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Rate of Chain Breakage at Apurinic Sites in Double-Stranded Deoxyribonucleic Acid†

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ABSTRACT: Several apurinic sites were introduced in the covalently circular, double-stranded DNA from phage PM2 by heating the DNA below the T_m at pH 5. The rate of chain cleavage at apurinic sites in such circular molecules, at pH 7.4 and physiological ionic strength, was subsequently measured by sedimentation velocity experiments. The presence of Mg^{2+} ions and/or amines increases the rate of chain breakage. Of several small molecules tested, putrescine (1,4-diaminobutane) was the most effective in this regard. In a Mg^{2+}-containing buffer, the average lifetime of the DNA chain at an apurinic site is 190 hr at 37°. The chain breaks are predominantly introduced at the 3′ side of the apurinic sugar moiety between this sugar and the phosphate residue. The DNA chain was more alkali resistant than expected at the apurinic sites; quantitative chain cleavage at such sites was only obtained after incubation for several hours at pH 12.8 and 25°.

Purine bases are released from DNA in acidic solutions (Feulgen, 1918; Tamm *et al.*, 1952a). Depurination of DNA also occurs at a very slow but physiologically significant rate at neutral pH (Greer and Zamenhof, 1962; Lindahl and Nyberg, 1972). Exposure of DNA *in vivo* or *in vitro* to alkylating agents or to nitrous acid leads to the formation of purine nucleotide derivatives with a labile purine–sugar glycosidic linkage, such as the nucleotide derivatives of 3-methyladenine, 7-methylguanine, and xanthine, and thus to great increases in the rate of DNA depurination (Lawley and Brookes, 1963; Burnotte and Verly, 1971).

The DNA chain is susceptible to alkaline hydrolysis at apurinic sites, and chain breakage at neutral pH occurs preferentially at such sites (Tamm *et al.*, 1953; Adamiec and Shugar, 1959). While such chain cleavage is still a slow reaction in neutral phosphate buffer at 37° (Laurence, 1963; Lawley *et al.*, 1969), the reaction is more rapid in the presence of Mg^{2+} ions (Tamm *et al.*, 1952b), primary amines (Tamm *et al.*, 1953; Strauss and Hill, 1970), high concentrations of basic proteins (McDonald and Kaufmann, 1954), or several aldehyde reagents (Livingston, 1964).

In the present work, small numbers of apurinic sites were introduced without accompanying chain breakage in circular DNA from phage PM2. Because of the unusual hydrodynamic properties of double-stranded, covalently closed circular DNA molecules (Vinograd *et al.*, 1965), it was possible to measure by sedimentation analysis the rate of introduction of a single-strand interruption in such a molecule under different solvent conditions. The results indicate that spontaneous chain cleavage at an apurinic site in double-stranded DNA would not occur in growing cells for several cell generations.

Materials and Methods

DNA. Phage PM2 and its *Pseudomonas* host were kindly provided by Dr. R. Espejo. PM2 [^{32}P]DNA preparations were made according to Masamune *et al.* (1971). The specific activity of this DNA was initially 40,000 cpm/μg. *Escherichia coli* DNA (mol wt 25 × 10^6) was prepared according to Marmur (1961).

Enzymes. Polynucleotide kinase was prepared from phage T4 infected *E. coli* according to Richardson (1965). A gel

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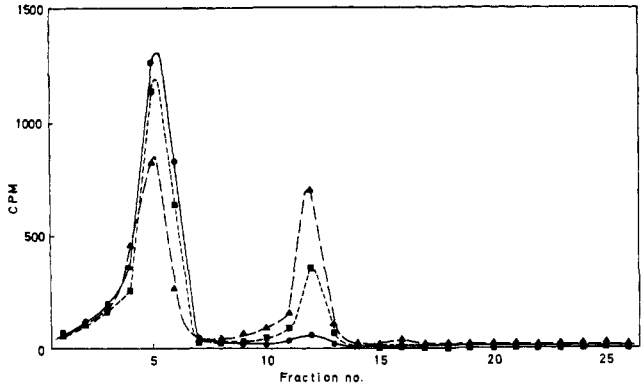

