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Enzymatic insertion of purine bases into depurinated DNA *in vitro*

(apurinic site/DNA repair/*Escherichia coli*/bacteriophage PM2)

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ABSTRACT **An enzymatic activity that inserts purines into depurinated DNA was found in a soluble enzyme extract of *Escherichia coli*. This activity brings about the insertion of adenine and guanine into the appropriate apurinic sites in double-stranded DNA by using the corresponding deoxyribonucleoside triphosphates as the purine donors. Magnesium ions are required for this activity, it is inhibited by caffeine, and it does not act on depurinated single-stranded DNA. The insertion activity described here may represent a step in a repair mechanism, "base-insertion repair," whereby apurinic sites (which may occur in double-stranded DNA either due to the removal of damaged purines with specific glycosylases or by spontaneous depurination) are directly filled with the correct missing purine base.**

Damage to DNA in cells frequently takes the form of the removal of purines from the sugar-phosphate backbone, which leads to depurinated DNA (apDNA). Depurination of DNA may occur via one of three known pathways. These are spontaneous hydrolysis of the purine-sugar glycosylic bond (1), enhanced spontaneous hydrolysis of alkylated purines in DNA due to labilization of the glycosylic bond caused by the alkylation (e.g., the depurination of 3-methyladenine or 7-methylguanine) (2, 3), and the removal of alkylated purines from DNA catalyzed by specific glycosylases as was shown, for example, for 3-methyladenine or *O*-6-methylguanine residues in DNA (4–6).

According to current views, the damage caused by depurination is repaired by excision repair (5, 7). This pathway is initiated by an endonuclease specific for apurinic sites in DNA which causes a single-strand break in the DNA at the vicinity of the apurinic site. Subsequently, excision, polymerization, and ligation steps occur, leading to restoration of the DNA integrity. Endonucleases specific for apurinic sites have been purified from various sources (5, 8–13), and the total repair of apurinic sites in DNA *in vitro* has been demonstrated with bacterial (14) and human enzymes (15).

In this manuscript we present evidence for the existence of an enzymatic activity in a soluble enzyme extract of *Escherichia coli* that directly and specifically inserts the correct missing adenine or guanine into the appropriate apurinic sites in calf thymus and PM2 DNAs. This activity may represent a mechanism, "base-insertion repair," for repair of apurinic sites in DNA.

MATERIALS AND METHODS

[8-^{3}H]dATP (29 Ci/mmol, 1 Ci = 3.7 × 10^{10} Bq), [8-^{3}H]dGTP (11 Ci/mmol), [*methyl*-^{3}H]dTTP (46 Ci/mmol), [5-^{3}H]dCTP (22 Ci/mmol), [*U*-^{14}C]dATP (500 Ci/mol), [*U*-^{14}C]dGTP (520 Ci/mol), [*U*-^{14}C]adenine (309 Ci/mol), [*U*-^{14}C]guanosine (545 Ci/mol), [2-^{3}H]adenosine (24 Ci/mmol), [*G*-^{3}H]deoxyadenosine (21 Ci/mmol), [8-^{3}H]deoxyguanosine (2.3 Ci/mmol), [2-^{3}H]ATP (16 Ci/mmol), [α-^{32}P]dATP (200 Ci/mmol), and [α-^{32}P]dGTP (200 Ci/mmol) were purchased from the Radiochemical Centre (Amersham, England). Unlabeled deoxyribonucleoside triphosphates (dNTPs) were purchased from Boehringer Mannheim. Nucleosides and bases were from Sigma. The chemical and radioactive purities of the dNTPs were checked by thin-layer chromatography on silica gel plates in methanol/water/concentrated ammonia (6:2:1, vol/vol) or by chromatography on Whatman DE-81 paper in 0.2 M ammonium bicarbonate/5 mM sodium borate. The dNTPs were further tested by measurement of polymerization incorporation of labeled dNTPs into PM2 DNA as described (16).

The distribution of ^{14}C label between the base and sugar moieties of [*U*-^{14}C]dATP and [*U*-^{14}C]dGTP was determined by removal of the triphosphate moiety with bacterial alkaline phosphatase, followed by acid hydrolysis in 2% HCl for 15 min at 100°C. The resulting base and deoxyribose were separated either by high-voltage paper electrophoresis on Whatman no. 3MM paper, at pH 1.9 and 2500 V for 25 min, or by chromatography on Whatman no. 1 paper in *n*-butanol/water/concentrated ammonia (86:13:1, vol/vol). The paper was cut into 1-cm strips and radioactivity was measured. Ninety seven percent of the initial radioactivity was found in the spots corresponding to the base and the sugar. Thirty percent of the ^{14}C label in both uniformly labeled dATP and dGTP is located at the base, whereas 70% of the label is in the deoxyribose.

