Coding Properties of Poly(deoxycytidylic acid) Templates Containing Uracil or Apyrimidinic Sites: In Vitro Modulation of Mutagenesis by Deoxyribonucleic Acid Repair Enzymes†

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ABSTRACT: Heat treatment of poly(deoxycytidylic acid)-[poly(dC)] induces the formation of dUMP residues, which code for dAMP when replicated by *Escherichia coli* DNA polymerases I and III. The specificity of dUMP coding properties is indicated by the quantitative relation between the dAMP incorporated and the frequency of dUMP residues in the heat-treated poly(dC). The dAMP incorporation is prevented by preincubation of uracil containing poly(dC) with uracil–DNA glycosylase. The excision of uracil by uracil–DNA glycosylase leads to the formation of apyrimidinic sites (AP sites), which are barely replicated in vitro under physiological conditions. However, the alteration of *E. coli* DNA polymerase I fidelity of replication by Mn^{2+} greatly stimulates the replication of AP sites. There is a preferential incorporation of dAMP, as compared to dTMP, opposite the AP sites. The dAMP incorporation is prevented by preincubation of poly(dC) containing AP sites with *Micrococcus luteus* AP endonuclease B. The results show a close association between DNA repair by base excision and the prevention of mutagenic processes in vitro. Furthermore, since the alteration of DNA polymerase fidelity allows some replication of the noncoding DNA lesion (AP site), this could imply a role in SOS-induced mutagenesis in vivo.

A major source of base-pair substitution during DNA synthesis is the mispairing due to base modification, such as the deamination of cytosine, yielding uracil, and of adenine, yielding hypoxanthine, as well as the oxygen alkylation of guanine and thymine (Drake & Baltz, 1976; Abbott & Saffhil, 1977; Singer, 1982). The physical and chemical agents capable of inducing such DNA lesions are called direct mutagens; they do not require the induction of SOS functions in *Escherichia coli* (Drake & Baltz, 1976; Radman et al., 1979). Cytosine deamination occurs at a detectable rate in vitro under physiological conditions and is believed to be a significant source of mutation in vivo (Lindahl & Nyberg, 1974; Duncan & Miller, 1980). This lesion is repaired by removal of the dUMP residues by a specific uracil–DNA glycosylase, which excises the uracil by cleaving the N-glycosylic bond between the base and the deoxyribose moiety, thus generating apyrimidinic sites (AP sites).[1] The phosphodiester bonds are then cleaved at the AP sites by specific AP endonucleases, the AP sites are excised, the gaps thus formed are filled by DNA polymerases, and the continuity of the phosphodiester backbone is restored by DNA ligase [for a review, see Laval & Laval (1980) and Lindahl (1982)].

In this paper, we report the coding properties of poly(dC) templates, containing either uracil or apyrimidinic sites (AP sites), as substrates for DNA synthesis by *E. coli* DNA polymerase I. We also evaluate the action of physiological DNA repair enzymes and of DNA polymerase fidelity on mutagenesis.

Materials and Methods

Materials

Enzymes. *E. coli* DNA polymerase I (EC 2.7.7.7) and *E. coli* DNA polymerase I "large fragment" (EC 2.7.7.7) (Setlow et al., 1972) were purchased from Boehringer. One unit of enzyme incorporates 10 nmol of dNMP into acid-insoluble material in 30 min at 37 °C, under the conditions described by Richardson et al. (1964).

E. coli DNA polymerase III was purified from *E. coli* strain HMS 50, *pol A*1, *endo I*, according to the method of Livingston et al. (1975). We used the phosphocellulose fraction, which was essentially free of uracil–DNA glycosylase and of polymerases I and II. One unit of *E. coli* polymerase III incorporates 10 nmol of dNMP into acid-insoluble material in 30 min at 30 °C.

Micrococcus luteus uracil–DNA glycosylase was a gift of Dr. J. P. Leblanc. One unit of enzyme releases, as acid-soluble material, 1 pmol of uracil per min at 37 °C, using native [^{3}H]uracil PBS-1 DNA as substrate (Leblanc et al., 1982).

The PBS-1 inhibitor of uracil–DNA glycosylase (Cone et al., 1980) was a gift of Dr. E. Friedberg. One unit of inhibitor inhibits 1 unit of *M. luteus* uracil–DNA glycosylase with PBS-1 DNA as substrate.

M. luteus AP endonuclease B was a gift of Dr. J. Pierre. One unit of enzyme releases 1 pmol of acid-soluble material from depurinated DNA in 30 min at 37 °C (Pierre & Laval, 1980a).

Pancreatic DNase I and venom phosphodiesterase were Worthington products.