FIGURE 1: Neutral sucrose gradient centrifugation of PM2 [^{32}P]DNA after incubation in 0.1 M NaCl–0.01 M sodium citrate (pH 5.0) at 70°. (●) No incubation; (■) 30 min; (▲) 60 min.

filtration step was added to the preparation procedure (Lindahl, 1971a). *E. coli* alkaline phosphatase, chromatographed, was purchased from Sigma. The phosphatase was further purified by heat treatment (Lindahl, 1971a) and DEAE-cellulose chromatography (Weiss *et al.*, 1968).

Other Materials. [^{32}P]H_3PO_4 (carrier free) was obtained from AB Atomenergi, Studsvik, Sweden. [γ-^{32}P]ATP (9 mCi/μmole) was obtained from NEN Chemicals. Hepes[1] and putrescine dihydrochloride were purchased from Sigma.

Analytical Procedures. Centrifugations in linear 5–20% sucrose gradients were done at 40,000 rpm and 5° in a SW 41 rotor in the Spinco Model L3-50 ultracentrifuge. Neutral sucrose gradients contained 0.02 M sodium phosphate–0.001 M EDTA (pH 7.0) and were centrifuged for 6 hr. Alkaline sucrose gradients contained 0.5 M NaCl–0.3 M NaOH–0.01 M EDTA, and were centrifuged for 3 hr. PM2 [^{32}P]DNA (0.2 ml; 0.9 μg/ml), mixed with 0.05 ml of 0.2 M EDTA (pH 7), was layered on top of the gradient. After completion of a run, 0.43-ml fractions were collected from the bottom of the tube, and their radioactivity was determined. Recoveries were >85%.

Heat treatments and pH measurements were performed as described (Lindahl and Nyberg, 1972). For pH measurements at alkaline pH values, freshly prepared solutions and an alkali-resistant Radiometer type B electrode were used, and the results were corrected for Na^+ errors according to data provided by the electrode manufacturer. Radioactivity was measured as Cerenkov radiation in 12 ml of H_2O (Haviland and Bieber, 1970) in a Packard liquid scintillation spectrometer. Preincubation of DNA in alkaline solution was done by the addition of one volume of 2 M glycine–NaOH (pH 13.1) to the DNA solution, followed by incubation at 25° for 4 hr.

Results

Isolation of Covalent DNA Circles with Apurinic Sites. PM2 [^{32}P]DNA was incubated in 0.1 M NaCl–0.01 M sodium citrate (pH 5.0) at 70°. At various times aliquots were removed, and their conformation was analyzed by neutral sucrose gradient centrifugation. Covalently closed circular PM2 DNA has a sedimentation coefficient of $s_{20,w}$ = 29 S under the solvent conditions used, while circular molecules containing one (or more) single-strand interruption have a sedimentation

[1] Abbreviation used is: Hepes, *N*-2-hydroxyethylpiperazine-*N*-2′-ethanesulfonic acid.

