Light-Induced Free-Radical Reactions of Purines and Pyrimidines in Deoxyribonucleic Acid. Effect of Structure and Base Sequence on Reactivity†

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ABSTRACT: The multiplicity of damages induced in cellular DNA by treatment with ultraviolet light or with some chemical agents interferes with the assignment of specific types of damages in the DNA to the various biological effects expressed in the treated cells. This problem was approached by developing a series of light-induced alkylation reactions with 2-propanol which led to a single-type chemically defined product of each of the bases in nucleic acids. In the work described here, these reactions were applied to synthetic deoxyribopolynucleotides and to the various forms of ϕX174 DNA in order to study the effect of sequence and secondary structure on the reactivity of the bases in these reactions. When a series of deoxyribodinucleoside monophosphates and ordered homo- and heterodeoxyribopolynucleotides was used, it was shown that purines having 5′-neighboring pyrimidines are more reactive than those having 5′-neighboring purines. It was thus concluded that the middle purines in the sequences Py-*Pu*-Py or Py-*Pu*-Pu are more reactive than those in the sequences Pu-*Pu*-Py or Pu-*Pu*-Pu. The effect of the secondary structure of DNA on the photoalkylation reaction has been studied by comparing the reactivities of the bases in the following ϕX174 DNA substrates: single-stranded DNA, whole phage particles, and double-stranded replicative forms (RFI and RFII). Generally, the bases in the single-stranded substrates have been found to react faster than those in the double-stranded forms. The presence of proteins has been found to suppress the rate of alkylation in the phage particles. The bases of the circular superhelical double-stranded form have been found to react faster than those in the relaxed form. The latter result is explained on the basis of the fact that closed circular superhelical DNA contains single-stranded regions which result from unwinding of the DNA duplex. The effect of sequence has been demonstrated by comparing the reactivities of guanines vs. adenines in the single-stranded and RF forms. Our results are compatible with a computer analysis of the frequency of occurrence, in the two forms of the DNA, of triplet sequences with a middle purine. This analysis shows that the abundance of guanine-containing reactive sequences Py-G-Py and Py-G-Pu in single-stranded DNA is higher than that of adenine-containing reactive sequences. In double-stranded DNA, the relative abundance of guanine-containing reactive sequences diminishes. This is consistent with our finding that the reactivity of guanine relative to that of adenine in double-stranded ϕX174 DNA is lower than that of the respective bases in the single-stranded form.

Exposure of living cells to radiation or some chemical agents has deleterious consequences which are caused mainly by damage induced in their DNA. This damage may cause local destruction or local alterations of the genetic information, which may then be expressed as mutations, initiation of carcinogenesis, or inactivation (Smith, 1976; Hanawalt et al., 1978). One of the main factors that determines these biological effects is the type of the induced damage and its location along the genome. However, attempts to elucidate the chemical basis of these biological effects are complicated by the multiplicity of products which are induced in the DNA subsequent to its exposure to radiation or chemical agents (Kittler & Löber, 1977; Singer, 1975). Under these circumstances, an assignment of a given chemical change to the consequent biological effect is made impossible.

We have approached this problem by utilizing selective photochemical and free radical reactions which cause a single type of damage to each of the base constituents of the nucleic acids. We have previously shown that purine and pyrimidine derivatives react with a variety of organic compounds, such as alcohols, amines, ethers and acetals, when irradiated with UV light of $\lambda > 260$ nm, with UV light of $\lambda > 290$ nm in the presence of a free radical photoinitiator, or with γ rays [for a review, see Elad (1976)]. The reactions with purine derivatives resulted in the substitution of the appropriate group for the H-8 atom of the purine system; with uracil and its derivatives, addition across the 5–6 double bond occurred, and with thymine and its derivatives, both the addition reaction and substitution of an appropriate group for a hydrogen at the C-5 methyl group occurred. The reactions of adenine and thymine with 2-propanol are typical examples and are presented in Figure 1.

Our previous studies of the photoalkylation of mono-, di-, and triribonucleotides enabled us to formulate some rules which govern the reactivity of the various bases. The reactivity of the purines was found to be dependent on their conformation at the glycosylic bond and on the presence or the absence of a 5′-phosphate group. The reactivity of uridine residues was strongly dependent on the nature of the nearest and the second neighbors and on the sequence of the oligonucleotide. This dependence was related to the involvement of the uridines in stacking interaction with purines—the stronger stacking interactions resulting in lower reactivity [Havron et al., 1976; Levneh (Noy) et al., 1978].