Polymers and Nucleotides. Poly(dC)$_{300}$ and oligo(dG)$_{12-18}$ were purchased from P-L Biochemicals, and poly(dG)·poly(dC) was from Miles. dNTPs were from Boehringer Mannheim. [^{3}H]dGTP (16.4 Ci/mmol) and [^{32}P]-α-dATP and [^{32}P]-α-dTTP (>600 Ci/mmol) were from New England Nuclear. The purity of dNTP was checked by thin-layer chromatography on PEI–cellulose [Merck; Darmstadt, West

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[1] Abbreviations: poly(dC), poly(deoxycytidylic acid); oligo(dG), oligo(deoxyguanidylic acid); dNTP, deoxyribonucleoside 5′-triphosphate; dNMP, deoxyribonucleoside 5′-monophosphate; AP sites, apurinic/apyrimidinic sites; Cl_3CCOOH, trichloroacetic acid; PEI, poly(ethylenimine); Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; Cl_3CCOOH, trichloroacetic acid; HPLC, high-performance liquid chromatography.

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Germany; as described by Herschfield & Nossal (1972)]. No contaminant was detected.

Methods

Preparation of Poly(dC) Templates Containing Various Lesions. (1) Poly(dC) Containing dUMP Residues. (A) Heat Deamination at Neutral pH. Poly(dC)$_{300}$ was dissolved in 10 mM Tris-HCl, pH 8.0, containing 20 mM KCl and 0.1 mM Na_2EDTA (buffer A). The final concentration was 1 mM dCMP residues. Unless otherwise stated, poly(dC) was heated at 95 °C in buffer A for 6 h. It was hybridized with oligo(dG)$_{12-18}$ at a molar ratio of dCMP/dGMP of 2:1. Annealing was achieved by heating the mixture at 55 °C for 10 min, followed by slow cooling to room temperature.

(B) Heat Deamination at Alkaline pH. Poly(dC) was heated at 70 °C in 1.0 M NaOH (Ullman & McCarthy, 1973). After the solution was cooled, it was neutralized with HCl, dialyzed against buffer A, and hydridized with oligo(dG).

(2) Poly(dC) Containing AP Sites. The dUMP residues were induced by heating poly(dC) at 95 °C. Unless otherwise stated, the length of the heat treatment was 6 h. After the solution was cooled, it was hybridized with oligo(dG) at a 2:1 ratio, as previously described. AP sites were introduced to the above substrate with the aid of uracil–DNA glycosylase from *M. luteus*; the reaction mixture (0.05 mL) contained 10 mM Tris-HCl, pH 6.8, 20 mM KCl, 100 μM poly(dC)·oligo(dG), and 0.5 unit of uracil–DNA glycosylase. The reaction was carried out at 37 °C for 30 min. The enzyme was inactivated by heating at 50 °C for 3 min.

(3) Nicking of Poly(dC) at AP Sites. M. luteus AP endonuclease B was used to nick phosphodiester bonds at AP sites. The reaction mixtures (0.05 mL) contained 50 mM Tris-HCl, pH 8.0, 2.5 mM $MgCl_2$, 100 μM poly(dC)·oligo(dG), and 2.5 units of AP endonuclease B. After the treatment, the templates were heated at 50 °C for 3 min in order to inactive the enzyme and dialyzed overnight against buffer A.

Replication Fidelity Assays. (1) Replication by DNA Polymerase I. The incubation mixture (0.1 mL) containing 50 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 μM [^{3}H]dGTP (15 cpm/pmol), 50 μM [^{32}P]-α-dATP (3000 cpm/pmol), 50 μM poly(dC)·oligo(dG) expressed in dCMP residues, and 0.2 unit of *E. coli* DNA polymerase I. The reaction was carried out at 30 °C for 15 min, and the incorporation of dGMP and dAMP into an acid-precipitable fraction was measured. In the case of dTMP incorporation, we added 100 μM poly(dC)·oligo(dG), expressed in dCMP residues, and 50 μM [^{32}P]-α-dTTP (5000 cpm/pmol). The reaction mixtures were directly spotted on GF/C (Whatman) filters. The filters were washed 3 times with an ice-cold solution of 5% Cl_3CCOOH and 2% tetrasodium pyrophosphate, and once in 5% Cl_3CCOOH. They were finally soaked in ethanol and dried. The radioactivity bound to the filter was measured by liquid scintillation counting. The counting efficiency was 45% for tritium. The blank values without either poly(dC) or polymerase I were subtracted; they did not exceed 0.1 pmol with any of the triphosphates.

(2) Replication by DNA Polymerase III. The replication fidelity assay (0.15 mL) contained 20 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 100 μM [^{3}H]dGTP (15 cpm/pmol), 50 μM [^{32}P]-α-dATP (3000 cpm/pmol), 100 μM poly(dC)·oligo(dG), and 0.1 unit of polymerase III. The reaction was carried out at 30 °C for 30 min. The mixture was spotted on filters and processed as described for polymerase I.