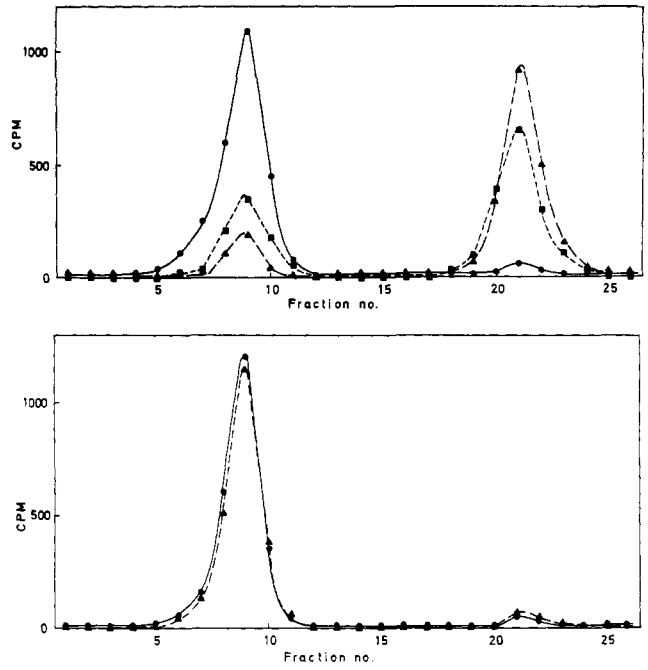

FIGURE 2: Alkaline sucrose gradient centrifugation of PM2 [^{32}P]-DNA. (a, top) DNA containing 1.9 apurinic sites per DNA molecule. (●) DNA not preincubated in alkali; (■) DNA preincubated for 30 min in 1 M glycine-NaOH (pH 12.8) at 25°; (▲) DNA preincubated for 16 hr. (b, bottom) DNA containing <0.1 apurinic site per molecule. Symbols as in part a.

coefficient of $s_{20,w}$ = 21 S (Böttger and Kuhn, 1971). The covalently closed circular PM2 DNA was slowly converted to a 21S form on heating at pH 5, but even after 60 min most of the DNA (~65%) remained in a 29S form (Figure 1). From the known rate of depurination of double-stranded DNA under these conditions, it was expected that 1 purine base would be released per PM2 DNA molecule in 4 min (Lindahl and Nyberg, 1972). The 29S DNA remaining after 60 min of heat treatment at pH 5 should therefore contain several apurinic sites.

To quantitate the number of apurinic sites introduced, PM2 [^{32}P]DNA was heated for 8 min at pH 5 as described. This treatment, which left >90% of the DNA in a 29S form when analyzed in neutral solution, was expected to produce 2 apurinic sites per molecule. The structure of the heated DNA was subsequently analyzed by gradient centrifugation in alkaline solution.

When the heated DNA was denatured by the addition of an equal volume of 0.5 M NaOH and immediately applied to the alkaline sucrose gradient, >90% of the material sedimented at 76 S, which is the sedimentation coefficient of the alkali-denatured form of covalently circular PM2 DNA (Figure 2). No alkali-labile sites could thus be detected in comparison with a control DNA preparation by this procedure. However, when the preheated DNA was incubated in 1 M glycine–NaOH (pH 12.8) at 25° prior to centrifugation, most of the DNA was converted to a slowly sedimenting form, while unheated control DNA remained in a 76S form (Figure 2). The preincubation in alkaline solution was therefore necessary to reveal the presence of apurinic sites in DNA by alkaline sucrose gradient centrifugation. Both after 4- and 16-hr preincubation in alkali, 15% of the PM2 DNA heated at pH 5, and >95% of the control DNA remained as covalently closed circular material. These results show that the PM2

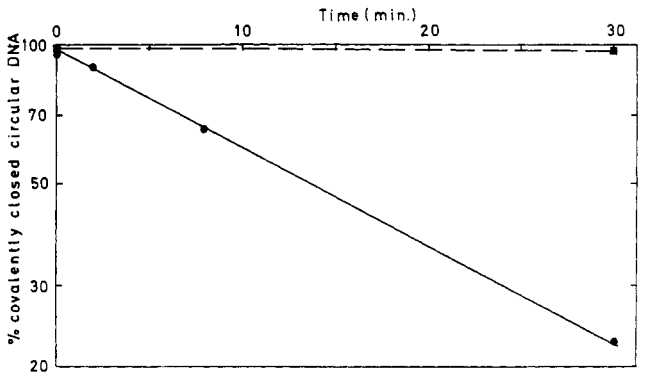

FIGURE 3: Rate of degradation of partly depurinated PM2 [^{32}P]-DNA in 0.1 M NaCl–0.05 M Hepes-KOH–0.01 M $MgCl_2$–0.0005 M EDTA (pH 7.4) at 70°. (●) DNA containing 14 apurinic sites per molecule; (■) DNA containing <0.1 apurinic site per molecule.

DNA heated at pH 5 for 8 min contained 1.9 alkali-labile sites per molecule, in good agreement with the expected value of 2 apurinic sites per molecule.