In the present paper we describe the reactivities of purines and pyrimidines in the various forms of ϕX174 DNA in the light-induced free-radical alkylation reactions with 2-propanol and evaluate the effect of the secondary and the tertiary structure of the DNA on the reactivity of the bases. We also present evidence for sequence selectivity of these reactions in deoxyribonucleoside monophosphates, ordered synthetic deoxyribopolynucleotides, and ϕX174 DNA.

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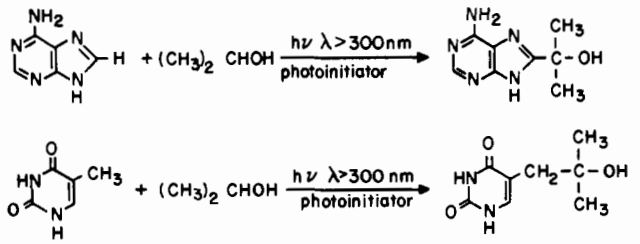

FIGURE 1: Photoalkylation of adenine and of thymine with 2-propanol.

Materials and Methods

[2-^{3}H]Adenosine (21 Ci/mmol; 1 Ci = 3.7 × 10^{10} Bq), [5′-^{3}H]guanosine (28 Ci/mmol), [*methyl*-^{3}H]thymidine (58 Ci/mmol), 5′-deoxy[U-^{14}C]adenosine 5′-monophosphate (574 mCi/mmol), deoxy[U-^{14}C]guanosine 5′-monophosphate (482 mCi/mmol), [2-^{14}C]thymidine (57.7 mCi/mmol), and deoxy[U-^{14}C]adenosine 5′-triphosphate (477 mCi/mmol) were purchased from The Radiochemical Centre, Amersham, England. Deoxy[8-^{14}C]guanosine 5′-triphosphate (42.4 mCi/mmol) was from New England Nuclear.

Other materials were as follows: nucleosides, nucleotides, and dinucleoside monophosphates (Sigma); deoxyribonucleoside triphosphates (Boehringer); 2-propanol (BDH); di-*tert*-butyl peroxide [(*t*-BuO)$_2$][1] (Merck-Schuchard, Munich); deoxyribonuclease I and snake venom phosphodiesterase (Worthington); alkaline phosphatase (Sigma); proteinase K (Merck); *M. luteus* DNA polymerase (P-L Biochemicals); poly(dA)·poly(dT), poly[d(A-T)]·poly[d(A-T)], poly(dG)·poly(dC), and poly[d(G-C)]·poly[d(G-C)] (Sigma); poly[d(T-G)]·poly[d(A-C)] (Boehringer).

Bacteriophage and Bacteria. In all preparations of DNA from phage φX174, the lysis-defective mutant φX174am3Cs70 was used. The hosts used were *E. coli* C-416 (pu$^-$, met$^-$, tryp$^-$, arg$^-$), *E. coli* H502 (thy$^-$, hcr$^-$, endo$^-$), *E. coli* C (BTCC 122), and *E. coli* C-4714 (amber suppressor). The latter was used to determine the titer of the bacteriophage.

DNA Preparations. Phage φX174 was prepared and purified, and its single-stranded DNA was extracted as described (Razin et al., 1970). Labeled φX174 DNAs containing [^{3}H]thymine [(1.5–2.0) × 10^5 cpm/μg], [^{3}H]adenine (5 × 10^5 cpm/μg), or deoxy[^{3}H]guanosine (1 × 10^5 cpm/μg) were prepared by the same procedure, using [*methyl*-^{3}H]thymidine, [2-^{3}H]adenosine, and [5′-^{3}H]guanosine, respectively. φX174 RFI DNA was prepared according to Eisenberg et al. (1975). Labeled φX174 RFI DNAs containing [^{3}H]thymine (7 × 10^4 cpm/μg), [^{3}H]adenine (1 × 10^5 cpm/μg), or deoxy[^{3}H]-guanosine (9 × 10^4 cpm/μg) were prepared by the same procedure. φX174 RFII DNA was prepared by limited DNase I digestion of φX174 DNA as described (Wang, 1974).