Preparation of Extract. The enzyme extract was prepared from *E. coli* H502 (*endoI*$^-$, *thy*$^-$, *uvrA*, *su*$^-$) strain. The procedures for bacterial growth and preparation of soluble enzyme extract have been described (16). The protein concentration was 10 mg/ml, as determined by the Lowry procedure (17).

DNA Preparations. Phage PM2 was prepared and purified (18), and its DNA was extracted (19). PM2 DNA labeled in adenine residues (0.5–1.0 × 10^5 cpm/μg) or in guanine residues (4–6 × 10^4 cpm/μg) was prepared by the same procedure, with [*U*-^{14}C]adenine or [*U*-^{14}C]guanosine, respectively. Calf thymus DNA was obtained from Worthington, and ϕX174 DNA, prepared according to Razin *et al.* (20), was a gift from E Livneh.

apDNA. PM2 apDNA was prepared as described (1), by incubating the DNA in 0.1 M NaCl/0.01 M sodium citrate, pH 5.0 at 70°C. Under these conditions one purine base is released per PM2 DNA molecule in about 4 min. The number of apurinic sites per molecule was determined by analysis of the purines released from purine-labeled DNA in the following way: after the depurination incubation, carrier adenine or guanine (10 μg) was added and the reaction mixture was placed onto a Whatman no. 1 paper and chromatographed in *n*-butanol/water/concentrated ammonia (86:13:1, vol/vol). The paper was then cut into 1-cm strips and radioactivity was measured. Unless otherwise stated, PM2 DNA was used that contained 10 apurinic sites each of adenine and guanine residues per molecule.

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Abbreviations: dNTP, deoxyribonucleoside triphosphate; apDNA, depurinated DNA.

Standard Reaction Mixture. The standard insertion reaction mixture (25 μl) contained 50 mM K_2HPO_4 (pH 7.4), 20 mM NaCl, 5 mM $MgCl_2$, and variable amounts of extract. Unless otherwise stated, mixtures contained 0.4 μg of PM2 DNA or 0.4 μg of PM2 DNA with 20 apurinic sites per molecule and 4 μM [8-^{3}H]dATP or [8-^{3}H]dGTP. Incubations were carried out at 37°C for 30 min.

Insertion Assay. Three different assay procedures were used to measure incorporation of label from dNTPs into apDNA:

(*i*) *DE-81 paper assay.* This assay was performed essentially as described (21). After the incubation was completed, the whole reaction mixture was applied to a 2-cm square of Whatman DE-81 paper. The paper squares (from several incubations) were washed four times by gentle agitation for 5 min in 100 ml of 0.3 M ammonium formate solution adjusted to pH 7.8, which contained 10 mM adenosine and 10 mM sodium pyrophosphate to reduce background. The paper squares were dehydrated by two washes in 95% ethanol and one in anhydrous ether. They were air dried and the amount of radioactivity incorporated into the DNA was measured.

(*ii*) *Trichloroacetic acid precipitation assay.* Carrier calf thymus DNA (20 μg) was added at the end of incubation and the DNA was precipitated by addition of 0.1 vol of 3 M sodium acetate (pH 5.5) and 3 vol of absolute ethanol that had been cooled to −20°C. After cooling at −20°C for 30 min and centrifugation, the supernatant was discarded and the DNA was dissolved in water (100 μl). Cold 5% trichloroacetic acid (2 ml) was added, and the mixture was allowed to stand for 10 min at 0°C. The mixture was then filtered through a Whatman GF/C filter and washed three times with 2-ml portions of cold 5% trichloroacetic acid. The filters were dried and radioactivity was determined.

(*iii*) *Agarose gel assay.* The reaction was terminated by extraction with an equal volume of phenol. Sixty percent sucrose solution (10 μl) containing bromphenol blue was added and the mixture was applied to the top of an agarose slab gel. The gels, 3 mm thick and 20 cm long, contained 0.7% agarose in 40 mM Tris·HCl/20 mM sodium acetate/2 mM EDTA at pH 7.7. The gels were run for 3 hr at 200 V at 4°C and were stained in ethidium bromide (0.1 μg/ml in the running buffer). In order to reduce the background radioactivity, we gently agitated the gels for 3 hr in 400 ml of the same buffer. Each of the visible DNA bands was cut out of the gel and solubilized in 5% perchloric acid (4 ml) by warming at 65°C for 30 min. The solution was neutralized with 2 M NaOH (1.6 ml), Triton/toluene-based scintillation liquid (6 ml) was added, and the radioactivity of the mixture was determined.