In order to measure the rate of chain breakage at apurinic sites in neutral solvents, PM2 [^{32}P]DNA was heated for 60 min at pH 5.0 as described, to introduce ~14 apurinic sites per molecule. The remaining 29S material was then isolated as in Figure 1, and the sucrose removed by dialysis against 0.2 M KCl–0.01 M Hepes-KOH–0.001 M EDTA (pH 6.8) at 2°. Such DNA was employed for the centrifugation experiments described below, and was used within 3 days. After preincubation of an aliquot of this material for 4 hr at 25° in 1 M glycine–NaOH, pH 12.8, >99% of the material sedimented slowly (at ~10 S), and no remaining covalently closed circular DNA was detected.

Chain Breakage at Apurinic Sites. Covalently closed circular PM2 [^{32}P]DNA, with 14 apurinic sites per molecule, was incubated at 70° in 0.1 M KCl–0.05 M Hepes-KOH–0.01 M $MgCl_2$–0.0005 M EDTA (pH 7.4) (at 70°). Aliquots were removed at various times and analyzed by neutral sucrose gradient centrifugation. Control DNA without apurinic sites was treated in the same fashion. The results are given in Figure 3. One chain break per molecule (63% conversion) was introduced in 20 min in the DNA containing apurinic sites, while very little cleavage (<2%) of the control DNA occurred under the same conditions. From these data, the rate constant for chain breakage at a single apurinic site in double-stranded DNA is estimated to be $k = 5.7 \times 10^{-5}$ sec^{-1} at 70° under the solvent conditions used.

Chargaff and his coworkers have previously reported that Mg^{2+} ions and primary amines (glycine, Tris) promote the rate of chain breakage at apurinic sites in DNA at neutral pH (Tamm *et al.*, 1952b, 1953). To evaluate the effect of such compounds on double-stranded DNA with a small number of apurinic sites, the same type of experiment as above was repeated in the presence of 0.05 M glycine, and also in Mg^{2+}-free buffers with and without glycine. The rate constants obtained are given in Table I. In agreement with the observations of Tamm *et al.* (1952b, 1953), both Mg^{2+} ions and glycine accelerated chain cleavage. When both compounds were present, their effects were additive. The relatively small effect of 0.05 M glycine in these experiments indicates that the presence of EDTA in the solvents employed, at a 100-fold lower concentration, should not have detectably influenced the rate of degradation.

TABLE I: Rate of Chain Breakage at an Apurinic Site in Double-Stranded DNA at 70° and pH 7.4.

Solvent	k (sec^{-1})
0.1 M KCl–0.05 M Hepes-KOH–0.01 M $MgCl_2$–0.0005 M EDTA (buffer B)	5.7×10^{-5}
Buffer B − $MgCl_2$	2.4×10^{-5}
Buffer B + 0.05 M glycine	7.8×10^{-5}
Buffer B − $MgCl_2$ + 0.05 M glycine	4.3×10^{-5}
Buffer B + 0.05 M lysine	4.1×10^{-4}
Buffer B + 0.05 M arginine	1.9×10^{-4}
Buffer B + 0.05 M histidine	3.4×10^{-4}
Buffer B + 0.01 M putrescine	1.5×10^{-3}

The effect of several positively charged amines on the rate of chain breakage was also determined in the same fashion (Table I). Buffers containing such compounds were readjusted to the same pH before use. The positively charged amino acids lysine and arginine were more effective than glycine in promoting chain breakage, as 0.05 M glycine only caused a 1.4-fold rate increase in a Mg^{2+}-containing buffer, while 0.05 M lysine caused a 7-fold increase, and 0.05 M arginine a 3-fold increase. Histidine, which acts as a tertiary amine due to its imidazole side chain, was almost as effective as lysine (6-fold rate increase at 0.05 M). The diamine putrescine had a much stronger promoting effect, and at a concentration of 0.01 M caused a 25-fold increase in the rate of chain breakage at apurinic sites.