Labeled Poly(deoxyribonucleotide) Preparations. [^{14}C]-Purine-labeled poly[d(A-T)]·poly[d(A-T)] (40 mCi/mmol), poly(dA)·poly(dT) (28 mCi/mmol), poly[d(T-G)]·poly[d(A-C)] (18 mCi/mmol of adenine or guanine), and poly[d(G-C)]·poly[d(G-C)] (42 mCi/mmol of guanine) were prepared according to Harwood et al. (1970), and poly(dG)·poly(dC) (42 mCi/mmol) was prepared according to Litman (1971), using the appropriate templates, ^{14}C-labeled deoxyribonucleoside triphosphates, and *M. luteus* DNA polymerase. The reaction mixtures were extracted with phenol, and the polynucleotides were purified on a Sephadex G-50 column (16 × 0.7 cm) in either 0.1 M potassium phosphate, pH 7.4, or 10 mM Tris-HCl, pH 7.5/2 mM EDTA/40 mM NaCl.

Irradiation Procedure. The irradiation mixture (2 mL) contained φX174 DNA or synthetic polynucleotides (5 μg/mL, ≥5 × 10^4 cpm/μg) in 0.1 M KH_2PO_4 (pH 7.4)/1.3 M 2-propanol/0.1 M (*t*-BuO)$_2$. Irradiations were carried out with a Wild Universal Unit (Wild Heerbrug, Switzerland) equipped with an Osram 200-W high-pressure mercury lamp. Samples in 3-mL spectrophotometric quartz cells were flushed with oxygen-free nitrogen for 10 min and irradiated at room temperature, at a distance of 150 mm from the light source, by using a Schott WG 320 cutoff filter (λ >305 nm). The incident light intensity was 7.3 × 10^{-5} einstein cm^{-2} min^{-1} (1 einstein = 1 mol of photons), determined as described [Livneh (Noy) et al., 1978]. Infrared radiation from the light source was filtered out by using a 20-mm path-length quartz cell containing double-distilled water.

Enzymatic Digestion of DNA. After the irradiation, samples were dialyzed against 0.25 mM Tris-HCl (pH 7.4) and concentrated 40-fold by lyophilization. Digestion with DNase I, venom phosphodiesterase, and alkaline phosphatase was carried out according to Baird & Brooks (1973). Samples were boiled for 10 min, lyophilized and extracted with methanol (two portions of 400 μL). The methanol was evaporated under reduced pressure, and the residue was dissolved in water (20 μL). One microgram of each of the appropriate modified deoxyribonucleosides was added as markers, and the samples were analyzed by high-pressure liquid chromatography (HPLC) as described below.

Separation of Deoxyribonucleosides. Modified and nonmodified deoxyribonucleosides were separated on a reversed phase, Lichrosorb RP-18 (10 μm) column (0.3 m in length and 4.6 mm in diameter) operated at 1000 psi and eluted with mixtures of methanol and 50 mM sodium acetate (pH 4.5). 8-(2-Hydroxy-2-propyl)deoxyadenosine was separated from deoxyadenosine by using 25% methanol in the acetate buffer, 8-(2-hydroxy-2-propyl)deoxyguanosine was separated from deoxyguanosine by using 15% methanol in the acetate buffer, and 5-(2-hydroxy-2-methylpropyl)deoxyuridine, 6-(2-hydroxy-2-propyl)-5,6-dihydrodeoxythymidine, and thymidine were separated by using 10% methanol in acetate buffer.

Irradiation of Deoxyribonucleoside Monophosphates. Deoxyribodinucleoside monophosphates were irradiated and the extent of alkylation of the adenines was determined by enzymatic digestion with venom phophodiesterase, followed by paper chromatography as described [Livneh (Noy) et al., 1978] or by acid depurination followed by paper chromatography as described (Havron et al., 1976).

Irradiation of Phage Particles. Labeled phage φX174 was prepared and purified as described (Razin et al., 1970). Phage particles (20 μg/mL, ≥1.25 × 10^4 cpm/μg) were irradiated under the same conditions used for the DNA and then dialyzed against a buffer containing 50 mM Tris-HCl, pH 8.1/0.1 mM EDTA. The coat proteins were digested with proteinase K (250 μg/mL) in the presence of 0.5% sodium dodecyl sulfate, for 1 h at 37 °C. The DNA was extracted twice with phenol and precipitated with ethanol. Analyses of the products were performed as described above.