Table 1. Incorporation of [^{3}H]dATP into PM2 apDNA

Exp.	dATP incorporation, pmol: DE-81 assay	Cl_3CCOOH assay	Gel assay
Standard reaction mixture (t = 30 min)			
+ apDNA	1.16	0.70	0.65
+ intact DNA	0.60	0.20	0.12
Net	0.56	0.50	0.53
Standard reaction mixture (t = 0 min)			
+ apDNA	0.50	0.10	0.05
+ intact DNA	0.40	0.10	0.05
Net	0.10	0	0
Standard reaction mixture without extract			
+ apDNA	0.45	0.10	<0.05
+ intact DNA	0.40	0.10	<0.05
Net	0.05	0	0
[^{3}H]dATP added at the end of incubation			
+ apDNA	0.47	0.10	<0.05
+ intact DNA	0.43	0.10	<0.05
Net	0.04	0	0
Standard reaction mixture without DNA	0.37	0	0

Samples were incubated under standard conditions. The reaction mixture contained 0.4 μg of PM2 apDNA or intact PM2 DNA, 4 μM [8-^{3}H]dATP, and 8 μg of protein. The samples were assayed by the indicated methods. [8-^{3}H]dGTP gave similar results (data not shown).

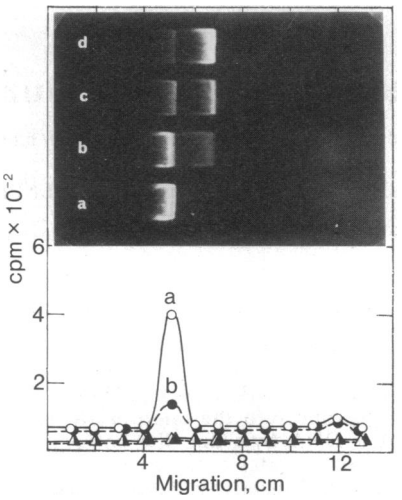

FIG. 1. Gel analysis of PM2 DNA after insertion incubation. Samples were incubated under standard conditions with 4 μM each of [*U*-^{14}C]dATP and [*U*-^{14}C]dGTP and with 4 μg of protein per sample. Gels were run, stained, and cut into 5-mm slices and radioactivity was measured. (*Upper*) Ethidium bromide-stained gel; (*Lower*) radioactivity distribution along the gel. Gel a (○), PM2 DNA (0.4 μg) with 10 apurinic sites per molecule; gel b (●), intact PM2 DNA (0.4 μg); gel c (△), as in gel a, without extract; gel d, (▲) as in gel b, without extract.

Isolation and Analysis of DNA after Insertion Reaction. The reaction in a standard mixture containing [*U*-^{14}C]dATP or [*U*-^{14}C]dGTP was terminated by phenol extraction. Carrier calf thymus DNA (20 μg) was added and the volume was adjusted to 1 ml by addition of water. This mixture was extensively dialyzed against 10 mM K_2HPO_4/1 M NaCl/10 mM adenosine at pH 7.4 and then against 1 mM K_2HPO_4 (pH 7.4).

Total depurination was performed by mild acid treatment (2% HCl at 100°C for 15 min). The sample was then lyophilized, chromatographed in *n*-butanol.water/ammonia (86:13:1, vol/vol), and analyzed as described.

Total enzymatic digestion of the isolated DNA was performed as described (22), with DNase I and snake venom phosphodiesterase (Boehringer Mannheim) at neutral pH. The digest was chromatographed on Whatman no. 1 paper in *n*-propanol/concentrated ammonia/water (6:3:1, vol/vol) and air dried. The paper was cut and radioactivity was measured. The strips on which radioactivity was found were washed several times with toluene, air dried, and eluted with water. After lyophilization, each sample was dissolved in 95 μl of 40 mM Tris·HCl (pH 7.8). Alkaline phosphatase (5 μg) was added and the mixture was incubated for 2 hr at 25°C. At the end of incubation, each sample was divided into two equal portions. The first portion was chromatographed in *n*-butanol/water/

concentrated ammonia (86:14:5, vol/vol), while the second portion was hydrolyzed by acid treatment (2% HCl for 10 min at 100°C) and chromatographed in the same solvent system. Both chromatograms were dried and cut, and radioactivity was measured.