The temperature dependence of the chain cleavage reaction was investigated by measuring the rate of degradation of covalently closed circular DNA with apurinic sites at 10° intervals between 40 and 70°. At all temperatures, the rate of degradation of control DNA was <5% of that obtained for DNA with apurinic sites. The activation energy for chain breakage at apurinic sites in double-stranded DNA was found to be 24.5 ± 1.5 kcal/mole. Eigner *et al.* (1961) have previously estimated the activation energy of chain breakage of single-stranded DNA in 0.0075 M sodium phosphate–0.001 M EDTA (pH 6.8) to be 25 ± 2 kcal/mole.

Termini at Cleaved Sites. The chain breakage at apurinic sites in DNA, occurring in alkaline solution, has been postulated to take place at the 3′ side of the apurinic sugar residue, either by β elimination (Brown and Todd, 1955) or by 3′,4′-cyclic phosphate formation (Tamm *et al.*, 1953). To evaluate the relative importance of these two cleavage mechanisms with double-stranded DNA at pH 7.4, native *E. coli* DNA was first heated at pH 5.0 to obtain ~1% depurination (Table II). The DNA was then incubated in a Mg^{2+}-containing buffer, at pH 7.4 and 70°, to achieve >95% chain breakage at the apurinic sites. Control DNA, not preincubated at pH 5.0, was treated in the same fashion. After dialysis, the DNA solutions were divided, and on one part the phosphate groups at the termini were removed by alkaline phosphatase treatment at 65°. All DNA solutions were subsequently incubated with [γ-^{32}P]ATP and polynucleotide kinase to label 5′-OH residues (Weiss *et al.*, 1968). Of the 5′ ends created by chain breakage at apurinic sites, <25% could be labeled without previous alkaline phosphatase treatment (Table II), showing that most 5′ termini had phosphate residues. Similar results were obtained when the buffer used for the incubation at pH 7.4 also contained 0.05 M glycine (Table II).

These results suggest that β elimination is a more important reaction than 3′,4′ cyclization at apurinic sites at pH 7.4, as the latter type of cleavage would directly generate termini with 5′-OH groups. The amount of ^{32}P incorporated shows that most of the termini had been labeled after the alkaline phosphatase treatment (Table II).

Chain breakage could conceivably also occur at the 5′ side of the apurinic sugar residue by a 4′,5′-cyclization event (Bayley *et al.*, 1961). In this case, the 5′ ends at the chain breaks would be blocked to polynuceleotide kinase. While the free sugar residue should remain in the DNA at neutral pH, it would be selectively released by treatment of the DNA with strong alkali (Jones *et al.*, 1968). To investigate if cleavage at the 5′ side of the apurinic site also occurred, DNA with chain breaks at apurinic sites was treated with 0.5 M NaOH for 1 hr at 37°, prior to the alkaline phosphatase treatment. A small increase (<35%) in the amount of available end groups was noted after such treatment, indicating that cleavage by 4′,5′-cyclic phosphate formation could only occur to a minor extent.

TABLE II: DNA End-Group Analysis.[a]

	pmoles of ^{32}P Inc at Cleaved Apurinic Sites/ μg of DNA	
	DNA Pretreated with Alkaline Phosphatase	DNA Not Treated with Alkaline Phosphatase
DNA with chain breaks induced in the presence of 0.01 M $MgCl_2$	21.0	5.6
Same DNA, after preincubation in 0.5 M NaOH	28.2	8.2
DNA with chain breaks induced in the presence of 0.05 M glycine and 0.01 M $MgCl_2$	34.7	3.7
Same DNA, after preincubation in 0.5 M NaOH	36.9	3.8