Results

Modification of φX174. Various forms of adenine-, guanine-, or thymine-labeled φX174 were irradiated with UV light

[1] Abbreviations: (*t*-BuO)$_2$, di-*tert*-butyl peroxide; HPLC, high-pressure liquid chromatography; ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; 8-hpAde, 8-(2-hydroxy-2-propyl)adenine; 8-hpGua, 8-(2-hydroxy-2-propyl)guanine; hp(m)Thy, 5-(2-hydroxy-2-methylpropyl)uracil; 8-hpdAdo(or Guo), 8-(2-hydroxy-2-propyl)deoxyadenosine (or deoxyguanosine); hp(m)dThd, 5-(2-hydroxy-2-methylpropyl)deoxyuridine; 6-hpdThd, 5-(2-hydroxy-2-methylpropyl)deoxyuridine; 6-hpdThd, 6-(2-hydroxy-2-propyl)-5,6-dihydrodeoxythymidine; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

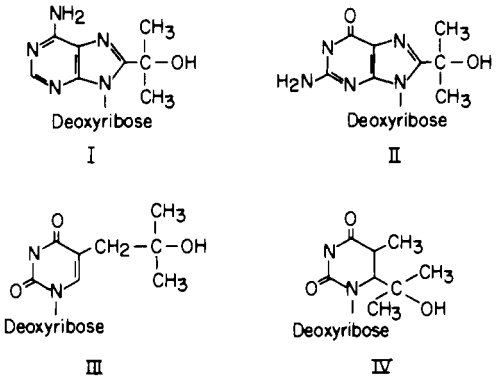

FIGURE 2: Products of the photoalkylation of deoxynucleosides with 2-propanol. I, 8-(2-hydroxy-2-propyl)deoxyadenosine (8-hpdAdo); II, 8-(2-hydroxy-2-propyl)deoxyguanosine (8-hpdGuo); III, 5-(2-hydroxy-2-methylpropyl)deoxyuridine [hp(m)dThd]; IV, 6-(2-hydroxy-2-propyl)-5,6-dihydrodeoxythymidine (6-hpdThd).

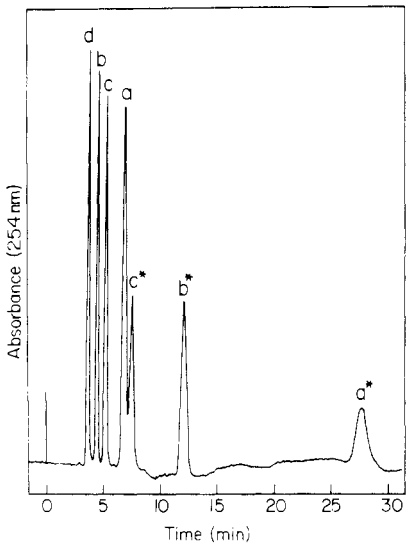

FIGURE 3: High-pressure liquid chromatography separation of native and modified nucleosides. Samples, prepared as described under Materials and Methods, were eluted with 20% methanol in 50 mM sodium acetate, pH 4.5; a, dAdo; b, dGuo; c, dThd; d, dCyd; a*, 8-hpdAdo; b*, 8-hpdGuo; c*, hp(m)dThd.

of $\lambda > 305$ nm in the presence of 2-propanol and $(t\text{-BuO})_2$ which served as a photoinitiator. Following irradiation, the DNA was digested with DNase I, venom phosphodiesterase, and alkaline phosphatase to yield a mixture of native and modified nucleosides. The modified nucleosides were chromatographically identical with those obtained by the irradiation of the free nucleosides, namely, 8-(2-hydroxy-2-propyl)deoxyadenosine (Figure 2, I), 8-(2-hydroxy-2-propyl)deoxyguanosine (Figure 2, II), and 5-(2-hydroxy-2-methylpropyl)deoxyuridine (Figure 2, III). [The thymine addition product 6-(2-hydroxy-2-propyl)-5,6-dihydrodeoxythymidine (Figure 2, IV), which appeared upon irradiation of the free nucleoside, did not appear in irradiated DNA.] The native and the modified nucleosides were separated by HPLC (Figure 3) and quantified by radioactivity counting. Each irradiated DNA sample contained 5×10^5–7.5×10^5 cpm in a single type of radioactive base; thus, the lowest detection limit was 0.01% of photoproducts per base. The ability to detect such small yields of products in the DNA enabled us to determine the initial rates of alkylation.