RESULTS AND DISCUSSION

Incorporation of Purines into apDNA. Incubation of PM2 apDNA with a soluble enzyme extract of *E. coli* in the presence of Mg^{2+} and labeled dATP or dGTP resulted in the incorporation of radioactivity into the DNA (Table 1). Three different assay procedures gave identical results for the net incorporation of radioactivity, which was calculated by subtracting the incorporation into intact PM2 DNA from the incorporation into PM2 apDNA. These procedures were adsorption of DNA on DEAE-cellulose paper, precipitation by trichloroacetic acid, and agarose gel electrophoresis.

Fig. 1 shows a typical agarose gel analysis of PM2 apDNA after incubation. The apDNA appears as the nicked circular form, whereas intact DNA, which was incubated under the same conditions, appears as a mixture of circular form I and the nicked circular form, the latter being the major component. In both cases a minor amount of smaller fragments appears. These fragments originate, mainly, from the extract itself, as they appeared in the gel when extract samples were run alone. However, we cannot exclude the possibility of very limited degradation of the DNA by the extract. Formation of the nicked circular form DNA is probably due to nucleolytic activity present in the extract. The absence of circular form I DNA from the incubated PM2 apDNA can be attributed to its smaller initial amount in the PM2 apDNA preparation, as compared with the intact PM2 DNA preparation (Fig. 1, gels c and d), or to its incision by endonucleases specific for apurinic sites in DNA. The label distribution along the gel shows clearly that the radioactivity is located only at the band of incubated apDNA (Fig. 1, *lower*). This band contains 0.25 pmol of [^{14}C]dNTP per 0.23 pmol of available apurinic sites. Incorporation into intact PM2 DNA amounts to only 0.07 pmol, and no significant label is associated with the small fragments. These data eliminate the possibility of end labeling of small fragments. We have also found that addition of DNase I to a standard reaction mixture containing intact PM2 DNA does not increase the incorporation of labeled dATP or dGTP into the DNA, indicating that random nicks on intact double-stranded regions do not contribute to the incorporation under our reaction conditions.

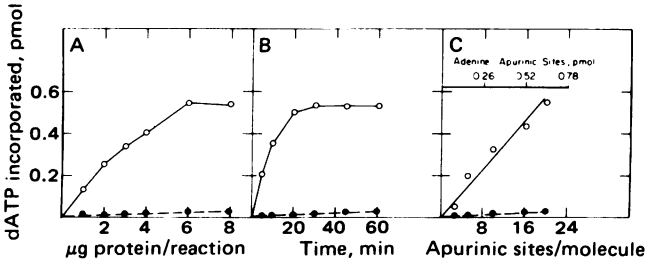

FIG. 2. Incorporation of [8-^{3}H]dATP and [α-^{32}P]dATP into PM2 apDNA, measured by DE-81 assay. (*A*) Samples were incubated under standard conditions with 4 μM dATP containing [8-^{3}H]dATP (4×10^5 cpm) and [α-^{32}P]dATP (4×10^5 cpm) and the indicated amounts of protein. (*B*) Standard incubation mixtures containing dATP as in *A* and 6 μg of protein were analyzed at the indicated time intervals. (*C*) Standard mixtures contained dATP as in *A*, 8 μg of protein, and 0.4 μg of PM2 DNA containing the indicated number of apurinic sites per molecule. O, Incorporation of [8-^{3}H]dATP; ●, incorporation of [α-^{32}P]dATP.

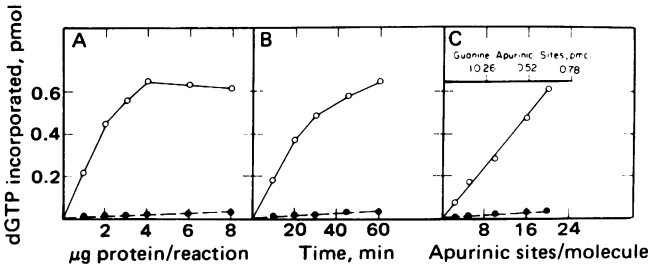

FIG. 3. Incorporation of [8-^{3}H]dGTP and [α-^{32}P]dGTP into PM2 apDNA, measured by DE-81 assay. (*A*) Samples were incubated under standard conditions with 4 μM dGTP containing [8-^{3}H]dGTP (2×10^5 cpm) and [α-^{32}P]dGTP (4×10^5 cpm) and the indicated amounts of protein. (*B*) Standard incubation mixtures containing dGTP as in *A* and 3 μg of protein were analyzed at the indicated time intervals. (*C*) Standard mixtures contained dGTP as in *A*, 6 μg of protein, and 0.4 μg of PM2 DNA containing the indicated number of apurinic sites per molecule. O, Incorporation of [8-^{3}H]dGTP; ●, incorporation of [α-^{32}P]dGTP.