[a] *E. coli* DNA (1 mg/ml) was incubated for 8 hr at 70° in 0.1 M NaCl–0.01 M sodium citrate (pH 5.0) to obtain ~1% depurination of the DNA (Lindahl and Nyberg, 1972). The DNA was subsequently dialyzed against 0.2 M KCl–0.01 M Hepes-KOH–0.001 M EDTA (pH 6.8) at 2°. An equal volume of 0.09 M Hepes-KOH–0.02 M $MgCl_2$ (pH 7.9) (at 25°) was added to both DNA preparations, and they were incubated at 70° (pH 7.4 ± 0.1 at 70°) for 15 hr. (In a separate experiment, an equal volume of 0.09 M Hepes-KOH–0.02 M $MgCl_2$–0.1 M glycine (pH 7.9) was instead added, followed by incubation at 70° for 10 hr.) This heat treatment caused chain breakage at >95% of the apurinic sites in DNA (see Table I), but caused <0.1% depurination of the control DNA (Lindahl and Nyberg, 1972). One-half of each of the two DNA solutions was brought to 0.5 M in NaOH, and further incubated at 37° for 2 hr. At the end of the latter incubation, these DNA solutions were neutralized with 3 M Hepes.

All DNA solutions were subsequently dialyzed extensively against 0.02 M NaCl–0.01 M Tris-HCl (pH 8.0) and their A_{260} was determined. One-half of each solution was treated with alkaline phosphatase (10 μg/ml) for 30 min at 65°. All DNA solutions were then incubated with polynucleotide kinase at 37°, in a total volume of 0.1 ml, in the following reaction mixture: 0.07 M Tris-HCl (pH 7.5), 0.01 M $MgCl_2$, 0.01 M 2-mercaptoethanol, 0.002 M K_2HPO_4, 2×10^{-5} M [γ-^{32}P]ATP (0.1 mCi/μmole), *E. coli* DNA (30 μg/ml), and polynucleotide kinase (5 units). After 20 min, an additional 5 units of enzyme was added to each tube, and the incubation was continued for 20 min. The reaction was stopped by chilling, followed by addition of several volumes of cold 5% trichloroacetic acid. The precipitates were collected on membrane filters (Schleicher and Schuell), washed with cold 5% trichloroacetic acid, dried, and analyzed for radioactivity. The amount of radioactivity incorporated in controls (containing DNA not pretreated at pH 5.0) was <20% of that of correspondingly treated DNA samples in all cases.

Discussion

This investigation of the stability of the DNA chain at an apurinic site was limited to solvent conditions that presumably are similar to those occurring *in vivo*, *i.e.*, to solvents of pH 7.4, with an ionic strength of ~0.15, and containing 0.01 M Mg^{2+} ions. Under such conditions, the average lifetime of the labile phosphodiester bond is 190 hr at 37°. It has been observed previously that a single depurination event in DNA resulted in a strand break after ~2000 hr in neutral phosphate buffer at 37° (Laurence, 1963; Lawley *et al.*, 1969). Some of the difference between these results and the present ones no doubt depends on the absence of Mg^{2+} ions in the earlier experiments. The possibility that an activation of a contaminating nuclease by Mg^{2+} ions occurred in the present experiments can be ruled out, as only DNA containing apurinic sites was degraded, and the rate of chain breakage was much more rapid at 70° than at 37°.

It has often been taken as proof for the absence of apurinic sites from DNA that a correct single-strand molecular weight has been obtained by alkaline sucrose gradient centrifugation (Strauss and Robbins, 1968; Friedberg *et al.*, 1969). However, in the present work it was possible to completely denature the macromolecular structure of PM2 DNA and obtain the correct sedimentation coefficient for the covalently circular molecule in an alkaline sucrose gradient even with DNA containing several apurinic sites (Figure 2). Preincubation of the DNA in alkaline solution was necessary to reveal these sites. Cape and Spencer (1968) and Strauss and Hill (1970) previously noted that prolonged alkaline treatment was necessary to quantitatively cleave the DNA chain at sites lacking bases. In the present work, it proved to be useful to preincubate DNA in 1 M glycine–NaOH (pH 12.8) for 4 hr at 25°, as this procedure was found to induce breakage at >98% of the apurinic sites, while <1% of the intact PM2 DNA molecules suffered any strand interruption by the same treatment.