The order of reactivity of the various bases in the deoxyribonucleoside form has been found to be adenine > guanine > thymine (Figures 4–6). It can be seen from Figures 4 and 5 that the reactivity of the purines in ssDNA is decreased by

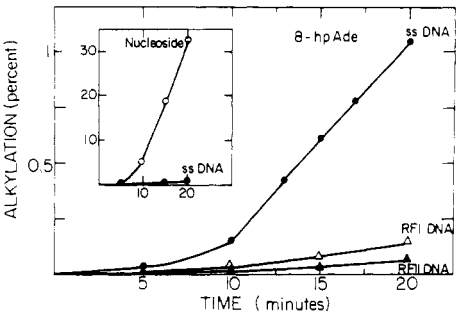

FIGURE 4: Time course of the photoalkylation of adenine with 2-propanol in various forms of DNA. (○) Deoxynucleoside; (●) ssDNA; (△) RFI DNA; (▲) RFII DNA.

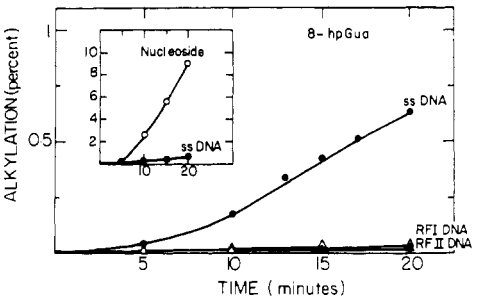

FIGURE 5: Time course of the photoalkylation of guanine with 2-propanol in various forms of DNA. (○) Deoxynucleoside; (●) ssDNA; (△) RFI DNA; (▲) RFII DNA.

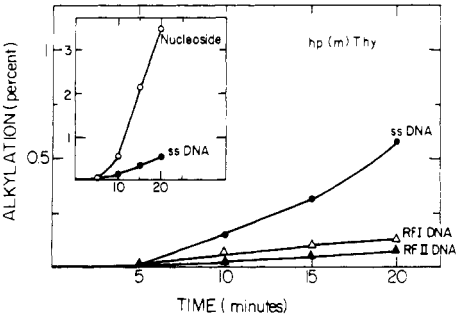

FIGURE 6: Time course of the photoalkylation of thymine with 2-propanol in various forms of DNA. (○) Deoxynucleoside; (●) ssDNA; (△) RFI DNA; (▲) RFII DNA.

Table I: Relative Reactivities[a] of Bases in the Various Forms of DNA

	Ade	Gua	Thy[b]
R_{ssDNA}/R_{RFII}	13.0	34.0	7.9
R_{RFI}/R_{RFII}	1.7	1.3	1.9
R_{ssDNA}/R_{RFI}	7.7	26.2	4.2
R_{ssDNA}/R_{phage}	3.5	4.0	1.9
R_{phage}/R_{RFI}	2.2	6.6	2.2

[a] Relative reactivity is defined as the ratio of the slopes of the linear parts of the time course curves (Figures 4–6) for the formation of a product from the respective substrates. Data for time points shorter than 5 min are not used because the onset of the alkylation reaction is delayed. This delay can be atributed, among other factors, to the time it takes to build up a sufficiently high concentration of free radicals in the reaction mixture (see Discussion). [b] Reactivity ratios correspond to the substitution at the C-5 methyl group.

an order of magnitude as compared to their reactivity in the deoxyribonucleosides. An additional decrease of about an order of magnitude is observed for these bases in the double-stranded RFI and RFII ϕX174 DNAs as compared to the ssDNA. Thus, the reactivity of adenines and guanines in RFII DNA is 13- and 34-fold lower, respectively, than that of the

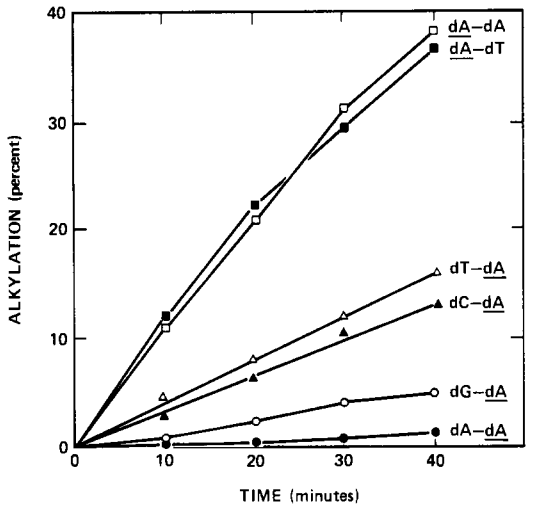

FIGURE 7: Time course of the photoalkylation of adenine in deoxyribodinucleoside monophosphates. (●) dA-d*A*; (○) dG-d*A*; (▲) dC-d*A*; (△) dT-d*A*; (■) *dA*-dT; (□) *dA*-dA.