The plots of the net incorporation as a function of added extract (Figs. 2*A* and 3*A*) and of time (Figs. 2*B* and 3*B*) are characteristic of enzymatic activity. This has been substantiated by further characterization of the activity. Thus, the insertion activity requires Mg^{2+}, and it is totally inhibited in the presence of high salt concentrations (1 M NaCl) or by using heated (60°C for 15 min) enzyme extract. Caffeine, which is an inhibitor of repair *in vivo* (23), inhibited the insertion activity by 20%.

The incorporation is an efficient process since it reaches high values of saturation. For example, 0.53 pmol of dATP was incorporated into a total amount of 0.64 pmol of available adenine apurinic sites (80%), whereas 0.65 pmol of dGTP was incorporated into 0.64 pmol of available guanine apurinic sites (100%). Furthermore, the saturation values of incorporation correlate linearly with the number of available apurinic sites in the DNA (Figs. 2*C* and 3*C*).

The substrates required for the insertion activity of the *E. coli* extract are double-stranded apDNAs. PM2 apDNA and calf thymus apDNA incorporate dATP and dGTP with similar efficiency. On the other hand, purine nucleotides were not incorporated into ϕX174 apDNA, indicating that single-stranded apDNAs are not substrates for the insertion activity. The amount of ϕX174 DNA that was degraded during the incubation into small pieces and thus escaped our assay procedure was less than 20% of the total starting DNA. This excludes the possibility that our failure to observe incorporation into single-stranded DNA was due to its degradation by nucleases present in the extract.

dATP and dGTP Are Donors for Insertion Activity. A scan of various nucleic acid base derivatives shows that only [^{3}H]-dATP and [^{3}H]dGTP, and to a smaller extent [^{3}H]dTTP and [^{3}H]ATP, are incorporated into apDNA (Table 2). Bases or nucleosides are not incorporated, while the small incorporation of [^{3}H]dTTP can be completely eliminated in the presence of an equimolar concentration of unlabeled dATP. ATP may serve as a donor too; however, this point has not been established. In order to determine whether dATP and dGTP are the donors for the insertion activity, we measured the incorporation of [^{3}H]dATP or [^{3}H]dGTP into apDNA in the presence of a 100-fold excess concentration of unlabeled adenine or guanine derivatives, respectively. Such an excess of an unlabeled presumed donor is expected to decrease considerably the specific activity of the labeled one, thereby reducing the incorporation of label into the DNA to an undetectable level. The data (Table 3) indicate that only the addition of the appropriate unlabeled dNTP completely abolishes the incorporation of label into the

Table 2. Incorporation of various base derivatives into PM2 apDNA

Labeled base derivatives	Net incorporation, pmol
[^{3}H]dATP	0.53
[^{3}H]dGTP	0.65
[^{3}H]dCTP	0
[^{3}H]dTTP	0.20
[^{3}H]ATP	0.35
[^{3}H]Ade, [^{3}H]Ado, [^{3}H]dAdo	0
[^{3}H]Gua, [^{3}H]Guo, [^{3}H]dGuo	0

Samples were incubated under standard conditions with 8 μg of protein per sample and the indicated labeled base derivatives and assayed by the DE-81 procedure.

DNA. Moreover, lowering the specific activity of the labeled dNTP by addition of the unlabeled one results in a linear decrease in the incorporation of radioactivity (data not shown). This indicates that dATP and dGTP are true donors for the insertion activity. The fact that none of the purine derivatives listed in Table 3, excluding the dNTPs, reduces the incorporation of label below the detection level rules out the possibility that either of these is an intermediate in the insertion reaction. The significantly reduced incorporation of the labeled dNTPs in the presence of an excess of the corresponding unlabeled dNDPs may be caused, at least in part, by conversion of the diphosphate derivatives into the corresponding triphosphates by enzymes present in the extract, thereby reducing the specific activity of the labeled dNTPs.

