MHz experiments. The T_1 and NOE experiments were performed in the usual fashion as described elsewhere.

Chemical shifts are reported here with an arbitrary origin. The chemical shifts of poly(A) have been given by Aldefer & Ts'o (1977) and those of tRNA most recently by Agris and co-workers (Thompson et al., 1979). The assignments of the tRNA and poly(A) spectra are from the above-mentioned articles (Aldefer & Ts'o, 1977; Thompson et al., 1979) and those of DNA as discussed elsewhere (Bolton & James, 1979).

EDTA was added to the DNA sample as an internal intensity standard for NMR. The ratio of the intensity of the EDTA peaks to that of the DNA ribose carbons was within ~10% of that calculated for the known amounts of DNA and EDTA in the sample. The intensities of the peaks were corrected for the pulse recycle time of the NMR experiments to account for the different relaxation rates of EDTA and nucleic acids. This experiment indicates that essentially all of the ribose carbons of DNA contribute to the observed ^{13}C NMR signals. In addition, optical melting of the DNA sample was examined after completion of the NMR experiments with no evidence for single-stranded regions being found.

Theory

The mathematical details of the model used to predict the dependence of the ^{13}C NMR relaxation of polynucleotides on molecular motion have been presented elsewhere (Bolton & James, 1979, 1980). In brief, the model describes the long-range motion of polynucleotides as a bending motion. The long-range bending motion, which also gives rise to the persistence length, produces an essentially isotropic reorientation of the carbon–hydrogen magnetic dipoles (Bolton & James, 1979, 1980). The local motion is analyzed in terms of rotation about an intramolecular bond (see Figure 1). For the ribose carbons, the angle of internal motion is taken as 70.5° (the complement of the tetrahedral angle) and the internuclear carbon–hydrogen distance as 0.109 nm. Small changes in the angle or distance are not expected to appreciably alter the correlation times deduced from the NMR relaxation data.

The local motion of the ribose might be more properly thought of as being rotational wobbling. The use of a wobble model would tend to give rise to slightly shorter correlation times than that of the simpler model used here. However, the wobble model contains an extra parameter to be determined, the extent of restriction of the rotation. For the accuracy of the correlation times used here (see Discussion), the simple free rotation model is adequate. It is unlikely, in any case, that the data are sufficient to determine the extra parameter with much accuracy.

Table I: Carbon-13 T_1 and NOE Values of DNA, tRNA, and Poly(A)

	T_1 values (s)		
carbon no.	DNA[a]	tRNA[b]	poly(A)[c]
1′	0.1	0.1	0.1
2′	0.1	0.1	0.1
3′	0.1	0.1	0.1
4′	0.1	0.1	0.1
5′	0.1	0.07	0.06

	NOE values				
		tRNA		poly(A)	
carbon no.	DNA,[a] 50.3 MHz	25.1 MHz	50.3 MHz	25.1 MHz	50.3 MHz
1′	1.2	1.1	1.1	1.2	1.2
2′	1.4	1.3	1.2	1.8	1.4
3′	1.2	1.3	1.2	1.5	1.2
4′	1.2	1.2	1.2	1.5	1.1
5′	1.6	1.5	1.2	1.6	1.3

[a] Sample as described under Materials and Methods. T_1 is accurate to about ±0.04 s, with NOE to ~20%. 1′ and 4′ carbons are not resolved, and data for the two are taken as the same. [b] Sample as described under Materials and Methods. T_1 is accurate to about ±0.02 s, with NOE to ~15%. [c] Sample as described under Materials and Methods. T_1 is accurate to about ±0.01 s, with NOE to ~10%.

Results

The ^{13}C NMR relaxation data for poly(A) obtained at 50.3 and 25.1 MHz are given in Table I, and a representative spectrum is shown in Figure 2. The spin–lattice relaxation time (T_1) values of the ribose carbons are all 0.1 s at 50.3 MHz except that of the 5′ carbon which is 0.06 s. It should be noted that the 5′ carbon is dipolar coupled to two directly bonded hydrogens; consequently, its T_1 value will be about half that of the other ribose carbons. Examination of the theoretical curves in Figure 3 for the dependence of T_1 on the internal and overall motion correlation times shows that the internal motion correlation times of the ribose carbons are on the order of 1–10 ns if T_1 is ~0.1 s. The T_1 values obtained in the present study are consistent with the values obtained previously for poly(A) at a lower frequency (15 MHz) by Komoroski (1973).