The alkali-catalyzed chain cleavage at apurinic sites is a complex reaction (Bayley *et al.*, 1961; Jones *et al.*, 1968). Alkali treatment at 100° of DNA with many apurinic sites results in the formation of oligonucleotides with phosphate groups at both the 3′ and the 5′ termini (Shapiro and Chargaff, 1964). At neutral pH, 3′ termini with a nucleotide end group having a 3′-OH residue could also only be generated to a very minor extent, as the 3′ termini at cleaved apurinic sites do not function as primers for the *E. coli* DNA polymerase (Kotaka and Baldwin, 1964; L. Skoog and T. Lindahl,

unpublished results). In experiments with polynucleotide kinase, it was observed here that the chain cleavage at pH 7.4 seems to take place at the 3′ side of the apurinic sugar residue, between this sugar and the adjacent phosphate residue. This result is consistent with the notion that chain breakage primarily is due to β elimination (Brown and Todd, 1955). However, the present results do not rule out the possibilities that degradation by cyclic phosphate formation also occurs to a minor extent, or that a double β-elimination event occurs, with simultaneous chain breakage at both sides of the apurinic sugar residue.

At an apurinic site in DNA, the deoxyribose residue occurs in equilibrium between the free aldehyde form and the furanose form (Overend, 1950). The effect of amines on the rate of chain cleavage probably depends on an interaction with these aldehyde groups (Tamm *et al.*, 1953). The presence of reactive aldehyde groups in the DNA chain presumably also accounts for the introduction of occasional cross-links with the opposite DNA strand at apurinic sites. However, as such cross-links only occur at a small proportion of the apurinic sites (Freese and Cashel, 1964), the partly depurinated DNA circles used in the present work should have contained <1 cross-link per molecule.

Several amines promoted the rate of chain breakage at apurinic sites in DNA. The amino acids lysine, histidine, and arginine were considerably more effective in this regard than glycine. The three former amino acids, but not glycine, also bind weakly to nucleic acids (Felsenfeld and Huang, 1960). Diamines bind much more strongly than the positively charged amino acids to nucleic acids, almost as tightly as Mg^{2+} ions (Felsenfeld and Huang, 1960). For this reason, the effect of a diamine was also investigated. Putrescine was chosen for this experiment, because it is the polyamine occurring in largest amounts in many bacteria, *e.g.*, in *E. coli* (Tabor and Tabor, 1964). This compound was found to cause a great increase (25-fold, at a concentration of 0.01 M) in the rate of chain breakage at apurinic sites. Small basic proteins that bind strongly to DNA, *e.g.*, lysozyme and cytochrome *c*, have previously been found to promote chain breakage at apurinic sites in DNA more effectively than Mg^{2+} ions (McDonald and Kaufmann, 1954), and the present results are in agreement with these earlier studies.

The different monoamines investigated here were added in concentrations that probably greatly exceed the ones occurring *in vivo*, as most of the physiological ionic strength (~0.15) is accounted for by the presence of K^+ ions. The total concentration of polyamines (putrescine, spermidine, and spermine) in most cells is also ~5-fold lower than the putrescine concentration employed here (Tabor and Tabor, 1964). However, it is difficult to accurately predict the rate of chain breakage at apurinic sites in DNA *in vivo*, as different types of cells contain polyamine pools of very different sizes, and in addition mammalian cells have basic proteins bound to at least 50% of their DNA (Clark and Felsenfeld, 1971). It appears from the present data that the chain breakage at apurinic sites in DNA would be a slow event (requiring 20–100 hr at 37°) under physiological conditions.

Though it is concluded from this work that chain cleavage at apurinic sites in DNA seems to be a slow reaction under "physiological" solvent conditions, cells may contain a special factor(s) that accelerates the rate of chain cleavage. Incubation of DNA containing apurinic sites with cell extracts, both from mammalian cells and from several bacteria, does indeed lead to very rapid breakage of the chain at these sites at 37°. The most active factor in a calf thymus extract is a heat-labile endonuclease of mol wt ~40,000 that specifically attacks DNA at these lesions (Lindahl, 1971b; S. Ljungquist and T. Lindahl, in preparation). In comparison, little promotion of the rate of chain breakage at apurinic sites was observed with low molecular weight cellular material, isolated by gel filtration. The *E. coli* endonuclease II apparently acts in a similar fashion, as it preferentially cleaves DNA at apurinic sites (Hadi and Goldthwait, 1971). Further, Verly and Paquette (1972a,b) have independently found nucleases in *E. coli* and rat liver that specifically attack at apurinic sites. The presence of such enzymes in living cells suggests that apurinic sites in DNA are corrected by an excision–repair mechanism similar to that used for the removal of pyrimidine dimers from DNA (Howard-Flanders and Boyce, 1966).