Base Moiety of dNTPs Is the Inserted Group. Measurements of the incorporation of the α-phosphate group of α-^{32}P-labeled dATP or dGTP into PM2 apDNA (Figs. 2 and 3; Table 4) show that no ^{32}P is incorporated whereas full incorporation of ^{3}H or ^{14}C takes place. This clearly indicates that the nucleoside monophosphate moiety of the dNTP is not the inserted entity. In order to determine whether the nucleoside or the base moiety of the dNTP is incorporated into the apDNA, we compared the incorporation of [8-^{3}H]dATP (or dGTP) with that of [*U*-^{14}C]dATP (or dGTP) into PM2 apDNA under identical conditions (Table 4). The label of the tritiated compound is located at H-8 of the base moiety, while the radioactivity of [*U*-^{14}C]dNTPs is distributed between the base and the

Table 3. Incorporation of [^{3}H]dATP or [^{3}H]dGTP into PM2 apDNA in the presence of respective unlabeled base derivatives

Standard reaction mixture	Net incorporation, pmol (%) [^{3}H]dATP	[^{3}H]dGTP
No additives	0.53 (100)	0.65 (100)
+ Ade	0.48 (91)	—
+ dAdo	0.35 (66)	—
+ dAMP	0.43 (81)	—
+ dADP	0.05 (10)	—
+ dATP	0 (0)	—
+ Gua	—	0.52 (80)
+ dGuo	—	0.44 (68)
+ dGMP	—	0.31 (48)
+ dGDP	—	0.05 (8)
+ dGTP	—	0 (0)

Samples were incubated under standard conditions in the presence of 6 μg of protein per sample and with 0.4 mM of the indicated additives and assayed by the DE-81 procedure. Specific activities were 5.3 × 10^3 cpm per pmol of dATP and 2.4 × 10^3 cpm per pmol of dGTP.

Table 4. Incorporation of labeled dNTPs into PM2 apDNA

Label	dATP, pmol DE-81 assay	dATP, pmol Cl_3CCOOH assay	dGTP, pmol DE-81 assay	dGTP, pmol Cl_3CCOOH assay
8-^{3}H	0.53	0.50	0.65	0.59
α-^{32}P	0.02	0.02	0.02	0.03
U-^{14}C	0.50*	0.55*	0.60*	0.63*

Samples were incubated under standard conditions with 6 μg of protein per sample and with 4 μM of the indicated labeled nucleotide. Specific activities were: DE-81 assay: [^{3}H]dATP, 5.3 × 10^3 cpm/pmol; [^{3}H]dGTP, 2.4 × 10^3 cpm/pmol; [^{14}C]dATP and [^{14}C]dGTP, 1.2 × 10^3 cpm/pmol; [α-^{32}P]dATP and [α-^{32}P]dGTP, 5 × 10^3 cpm/pmol. Trichloroacetic acid assay: [^{3}H]dATP, 5.8 × 10^4 cpm/pmol; [^{3}H]-dGTP, 2.6 × 10^4 cpm/pmol; [^{14}C]dATP and [^{14}C]dGTP, 1.5 × 10^3 cpm/pmol; [α-^{32}P]dATP and [α-^{32}P]dGTP, 5 × 10^3 cpm/pmol.

* Calculated on the basis of the *partial* specific activity corresponding to the base moiety of the [*U*-^{14}C]dNTPs (30% of the total specific activity). When calculated on the basis of the total specific activity of the dNTPs, the apparent incorporation is 0.17 and 0.20 pmol for dATP and dGTP, respectively. These values agree with the measured distribution of ^{14}C in the base and sugar moieties of the [*U*-^{14}C]dNTPs, which are 30% and 70%, respectively.

sugar moieties. Therefore, if only the base moiety is inserted, an apparent reduction in incorporation should be observed when [*U*-^{14}C]dNTPs are used. We determined the distribution of ^{14}C in [*U*-^{14}C]dATP and [*U*-^{14}C]dGTP and found that 30% of the label is located in the base moiety and 70% is located in the sugar moiety of both dATP and dGTP. Our results indicate that only the base moiety is inserted. It can be seen (Table 4) that incorporation (pmol) from both [*U*-^{14}C]dATP and [*U*-^{14}C]dGTP is equal to the incorporation of the tritiated compounds only if we assume insertion of the base moiety alone.