The ^{13}C nuclear Overhauser effect (NOE) produced by proton irradiation can be particularly instructive in monitoring the internal motion correlation time on the time scale indicated by the T_1 results. As shown in Figure 4, the NOE is very sensitive to the internal motion correlation time over the range of ~0.5–4 ns. In particular, the field strength dependence of the NOE as illustrated by the curves in Figure 4 can be

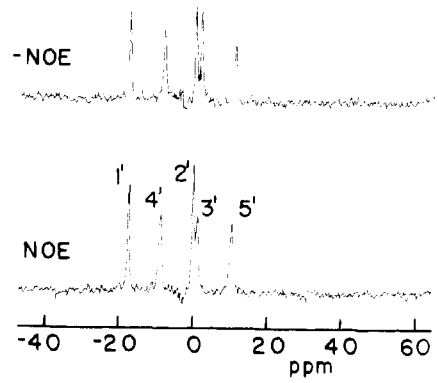

FIGURE 2: Natural abundance 50.3-MHz ^{13}C NMR spectra of the ribose carbons of 36 mg/mL poly(A) in 0.1 M NaCl, 2 mM EDTA, and 10 mM phosphate buffer, pH 7. The spectra illustrate the $^{13}C\{^1H\}$ nuclear Overhauser effect experiment. Each spectrum is the result of 1500 accumulations with an acquisition time of 0.20 s and a delay time of 0.61 s.

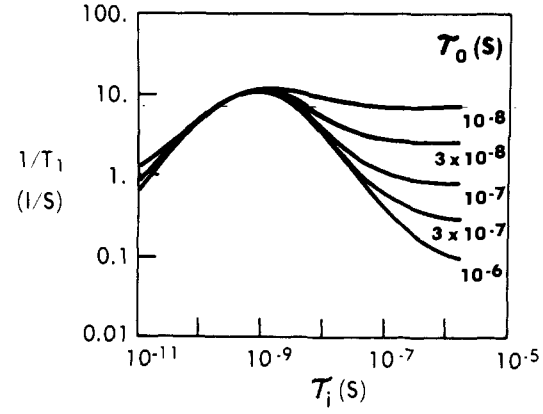

FIGURE 3: Theoretical dependence of the spin–lattice relaxation rate at 50.3 MHz for tetrahedral ^{13}C relaxed by dipolar coupling to a single proton 0.109 nm distant. The relaxation rate was calculated as a function of the internal motion correlation times τ_i for a series of long-range bending motion correlation times τ_0 ranging from 10^{-8} to 10^{-6} s. The angle between the C–H vector and the axis of rotation is 70.5°.

useful in determining internal motion correlation times. The NOE results observed at 25.1 and 50.3 MHz for poly(A) are listed in Table I. It is noted that the NOE values indicate internal motion correlation times of at least 1 ns, in agreement with the T_1 data. It should also be noted that the NOE values determined for the various ribose carbons cover a range. Since the NOE is not in general particularly sensitive to the number of protons dipolar coupled to the carbon or to the proton–carbon internuclear distance, the NOE data can be particularly informative.

A typical NOE experiment for yeast tRNA is shown in Figure 5. The T_1 and NOE data for tRNA are similar to those obtained for poly(A). For tRNA, as for poly(A), a range of values for the NOE is observed and the T_1 values are ~0.1 s. Komoroski & Allerhand (1972) previously measured T_1 values but not NOE values for yeast tRNA at 15 MHz; their results are consistent with our values obtained at higher field.

The NMR data obtained for DNA were of somewhat lower quality than those obtained for tRNA and poly(A) due to decreased signal to noise ratio. An NOE experiment is shown in Figure 6. The DNA data are roughly in the range observed for tRNA and poly(A), with a dispersion in the NOE values of the various ribose carbons also being observed. Useful DNA data at 25.1 MHz could not be obtained due to signal to noise considerations.