Enzymatic Hydrolysis of the Newly Synthesized Products. The poly(dC)·oligo(dG) templates were replicated by *E. coli* DNA polymerase I, as previously described. The reaction products were extensively dialyzed against 1 mM Na_2EDTA until all the acid-soluble materials were removed. The 3′→5′ exonucleolytic degradation mixtures (0.1 mL) contained 50 mM Tris-HCl, pH 8.0, 5 mM $MgCl_2$, 1 mM dithiothreitol, 3 units of *E. coli* DNA polymerase I (large fragment), and 1 nmol of neosynthetized products. In the absence of dXTP, the large fragment of *E. coli* polymerase I (lacking the 5′→3′ exonuclease activity) acted as a 3′→5′ exonuclease, hydrolyzing double-stranded and single-stranded DNA (Kornberg, 1974). The degradation was carried out at 30 °C, and the release of acid-soluble material was measured. The reaction was stopped by adding 0.05 mL of cold calf thymus DNA (0.5 mg/mL), followed 2 min later by 0.2 mL of cold 0.8 N perchloric acid. After 5 min at 0 °C, the mixture was centrifuged at 6000*g* for 15 min at 4 °C. The radioactivity of the supernatant was determined by liquid scintillation counting.

Alkaline $CsSO_4$ Density Gradient Centrifugation of Newly Synthesized Products. The reaction products obtained as described above were centrifuged in a density gradient according to a modification of the method described by Inman & Baldwin (1964). The density gradient (4.5 mL) contained 2.9 g of $CsSO_4$ diluted in buffer A supplemented with 15 mM K_3PO_4, 0.12 N KOH, the dialyzed reaction products, and poly(dG)·poly(dC) marker molecules. The initial density was 1.5260 g/cm^3. The centrifugation was performed at 35 000 rpm in an SW 50.1 Beckman rotor for 66 h at 15 °C. Fractions were collected from the bottom, the acid-insoluble radioactivity was measured, and the position of the marker molecules was monitored by the absorbance at 254 nm.

Quantification of dUMP Residues by HPLC. Heat-treated poly(dC) (25 μL of a 5 mM solution) was digested by the combined action of pancreatic DNase I and venom phosphodiesterase, as described by Lindahl & Nyberg (1974). The mononucleotides were separated by using high-performance liquid chromatography and a C_{18} μBondapak column (Waters; Milford, MA) as described by Leblanc et al. (1982). The mobile phase consisted of 50 mM ammonium phosphate buffer (pH 4.0) containing 2% methanol. The amounts of dCMP and dUMP were measured by the optical density at 254 nm. The order of elution was dCMP (3.75 mL) and dUMP (5.25 mL).

Results

The incorporation of dGMP and dAMP by *E. coli* DNA polymerase I was measured by using, as template, poly(dC) previously heated under conditions which allow the specific deamination of cytosine, yielding uracil. Figure 1 shows that after a given time of incubation with polymerase I, the incorporation of dAMP is proportional to the time of exposure of the template at 95 °C, while the dGMP incorporation remains constant. The ratio of dAMP/dGMP incorporation is, therefore, a linear function of the time at 95 °C. The low dAMP incorporation with untreated poly(dC) will be discussed later. The rate of formation of dAMP-coding residues, calculated from the results of Figure 1, is 1.9×10^{-7} residues/s.

Similar experiments were performed with DNA polymerase III, the enzyme responsible for DNA replication in vivo (Kornberg & Gefter, 1972). Polymerase III incorporates dAMP/dGMP at the same rate as polymerase I when heat-treated poly(dC) is the template. (In our experiments, polymerase I incorporated 1722 pmol of dGMP and 8.02 pmol of dAMP, whereas polymerase III incorporated 410 pmol of dGMP and 1.98 pmol of dAMP.)

A direct measurement of the dUMP content of heat-treated poly(dC) was performed by HPLC. The amount of

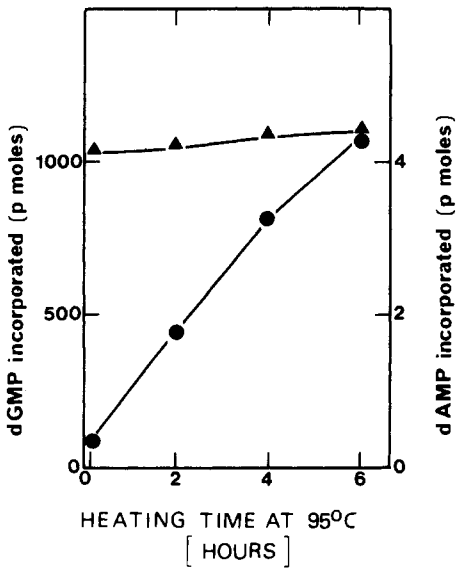

FIGURE 1: Incorporation of dGMP and dAMP by *E. coli* DNA polymerase I by using as template poly(dC) heated at 95 °C for increasing lengths of time. The poly(dC) was heated under neutral conditions before annealing and replication by polymerase I. The incorporation of dGMP (▲) and dAMP (●) into acid-insoluble fractions was measured. For details, see Materials and Methods.

dUMP/dCMP increased linearly with time. At 95 °C, and under our experimental conditions, the rate of formation of dUMP residues was 2.2×10^{-7} deaminations/s. This is in good agreement with the value calculated from the data of Figure 1.