Total depurination and chromatographic analysis of the products of isolated PM2 apDNA from the standard incubation mixture were performed in order to demonstrate that adenine and guanine had been incorporated into the DNA (Fig. 4A). The amount of adenine and guanine recovered from the radioactive bands was 84 and 72% of the respective available

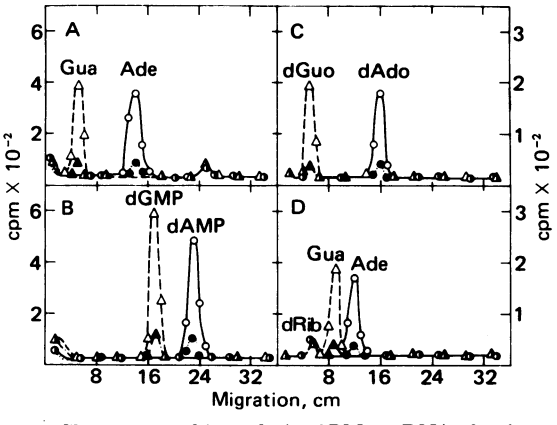

FIG. 4. Chromatographic analysis of PM2 apDNA after insertion incubation. Four standard reaction mixtures (*A–D*) were incubated with 4 μM [*U*-^{14}C]dATP or [*U*-^{14}C]dGTP and with 30 μg of protein per sample. After the incubation, the DNA was isolated and analyzed. (*A*) Acid hydrolysis of DNA; (*B*) total enzymatic digestion of DNA; (*C*) alkaline phosphatase treatment of the radioactive substance eluted from chromatogram in *B*; (*D*) alkaline phosphatase treatment followed by acid hydrolysis of the radioactive substance eluted from chromatogram in *B*. ○, PM2 apDNA incubated with [*U*-^{14}C]dATP; ●, intact PM2 DNA incubated with [*U*-^{14}C]dATP; △, PM2 apDNA incubated with [*U*-^{14}C]dGTP; ▲, intact PM2 DNA incubated with [*U*-^{14}C]dGTP.

Table 5. Incorporation of [^{3}H]dATP or [^{3}H]dGTP into PM2 apDNA in the presence of excess unlabeled dNTPs

Standard reaction mixture	Net incorporation, pmol (%)	
	[^{3}H]dATP	[^{3}H]dGTP
No additives	0.53 (100)	0.65 (100)
+ 0.4 mM dATP	0 (0)	0.68 (105)
+ 0.4 mM dGTP	0.56 (106)	0 (0)
+ 0.4 mM dCTP	0.55 (104)	0.33 (51)
+ 0.4 mM dTTP	0.56 (106)	0.25 (38)

Samples were incubated under standard reaction conditions with 8 μg of protein per sample and the indicated unlabeled nucleotides. Assayed by the DE-81 procedure; identical values were obtained by the trichloroacetic acid assay.

apurinic sites in the analyzed sample of PM2 apDNA. It can also be seen from Fig. 4*A* that a negligible amount of net radioactivity is found at the origin of the chromatogram, where the apDNA is located. Since [*U*-^{14}C]dNTPs were used in the experiment, this result means that the sugar moiety was not incorporated into the apDNA. Incorporation of both sugar and base should have resulted in about 2000 cpm at the origin, whereas we found only 150 cpm. This result provides further evidence for our conclusion that the base moiety is transferred from the dNTP into apurinic sites on the DNA.

In order to demonstrate that the inserted base binds to a sugar moiety in the apDNA and that the bond thus formed is the regular glycosylic bond, we isolated the apDNA after incubation and performed total enzymatic digestion. Fig. 4*B* shows the appearance of a single product, which comigrates with a marker of dAMP (dGMP). This product was extracted from the paper, treated with alkaline phosphatase, and rechromatographed. Again (Fig. 4*C*), a single product appeared, which comigrated with a marker of deoxyadenosine (deoxyguanosine). These results clearly indicate that the inserted purine base binds to a sugar moiety in the depurinated DNA with the formation of the regular N9—C1′ glycosylic bond, thus supporting our suggestion of insertion of purines into apurinic sites. Finally, we performed an acid hydrolysis of the sample after the phosphatase treatment and found (Fig. 4*D*) that only one major product appeared, which comigrated with a marker of adenine (guanine), while only a minor amount of labeled deoxyribose appeared. Because the deoxyribose moiety constitutes 70% of the label of [*U*-^{14}C]dNTP donors, the negligible amount found in the degradation products of the DNA further supports our suggestion that only the base moiety is inserted into the apDNA.