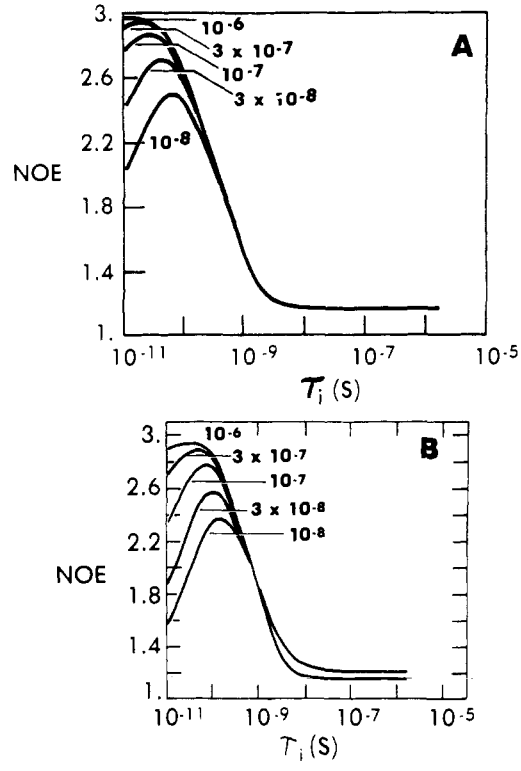

FIGURE 4: (A) Theoretical value of the $^{13}C\{^1H\}$ nuclear Overhauser effect for tetrahedral carbon (ϕ = 70.5°) at 50.3 MHz. The NOE is presented as a function of the internal motion correlation time τ_i for a series of slower motion correlation times τ_0 ranging from 10^{-8} to 10^{-6} s. (B) Same as for (A) with calculations done at 25.1 MHz.

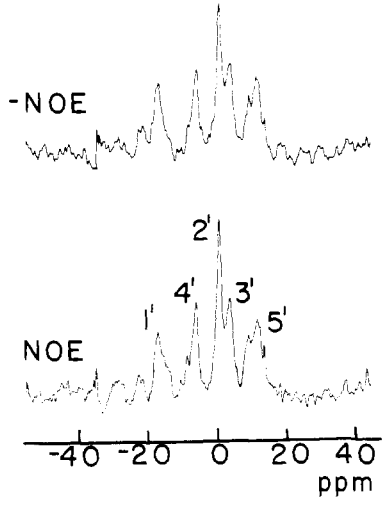

FIGURE 5: 50.3-MHz $^{13}C\{^1H\}$ nuclear Overhauser effect experiment for the ribose carbons of yeast tRNA (36 mg/mL) in 0.1 M NaCl, 2 mM EDTA, 10 mM $MgCl_2$, and 10 mM phosphate buffer, pH 7. Each spectrum is the result of 4600 transients with an acquisition time of 0.20 s and a delay time of 0.61 s.

The NMR relaxation behavior of the carbon nuclei in the aromatic bases of the nucleic acids was not investigated in detail. Values of T_1 and NOE for the protonated carbons of the adenine moiety of poly(A) were comparable to those of the ribose (data not shown). However, for both DNA and the native form of tRNA, the base carbons were not observed with nearly the same intensity as the ribose carbons. In fact, for DNA there was no discernible signal from any of the base carbons, including the methyl carbon of thymidine. This lack of observation of a full signal from the aromatic carbons is attributed to slow internal motion of the bases of tRNA and

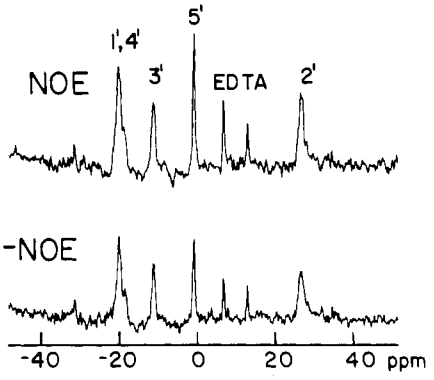

FIGURE 6: Natural abundance 50.3-MHz ^{13}C NMR spectra of the ribose carbons from 43 mg/mL calf thymus DNA in 0.1 M NaCl and 10 mM phosphate buffer, pH 7, illustrating a nuclear Overhauser effect experiment. Each spectrum is the average of 14 400 accumulations with an acquisition time of 0.20 s and a delay time of 0.51 s.