When heat-treated poly(dC) was incubated with uracil–DNA glycosylase before replication by DNA polymerase I, the incorporation of dAMP decreased as the amount of uracil–DNA glycosylase increased (Figure 2A). This shows that this specific DNA repair enzyme, which is 2-fold more active on single-stranded than on double-stranded DNA (Leblanc et al., 1982), prevents the mutagenic incorporation of dAMP. This effect is abolished when uracil–DNA glycosylase is incubated in the presence of its specific inhibitor, which is induced in *Bacillus subtilis* by phage PBS-I (Figure 2B). These results confirm that the dAMP-coding lesion induced by heat treatment is a dUMP residue.

When dUMP residues occur in DNA, they can either be replicated by DNA polymerases, leading to a mutagenic incorporation of dAMP as shown above, or be excised by specific DNA repair enzymes before replication. The recognition by *E. coli* polymerase I of the AP sites which appear when heat-treated poly(dC) is preincubated with uracil–DNA glycosylase is dealt with now.

Table IA shows the results obtained by using heat-treated poly(dC) as template. When DNA polymerase I was activated by Mg^{2+}, the dAMP incorporation was greatly reduced by preincubation of the template with uracil–DNA glycosylase alone, whereas AP endonuclease alone did not have any effect. Pretreatment of the template with both uracil–DNA glycosylase and AP endonuclease had about the same effect as treatment with uracil–DNA glycosylase alone. When polymerase I was activated by Mn^{2+}, the dAMP incorporation was slightly reduced by either uracil–DNA glycosylase or AP endonuclease alone. It was, however, further decreased by the combined action of the two enzymes. These results suggest that the Mn^{2+}-activated polymerase I incorporates dAMP opposite the AP sites generated by uracil excision. So that this hypothesis could be tested, poly(dC) containing dUMP residues was successively incubated with saturating amounts

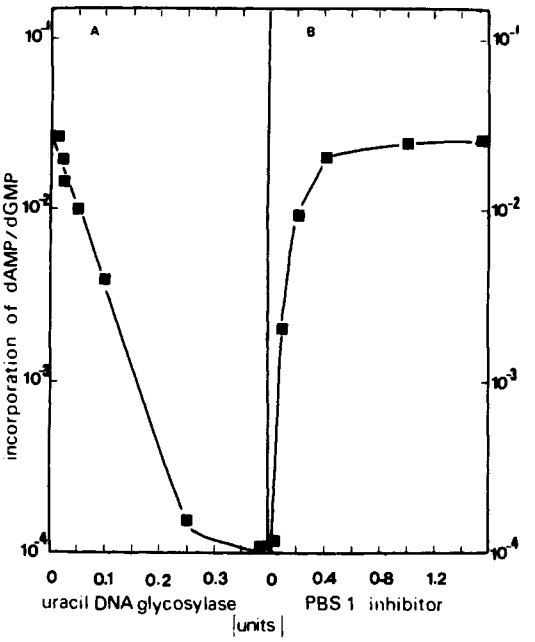

FIGURE 2: Enzymatic characterization of the dAMP-coding residues induced in poly(dC) by heat treatment. (A) dAMP incorporation when heat-treated poly(dC) is preincubated with uracil–DNA glycosylase. Poly(dC) was heated for 1 h at alkaline pH. The reaction was carried out in two steps. The excision of uracil by uracil–DNA glycosylase was performed in the presence of increasing amounts of uracil–DNA glycosylase. The polymerase fidelity assay was then performed by adding the polymerization mixture (see Methods). The ratio of dAMP/dGMP incorporated was measured (■). (B) Influence of the PBS-1 inhibitor of uracil–DNA glycosylase and uracil–DNA glycosylase on the incorporation of dAMP by *E. coli* DNA polymerase I. Increasing amounts of the PBS-1 inhibitor of uracil–DNA glycosylase were added during the first step of the reaction, 2 min before the addition of 0.5 unit of uracil–DNA glycosylase. The reaction was then carried out as described above. The ratio of dAMP/dGMP incorporated was determined (■).

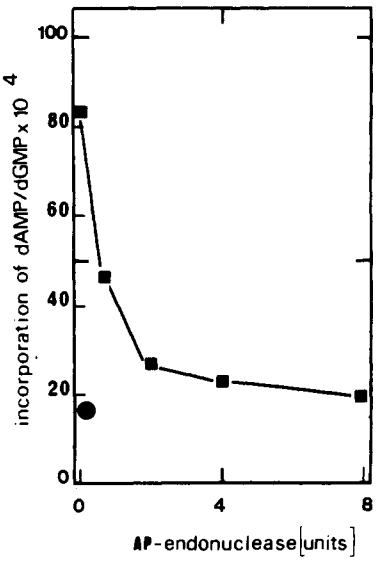

FIGURE 3: Enzymatic characterization of AP sites as dAMP-coding lesions in poly(dC), in the presence of Mn^{2+}-activated polymerase I. The poly(dC) template containing AP sites was prepared as described under Methods. It was further treated with increasing amounts of AP endonuclease B. The template was then used for a replication fidelity experiment, performed as described under Methods except that 0.25 mM $MnCl_2$ was added instead of $MgCl_2$. The ratio of dAMP/dGMP incorporated was measured (■). (●) dAMP/dGMP incorporated with an unheated poly(dC) template.