Incorporation Is Specific. Measurements in the presence of competing dNTPs were performed in order to find out whether the entering dATP or dGTP can be replaced by other dNTPs. Experiments were performed at saturation levels of protein in the presence of a 100-fold excess concentration of unlabeled dNTPs. It was found (Table 5) that the saturation level of incorporation of [^{3}H]dATP into PM2 apDNA remained unaltered in the presence of a large excess of each of the other dNTPs, indicating the specific insertion of adenine into adenine apurinic sites. For [^{3}H]dGTP, the level of incorporation was not changed by a 100-fold concentration of dATP, but it was reduced by the same excess of dCTP or dTTP. However, dCTP could not be incorporated at all into apDNA (Table 2), and the low level of incorporation of dTTP (Table 2) could be reduced completely in the presence of dATP.

The specificity of insertion and the fact that double-stranded DNA is required for this activity suggest that the insertion proceeds through base pairing with pyrimidines that are located on the intact strand opposite the apurinic sites.

In conclusion, we have demonstrated the enzymatic insertion of adenine and guanine into double-stranded apDNA *in vitro*, by using soluble enzyme extracts of *E. coli*. The activity that was studied uses dATP and dGTP as donors and specifically inserts the base moiety from the donors into apDNA. The inserted purine base binds to a deoxyribose moiety in the apDNA, forming the regular N9—C1′ glycosylic bond. The activity described here may represent a step in a repair mechanism, "base-insertion repair," for the repair of apurinic sites in DNA. These apurinic sites, which may occur in double-stranded DNA either due to removal of damaged purines with specific glycosylases or by spontaneous depurination, may be repaired by direct insertion of the correct missing purine into the apurinic site.

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1. Lindahl, T. & Nyberg, B. (1972) *Biochemistry* **11**, 3610–3618.
2. Lawley, P. D. & Brookes, P. (1963) *Biochem. J.* **89**, 127–138.
3. Strauss, B. & Hill, T. (1970) *Biochim. Biophys. Acta* **213**, 14–25.
4. Kirtikar, D. M. & Goldthwait, D. A. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 2022–2026.
5. Laval, J. (1977) *Nature (London)* **269**, 829–832.
6. Riazuddin, S. & Lindahl, T. (1978) *Biochemistry* **17**, 2110–2118.
7. Friedberg, E. C., Cook, K. H., Duncan, J. & Mortelmans, K. (1977) in *Photochemical and Photobiological Reviews*, ed. Smith, K. C. (Plenum, New York), Vol. 2, pp. 263–322.
8. Ljungquist, S. & Lidahl, T. (1974) *J. Biol. Chem.* **249**, 1530–1535.
9. Verly, W. G. & Rassart, E. (1975) *J. Biol. Chem.* **250**, 8214–8219.
10. Teebor, G. W. & Duker, N. J. (1975) *Nature (London)* **258**, 544–547.
11. Linsley, W. S., Penhoet, E. E. & Linn, S. (1977) *J. Biol. Chem.* **252**, 1235–1242.
12. Kuebler, J. P. & Goldthwait, D. A. (1977) *Biochemistry* **16**, 1370–1377.
13. Ljungquist, S. (1977) *J. Biol. Chem.* **252**, 2808–2814.
14. Verly, W. G., Gossard, F. & Crine, P. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 2273–2275.
15. Bose, K., Karran, P. & Strauss, B. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 794–798.
16. Weiner, J. H., Brutlag, D., Geider, K., Schekman, R., Wickner, W. & Kornberg, A. (1974) in *DNA Replication*, ed. Wickner, R. B. (Dekker, New York), pp. 187–194.
17. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
18. Hinnen, R., Schäfer, R. & Franklin, R. M. (1974) *Eur. J. Biochem.* **50**, 1–14.
19. Espejo, R. T., Canelo, E. S. & Sinsheimer, R. L. (1969) *Proc. Natl. Acad. Sci. USA* **63**, 1164–1168.
20. Razin, A., Sedat, J. W. & Sinsheimer, R. L. (1970) *J. Mol. Biol.* **53**, 251–259.
21. Brutlag, D. & Kornberg, A.(1972) *J. Biol. Chem.* **247**, 241–248.
22. Knippers, R., Razin, A., Davis, R. & Sinsheimer, R. L. (1969) *J. Mol. Biol.* **45**, 237–263.
23. Timson, J. (1977) *Mutat. Res.* **47**, 1–52.