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Studies on the Interactions of Nucleotides, Polynucleotides, and Nucleic Acids with Dihydroxyboryl-Substituted Celluloses†

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ABSTRACT: Nucleotides and polynucleotides which contain a free 2′,3′-diol group are capable of forming specific complexes with the two cellulose derivatives: *N*-(*m*-dihydroxyborylphenyl)carbamylmethylcellulose and *N*-[*N*′-(*m*-dihydroxyborylphenyl)succinamyl]aminoethylcellulose. In chromatography on columns prepared from these celluloses the retention volume of a particular mono- or polynucleotide depends on (i) the pH of the elution solvent, (ii) the ionic strength and the nature of the cations in the elution solvent, (iii) the number of negative charges in the vicinity of the diol group, and (iv) the nature of the nucleotide bases in the vicinity of the diol group. In the case of those polynucleotides which do not possess a 2′,3′-diol group a method has also been devised for the binding of these molecules to the substituted celluloses. The method involves the derivatization of the terminal phosphate group of the polymer with sorbitol or *N*-methylglucamine. The incorporated polyhydroxy group then serves as the moiety which undergoes complex formation with the cellulose-bound dihydroxyboryl groups. These methods can be applied to the study of the primary structure of nucleic acids and to the development of procedures for the chemicoenzymatic synthesis of polynucleotides of defined sequence.

Ribonucleosides and certain sugars and other polyols have been shown to form specific complexes with two new cellulose derivatives which contain covalently bound dihydroxyboryl groups (Weith *et al.*, 1970). One of the derivatives, *N*-(*m*-dihydroxyborylphenyl)carbamylmethylcellulose was prepared by reaction of the azide of carboxymethylcellulose with *m*-aminobenzeneboronic acid while the other, *N*-[*N*′-(*m*-dihydroxyborylphenyl)succinamyl]aminoethylcellulose was obtained by the condensation of *N*-(*m*-dihydroxyborylphenyl)succinamic acid with aminoethylcellulose in the presence of *N*-cyclohexyl-*N*′-β-(4-methylmorpholinium)-ethylcarbodiimide. The complexes formed with these cellulose derivatives are thought to consist of cyclic structures resulting from the reaction of a pair of hydroxyl groups on the polyol (the 2′,3′-diol groups in the case of ribonucleosides) with the dihydroxyboryl groups on the cellulose. Accordingly, these complexes are considered to be analogous to those structures known to be formed by certain polyols in aqueous borate solutions. In anticipation of the use of these new cellulose derivatives in chemical studies on nucleic acids the present work was directed to the investigation of the various factors which might affect the complex-forming capacity of nucleotides, polynucleotides, and nucleic acids, and a preliminary report of the results has been published (Rosenberg *et al.*, 1970).

Nucleotides. A number of nucleosides and nucleoside mono-, di-, and triphosphates have been chromatographed at pH 7.5 on columns of DBCM-cellulose[1] and their retention volumes measured. A comparison of these volumes in Table I shows that there are substantial differences in the degree of binding to the cellulose exhibited by the nucleotides and their parent nucleosides. For example, while there is a moderate amount of binding of the nucleoside, uridine, in the presence of the dilute buffer solution, there does not appear to be any complex formation between the cellulose and the various phosphate derivatives of this nucleoside since, under the same conditions, these derivatives can be eluted in volumes equal to the void volumes of the columns. However, the inclusion of 1 M sodium chloride in the elution solvent

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[1] Abbreviations used are: DBCM-cellulose, *N*-(*m*-dihydroxyborylphenyl)carbamylmethylcellulose; DBAE-cellulose, *N*-[*N*′-(*m*-dihydroxyborylphenyl)succinamyl]aminoethylcellulose.