of uracil–DNA glycosylase and increasing amounts of AP endonuclease B. Figure 3 shows that the dAMP incorporation

Table I: Incorporation of dGMP and dAMP by *E. coli* DNA Polymerase I during the Replication of Untreated or Heat-Treated Poly(dC) Incubated in the Presence or Absence of DNA Repair Enzymes

activating ion	enzyme added: uracil-DNA glycosylase	enzyme added: AP endonuclease	dGMP incorp (pmol)	dAMP incorp (pmol)	dAMP/dGMP × 10^4
		(A) Heat-Treated Poly(dC)[a]			
Mg^{2+}	−	−	886	4.86	54.9
	+	−	844	0.30	3.6
	−	+	866	4.97	57.4
	+	+	813	0.22	2.7
Mn^{2+}	−	−	728	6.11	83.9
	+	−	712	4.31	60.6
	−	+	712	5.05	70.9
	+	+	718	1.80	25.1
		(B) Unheated Poly(dC)[b]			
Mg^{2+}	−	−	1010	0.48	4.7
	+	−	800	0.24	3.0
	−	+	967	0.38	4.0
	+	+	1014	0.25	2.4
Mn^{2+}	−	−	881	2.05	23.0
	+	−	700	1.43	20.0
	−	+	687	1.07	16.0
	+	+	820	1.22	15.0

[a] The poly(dC) was heated at 95 °C for 6 h and then submitted to various enzymatic treatments before replication. The replication fidelity assays were performed by using either 1.5 mM $MgCl_2$ or 0.25 mM $MnCl_2$. For details, see Methods. [b] Unheated poly(dC) was used as template. The enzymatic treatments and replication fidelity assays were as described for heat-treated poly(dC).

Table II: Incorporation by *E. coli* Polymerase I of dTMP and dGMP in Poly(dC) Templates Containing dUMP Residues or Apyrimidinic Sites[a]

activating ion	uracil-DNA glycosylase	dGMP incorp (pmol)	dTMP incorp (pmol)	dTMP/dGMP × 10^4
Mg^{2+}	−	4272	0.13	0.31
	+	3221	0.11	0.34
	+[b]	4151	0.13	0.32
Mn^{2+}	−	3036	0.91	3.0
	+	2808	1.49	5.3
	+[b]	2016	0.36	1.7

[a] The modified poly(dC) templates used were the same as those described in Table I. The replication fidelity assays were performed by using either 1.5 mM $MgCl_2$ or 0.25 mM $MnCl_2$. In a control experiment, the ratio of incorporated dAMP/dGMP was 55 × 10^{-4}. [b] Values obtained with unheated poly(dC).

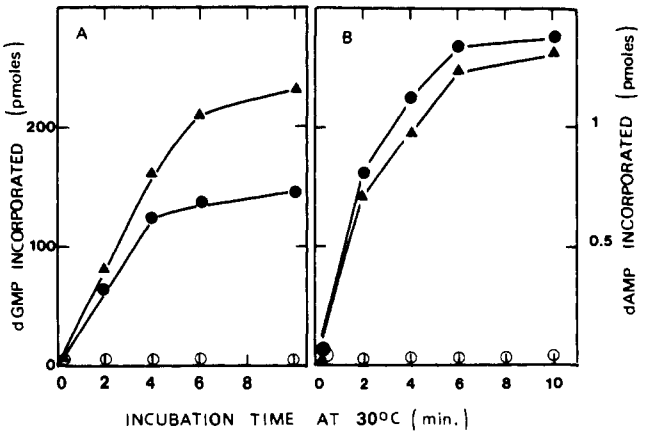

FIGURE 4: Kinetics of incorporation of dAMP and dGMP by *E. coli* DNA polymerase I by using poly(dC) templates containing either uracil or AP sites. The poly(dC) templates containing dUMP residues or AP sites were replicated by *E. coli* DNA polymerase activated by either $MgCl_2$ or $MnCl_2$, respectively. The kinetics of incorporation of dGMP (▲) and dAMP (●) were measured, as well as the incorporation of dAMP (○) in the absence of dGTP in the reaction mixture. Each point is one-fifth of the incubation mixture described in Figure 1. (A) Nucleotide incorporation by using as template poly(dC) containing dUMP residues. The DNA polymerase was activated by $MgCl_2$. (B) nucleotide incorporation by using as template poly(dC) containing AP sites. The DNA polymerase was activated by $MnCl_2$.

by polymerase I using, as template, poly(dC) containing AP sites decreases as the AP endonuclease increases. Pretreatment of the template with AP endonuclease B, which nicks preferentially double-stranded DNA, or with Lys-Trp-Lys, which nicks single-stranded DNA, prevents up to 95% of the dAMP incorporation when compared with the incorporation into unheated template. These results suggest that the alteration of the DNA polymerase fidelity by Mn^{2+} greatly stimulates the mutagenic incorporation of dAMP at AP sites.