DNA. Slow internal motion gives rise to a relatively long T_1 value (see Figure 3) and, with the short pulse recycle times used here (typically 0.5 s), the aromatic carbons are saturated. An internal motion correlation time of ~20 ns would yield a value for T_1 of 1 s for a protonated carbon of DNA. A T_1 value this long would preclude observation of a signal with the conditions used here. For tRNA, the maximum value for T_1 would be ~0.3 s due to restricted internal motion. Signals from the aromatic carbons were observed for tRNA but not with full intensity. Examination of the spectra obtained for relatively long delay times, 0.7 s, suggested that the T_1 values of some of the aromatic carbons of tRNA are on the order of 0.3 s. Without any further information, the data reveal that the motion of the bases of DNA and tRNA is restricted relative to that of the ribose carbons and that the internal motion correlation time of the bases appears to be 20 ns or longer for DNA and tRNA.

Discussion

Without recourse to a detailed model of the internal molecular motion of the riboses and bases of polynucleotides, several deductions can be made from the ^{13}C NMR relaxation of DNA, tRNA, and poly(A). The internal motion of the bases is restricted relative to that of the ribose for DNA and tRNA by at least an order of magnitude. The time scale for internal motion experienced by the ribose carbons is on the order of 1–7 ns, and there is some difference in the rates of local motion experienced by the different ribose carbons. For tRNA, the internal motion of the ribose is restricted somewhat relative to DNA. But the internal motion of the ribose moiety of poly(A) is somewhat less restricted than that of DNA. The 1′, 3′, and 4′ carbons tend to experience about the same local motion as monitored by NMR, with the 5′ carbon experiencing greater freedom. For tRNA, the local motion of the 2′ carbon is comparable to that of the other ribose carbons, but for DNA and poly(A) the 2′ carbon experiences greater mobility than the other ribose ring carbons.

A quantitative comparison of the internal motion correlation times can be obtained, within the limitations of the model used, and the correlation times are given in Table II. Quantitation of the data does not add appreciably to the interpretation of the data given below.

The difference in the local motion of the ribose and base moieties of DNA and tRNA is attributed to steric effects. For the bases to undergo appreciable motion, it appears likely that there must be concerted motion of adjacent bases. The ribose moiety can apparently undergo conformational fluctuations

Table II: Internal Motion Correlation Times (Nanoseconds) of DNA, tRNA, and Poly(A) Deduced from NMR Relaxation Data

carbon no.	DNA[a]	tRNA	poly(A)
1′	6	7	7
2′	2	6	1
3′	6	6	4
4′	6	7	4
5′	0.9	4	3

[a] 1′- and 4′-carbon signals are not resolved.

without such severe restrictions, which is in agreement with an examination of molecular models. The observation that the 5′ position exhibits the greatest motional freedom for tRNA and DNA is reasonable since this carbon is the only ribose carbon not contained in the ribose ring.

The NMR results of different investigations observing several nuclei at a variety of field strengths are consistent with the following model of the motions of nucleic acids. There is long-range bending motion of double-stranded polynucleotides which gives rise to the persistence length which can be observed hydrodynamically (Eisenberg, 1974) or by NMR (Bolton & James, 1979, 1980). NMR can be used to monitor the bending motion by line width, T_2, or $T_{1\rho}^{off}$ determinations (James et al., 1977). Due to insufficient information concerning the chemical shift inequivalence, the line width data are considered to be the least reliable. The experimental difficulties and artifacts associated with T_2 measurements can give rise to problems. The existing results indicate that the correlation time for the bending motion of double-stranded RNA or DNA at ~37 °C in 0.1 M NaCl at neutral pH is on the order of 500 ns.

The internal motion experienced by the ribose and phosphate moieties of polynucleotides is not severely restricted, with internal motion correlation times ranging from about 0.5 to 7 ns and with the greatest conformational flexibility being exhibited by the phosphate (Bolton & James, 1979, 1980; Klevan et al., 1979). The bases of DNA and tRNA appear to have internal motion correlation times of at least 20 ns on the basis of ^{13}C NMR results. The rate of decay of the fluorescence anisotropy of ethidium intercalated between the base pairs of DNA has been used to show that the correlation time of the internal motion of the bases is on the order of the fluorescence lifetime of ethidium, i.e., ~25 ns (Wahl et al., 1970; Barkley & Zimm, 1979). We have recently surveyed the NMR results, including ^{31}P and ^{1}H studies, pertaining to conformational flexibility (Bolton & James, 1979). NMR and fluorescence anisotropy decay are the only experiments of which we are aware that monitor the local motion of polynucleotides. The correlation times observed for the ribose carbons are in agreement with calculations which have predicted that the ribose of RNA and DNA is conformationally flexible even for thermodynamically stable double helixes.