Table IB also shows that the unheated poly(dC) template contains a low level of dUMP residues (estimated from our data to be about 1 dUMP residues per 2000–5000 nucleotides, depending on the batch used) as well as AP sites. The fact that the cleavage of DNA at AP sites at their 5′ side by AP endonuclease B (Pierre & Laval, 1980b), as well as the cleavage at the 3′ side by NaOH (Lindahl & Anderson, 1972) or by the tripeptide Lys-Trp-Lys (Pierre & Laval, 1981) (data not shown), prevents dAMP incorporation suggests that the AP sites which remain at the 5′ end of the polymer are not replicated. It suggests that these sites have to be inserted in a poly(dC) chain in order to be replicated. It should be noticed that the dGMP incorporation is weakly affected by the various treatments. Neither dUMP residues nor AP sites stimulate dTMP incorporation in relation to dGMP, when polymerase I is activated by Mg^{2+} (Table II). When polymerase I is activated by Mn^{2+}, the AP sites slightly stimulate the incorporation of dTMP (Table II). This effect is prevented when the poly(dC) templates containing AP sites are preincubated with AP endonuclease B (data not shown). By comparing the results of Tables I and II, it appears that the dTMP incorporation opposite AP sites is less than 10% that of dAMP. We have also investigated the possible incorporation of the other dNMPs opposite the AP sites by competition experiments. The addition of any other dNTP in the reaction mixture slightly decreases the dAMP incorporation. The efficiency order seems to be dGTP > dCTP > dTTP.

The characterization of the products obtained by replication

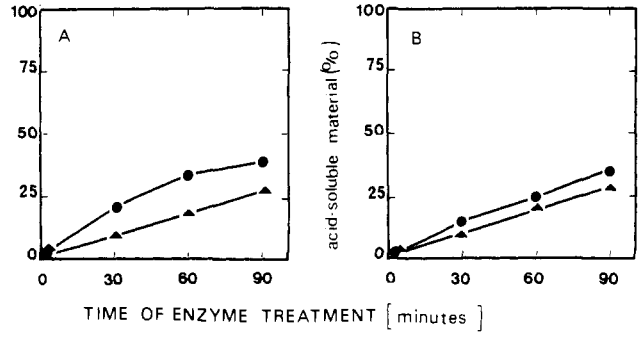

FIGURE 5: Enzymatic hydrolysis of products synthesized by polymerase I by using poly(dC) templates containing either uracil or AP sites. The products synthesized by DNA polymerase, as in Figure 4, were submitted to exonucleolytic degradation as described under Methods. The release of acid-soluble [^{3}H]dGMP (▲) and [^{32}P]dAMP (●) was measured. (A) Polynucleotides obtained after replication of poly(dC) containing dUMP residues by $MgCl_2$-activated polymerase I. The reaction mixture contained 14800 cpm of [^{3}H]dGMP and 17300 cpm of [^{32}P]dAMP incorporated into acid-insoluble material. (B) Polynucleotides obtained after replication of poly(dC) containing AP sites by $MnCl_2$-activated polymerase I. The reaction mixture contained 15630 cpm of [^{3}H]dGMP and 18200 cpm of [^{32}P]dAMP incorporated into acid-insoluble material.

of poly(dC) containing either dUMP residues or AP sites shows that the incorporation of dAMP and dGMP follows similar kinetics (Figure 4A,B). The dAMP incorporation is entirely dependent on the presence of dGTP in the reaction mixture (Figure 4A,B). The hydrolysis of the newly synthesized polynucleotides by a 3′→5′ exonuclease is shown in Figure 5A,B. The dGMP and dAMP incorporated opposite the uracil (Figure 5A) or the AP sites (Figure 5B) by DNA polymerase I are released by the exonuclease at the same rate. Analysis of the products by density centrifugation shows that dAMP bands with dGMP in alkaline $CsSO_4$ density grandients, with marker poly(dG) added (Figure 6A,B). These results, as well as the kinetics of incorporation of dAMP (Figure 4), strongly suggest that dAMP is randomly incorporated into newly synthesized poly(dG) chains through phosphodiester bonds, opposite either dUMP or AP sites.

Discussion

Heat treatment of poly(dC) induces the formation of dUMP residues, which seem to be quantitatively replicated in vitro by *E. coli* DNA polymerases I and III, with specific dAMP incorporation. The results suggest that cytosine deamination yielding uracil is a premutagenic DNA modification leading to GC–AT transitions. This, in turn, suggests that one of the biological roles of uracil–DNA glycosylase is to prevent this type of mutation. These in vitro results are in agreement with observations showing that *E. coli* mutants, which incorporate dUMP at high rates (Warner & Duncan, 1978), are normally viable and that mutants deficient in uracil–DNA glycosylase are GC–AT mutators (Duncan & Miller, 1980).

Our results show that the AP sites, which result from uracil excision, induced a barely detectable mutagenic incorporation of dAMP by a Mg^{2+}-activated polymerase I. The alteration of the base selection fidelity mechanism of polymerase I by Mn^{2+} (Sirover & Loeb, 1976) greatly stimulates the in vitro replication of AP sites with incorporation of any of the four dNMPs opposite them. The premutagenic role of AP sites as the source of transitions and transversions is confirmed by the fact that AP endonuclease prevents the Mn^{2+}-stimulated incorporation of noncomplementary dAMP and dTMP. However, the dNMPs are not randomly incorporated opposite AP sites, dAMP being preferentially incorporated as compared to dTMP. Our results and those reported by Shearman &

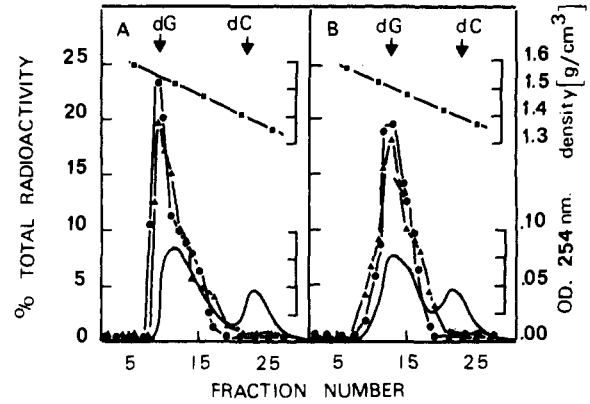

FIGURE 6: Alkaline $CsSO_4$ density gradient centrifugation of polynucleotides synthesized by polymerase I by using poly(dC) templates containing either uracil or AP sites. The polynucleotides were prepared as in Figure 5 and analyzed by alkaline $CsSO_4$ density gradient centrifugation. The gradients were collected, and the acid-insoluble radioactivity was measured. (●) (^{32}P)dAMP; (▲) (^{3}H)dGMP. The absorbance of poly(dC) and poly(dG) markers at 254 nm (—) and the density (●) were also measured. (A) Polynucleotides obtained after replication of poly(dC) containing dUMP residues by $MgCl_2$-activated polymerase I. 8950 cpm of [^{3}H]dGMP and 10034 cpm of [^{32}P]dAMP were placed in the gradient. (B) Polynucleotides obtained after replication of poly(dC) containing AP sites by $MnCl_2$-activated polymerase I. 7500 cpm of [^{3}H]dGMP and 9370 cpm of [^{32}P]dAMP were placed in the gradient.

Loeb (1979) show that AP sites induce different patterns of dNMP incorporation, depending on the template in which the AP sites are introduced. A possible explanation is that the nature of the nucleotide sequence surrounding the AP site directs the type of incorporation rather than the AP site itself. Alternatively the specificity of dAMP incorporation opposite AP sites may be due to the DNA polymerase itself, as suggested by Strauss et al. (1982). Using poly(dC) as template, we have observed an inhibition of DNA synthesis by polymerase I when the proportion of AP sites exceeds 1% of the number of dCMP residues. The apparent lack of inhibition of DNA synthesis by AP sites in our experiments (0.5% modification) may be due to the presence of multiple oligo(dG) primers per poly(dC) molecule. Similar results have been obtained by using poly(dC) templates containing pyrimidine dimers (Lecomte et al., 1981). These in vitro results indicate that AP sites are mostly inactivating DNA lesions under constitutive conditions of replication but that they may be highly mutagenic under conditions which decrease replication fidelity.

Our data emphasize the key role played by base excision DNA repair enzymes in the maintenance of the integrity of the genetic information, and in the survival of the cell. They also suggest that the replication of noninstructive DNA lesions may be related with a diminished DNA polymerase fidelity. Lackey et al. (1982) have found in SOS-induced bacteria a new form of DNA polymerase I. Its fidelity during the polymerization reaction is lower than that of the constitutive enzyme. Schaaper & Loeb (1981) have shown that AP sites induced in *φX174* DNA are mutagenic only when infecting SOS-induced bacteria. As a working hypothesis, we propose that the alteration of base selection, possibly associated with the inhibition of the 3′→5′ exonuclease (Villani et al., 1978), could mimic the action of the *E. coli* gene products responsible for SOS mutagenesis (Kato & Shinoura, 1977).