The observation that the local motion correlation times of the ribose carbons of DNA, tRNA, and poly(A) are roughly the same indicates that the internal motion is not strongly coupled to the conformation of the polynucleotide. The only possible exception to this might be the 2′ carbon. Poly(A) might be thought of as being somewhat comparable to highly denatured tRNA. In this view, the 2′ carbon is much less restricted in the denatured form than in the native form. Also, the major differences between tRNA and DNA are the correlation times of the 2′ and 4′ carbons. The difference in the motion of the 4′ carbon is not clear since, for DNA, the 1′- and 4′-carbon signals overlap. The restriction of the local motion of the 2′ position in native tRNA is consistent with

the intramolecular water bridge between the 2′-OH and 3′-phosphate proposed elsewhere (Bolton & Kearns, 1978, 1979) which might be expected to hinder the local motion at the 2′ position.

There is now a fair amount of evidence for the presence of conformational fluctuations of the backbone, phosphate and ribose of polynucleotides on the time scale of nanoseconds. Thus, the concept of the "rigid nucleotide" should be limited to a description of the equilibrium conformation of nucleic acids and not to the conformational rigidity of polynucleotides.

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Escherichia coli Ribonucleic Acid Polymerase Binding to the Deoxyribonucleic Acid of the Echinoid *Paracentrotus lividus*: Properties of the Complexes and Distribution of Stable Binding Sites†

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ABSTRACT: We describe the properties of the complexes that form between *Escherichia coli* RNA polymerase and *Paracentrotus lividus* DNA: dissociation kinetics, temperature dependence of the complex formation, resistance to heparin, and range of RNA polymerase–DNA weight/weight ratios that give rise to the stable binding events. The amount and distribution of the sites that form stable binding [class A sites as defined by Hinkle & Chamberlin [Hinkle, D., & Chamberlin, M. J. (1972) *J. Mol. Biol. 70*, 157]] with *E. coli* RNA polymerase were determined by the analysis of the dissociation of complexes formed by the enzyme on DNA fragments of various length. The *P. lividus* appears to form 3.1×10^5 stable ($t_{1/2} \geq 15$ min) complexes per haploid genome; the great majority of these complexes shows a short-range distribution (1000–2000 base pairs). The observed attributes of the stable binding sites of *P. lividus* DNA for *E. coli* RNA polymerase (amount, distribution, and quantitative ability to start in vitro RNA chains) point to the conclusion that *E. coli* and sea urchin DNA are nearly indistiguishable by the criteria adopted. The behavior of the sea urchin stable binding sites for the *E. coli* enzyme is not consistent with the expected behavior of the in vivo promoters.

It has been shown that *Escherichia coli* RNA polymerase can form stable complexes with DNA at sites [class A sites as defined by Hinkle & Chamberlin (1972)] which correspond to genetically identified promoters; the evidence obtained is limited to the interaction of procaryotic RNA polymerase with bacteriophagic, genetically well-defined genomes and with a few bacterial promoters [for a review, see Chamberlin (1976)]. We (Pedone et al., 1978) have described the distribution of class A sites on the relatively more complex genome of *E. coli*, on the assumption that such an analysis provides a rapid and detailed description of the procaryotic genome under consideration; the validity of such an assumption is based (a) on the observed correspondence between known bacterial and bacteriophagic promoter sites and stable binding sites (Chamberlin, 1976), (b) on the similarities of the properties of the stable complexes we (Pedone et al., 1978) have described in the *E. coli* system with the properties of complexes formed on genetically identified promoters, and (c) on the correspondence between the number and distribution of stable binding sites and the available knowledge of genetic complexity and organization of the *E. coli* genome.

In the present communication we describe the analysis of a much more complex genome (the echinoid *Paracentrotus lividus*) carried out with the same methodological approach that we have used for the *E. coli* genome (Pedone et al., 1978) and discuss the validity of the use of this bacterial enzyme as a tool in the analysis of eucaryotic genomes and in the study of their organization.

Material and Methods

Materials, the preparation of the DNA fragments, and the nitrocellulose filter assay conditions for the binding of RNA polymerase to DNA are as described in Pedone et al. (1978). Purification of *E. coli* RNA polymerase was also performed

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