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References

Abbott, P. J., & Saffhil, R. (1977) *Nucleic Acids Res. 4*, 761–769.
Cone, R., Bonura, T., & Friedberg, E. C. (1980) *J. Biol. Chem. 255*, 10354–10358.
Drake, J. W., & Baltz, R. H. (1976) *Annu. Rev. Biochem. 45*, 11–37.
Duncan, B. K., & Miller, J. H. (1980) *Nature (London) 287*, 569–571.
Herschfield, M. S., & Nossal, N. G. (1972) *J. Biol. Chem. 247*, 3393–3404.
Inman, R. B., & Baldwin, , R. L. (1964) *J. Mol. Biol. 8*, 452–469.
Kato, T., & Shinoura, Y. (1977) *Mol. Gen. Genet. 156*, 121–131.
Kornberg, A. (1974) in *DNA Synthesis*, pp 5369–5372, Freeman, San Francisco, CA.
Kornberg, T., & Gefter, M. L. (1972) *J. Biol. Chem. 247*, 5369–5372.
Lackey, D., Wald-Krauss, S., & Linn, S. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 330–334.
Laval, J., & Laval, F. (1980) *IARC Sci. Publ. 27*, 55–72.
Leblanc, J. P., Martin, B., Cadet, J., & Laval, J. (1982) *J. Biol. Chem. 257*, 3477–3483.
Lecomte, P., Boiteux, S., & Doubleday, O. P. (1981) *Nucleic Acids Res. 9*, 3491–3501.
Lindahl, T. (1982) *Annu. Rev. Biochem. 51*, 61–87.
Lindahl, T., & Anderson, T. (1972) *Biochemistry 11*, 3618–3623.
Lindahl, T., & Nyberg, B. (1974) *Biochemistry 13*, 3405–3410.
Livingston, D. M., Hinkle, D., & Richardson, C. C. (1975) *J. Biol. Chem. 250*, 461–469.
Pierre, J., & Laval, J. (1980a) *Biochemistry 19*, 5018–5024.
Pierre, J., & Laval, J. (1980b) *Biochemistry 19*, 5027–5031.
Pierre, J., & Laval, J. (1981) *J. Biol. Chem. 256*, 10217–10220.
Radman, M., Villani, G., Boiteux, S., Kinsella, A. R., Glickman, B. W., & Spadari, S. (1979) *Cold Spring Harbor Symp. Quant. Biol. 43*, 937–945.
Richardson, C. C., Schildkraut, C. L., Aposhian, H. V., & Kornberg, A. (1964) *J. Biol. Chem. 239*, 222–232.
Schaaper, R. M., & Loeb, L. A. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 1773–1777.
Setlow, P., Brutlag, D., & Kornberg, A. (1972) *J. Biol. Chem. 247*, 224–231.
Shearman, C. W., & Loeb, L. A. (1979) *J. Mol. Biol. 128*, 197–218.
Singer, B. (1982) *Annu. Rev. Biochem. 51*, 655–693.
Sirover, M. A., & Loeb, L. A. (1976) *Science (Washington D.C.) 194*, 1434–1437.
Strauss, B., Rabkin, S., Sagher, D., & Moore, P. (1982) *Biochimie 64*, 829–833.
Ullman, J. S., & McCarthy, B. J. (1973) *Biochim. Biophys. Acta. 294*, 396–404.
Villani, G., Boiteux, S., & Radman, M. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 3037–3041.
Warner, H. R., & Duncan, B. K. (1978) *Nature (London) 272*, 32–34.

Nuclear Matrix-Bound Deoxyribonucleic Acid Synthesis: An in Vitro System†

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ABSTRACT: A nuclear matrix-bound in vitro replicational system that utilizes α-polymerase and matrix-attached DNA has been isolated from regenerating rat liver and characterized for in vitro requirements. β-Polymerase is a minor component of the matrix in vitro system. Evidence is presented for the native association of α-polymerase with the nuclear matrix and for the parallel induction of matrix-bound α-polymerase and in vivo DNA synthesis during the proliferative response following partial hepatectomy. Our data suggest that the attachment of α-polymerase to the matrix may be an important step in the assembly of functional replisomes. We also demonstrate prereplicative and late-replicative rises in nuclear and matrix-bound β-polymerase activity and discuss these results in terms of possible genome screening.

Many cellular functions involve the regulation and integration of macromolecular assemblies, for example, the pyruvate dehydrogenase complex of *Escherichia coli*, the fatty acid synthetase of yeast, mitochondrial oxidative phosphorylation, and protein synthesis (Stryer, 1981). Significant advances have been made to define the components of the macromolecular assemblies or replisomes responsible for procaryotic DNA replication (Kornberg, 1980). It is intriguing to consider whether eucaryotic DNA replication involves mechanisms analogous to those in procaryotes. For example, Jacob et al. (1963) proposed that procaryotic DNA is synthesized on membrane-bound replisomes while Berezney & Coffey (1975, 1976) suggested that eucaryotic replication occurs in association with the matrix of the cell nucleus.

Although the precise spatial localization of functional replisomes remains to be clarified in both procaryotic and eucaryotic cells, a number of recent findings support the concept that DNA replication is a matrix-bound process in eucaryotes.

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