corresponding bases in ssDNA (Figures 4 and 5, Table I). Considering the dsDNA, it should be noted that the reactivity of the purines in the RFI form was higher than that in the RFII form (Table I). As mentioned above, irradiation of thymidine yields both photoproduct III (Figure 2), which results from substitution of the methyl group, and photoproduct IV (Figure 2), which results from addition of 2-propanol across the 5–6 double bond. In irradiated ssDNA, only traces of photoproduct IV appeared (the limit of detection being >0.01%), while in dsDNA, such photoproduct could not be detected at all. The reactivity of thymines in the substitution reaction was 8-fold higher in ssDNA, as compared to RFII DNA, and 2-fold higher in RFI DNA, as compared to RFII DNA (Table I).

The reactivities of the various bases were examined also in intact phage particles. Generally, they were lower than those in ssDNA, but higher than those in RFI DNA (Table I). Thus, adenines and guanines in the whole phage were found to be 3.5- and 4-fold less reactive than those in ssDNA, respectively, but 2.2- and 6.6-fold more reactive than the respective bases in RFI DNA. The phage thymines were 1.9-fold less reactive than those in the naked ssDNA and 2.2-fold more reactive than those in RFI DNA. It should be noted that dark incubation in the reaction mixture was found to have no influence on the infectivity of the phage, indicating that the phage remained intact under these conditions.

Modification of Purines in Deoxyribodinucleoside Monophosphates and Ordered Deoxyribopolynucleotides. The rates of photoalkylation with 2-propanol of adenine residues in deoxyribonucleoside monophosphates are shown in Figure 7. Generally, 5′-terminal adenines were more reactive than 3′-terminal adenines, and their rates of alkylation were independent of their 3′ neighbors. This behavior was similar to that of the analogous adenines in ribodinucleoside monophosphates and ribotrinucleoside diphosphates [Havron et al., 1976; Livneh (Noy) et al., 1978]. However, unlike the case of the ribo series, the reactivities of 3′-terminal adenines in the deoxy series depended on their 5′ neighbors. Thus, the adenines in dT-dA and dC-dA were 18- and 15-fold, respectively, more reactive than the 3′-terminal adenine of dA-dA, whereas the adenine of dG-dA was only 5-fold more reactive than the 3′-terminal adenine of dA-dA.

These results raised the possibility that a sequence dependence of the reactivity of purines exists in DNA. To test this possibility, we determined the reactivities of purines in ordered deoxyribopolynucleotides. The results are summarized in Table III. It can be seen that when adenines are flanked by pyrimidines to form the sequence Py-A-Py [the sequences T-A-T in poly[d(A-T)]·poly[d(A-T)] and the sequence C-A-C in poly[d(T-G)]·poly[d(A-C)]], the yields of 8-hpAde were 3- and 2-fold higher, respectively, than those of the adenines in poly(dA)·poly(dT), in which each adenine is flanked by purines. This effect is even more profound in guanine-containing polynucleotides in which the reactivities of guanines in the sequences Py-G-Py were 13- and 40-fold higher than those in the sequences Pu-G-Pu.

Table II: Relative Reactivities[a] of Adenine and Guanine in the Various Forms of DNA

	R_{Gua}/R_{Ade}
nucleoside	0.21
ssDNA	0.52
RFII	0.19
RFI	0.15
phage	0.62

[a] See footnote *a* to Table I.

Discussion

Reaction Mechanism. The reactions described involve free-radical intermediates and proceed through photolysis of the peroxide to yield oxy radicals which abstract the hydrogen atom from the C-2 of 2-propanol to give ketyl radicals Ċ-$(CH_3)_2OH$. These are subsequently scavenged by the purine or the pyrimidine bases to give adducts I–III (Figure 2). The reactions with the purines proceed through an initial attack of the ketyl radical at C-8, and with thymine the initial attack occurs at the C-5 methyl (Frimer et al., 1976). It is noteworthy that in dsDNA all the targets for the ketyl radicals point toward the major groove of the duplex DNA and are not involved directly in base-pairing interactions.

Reactivity of Purines. In the polymeric substrates, the reactions of both adenine and guanine in ssDNA were found to be the fastest (Table I). In dsDNAs, relaxation of superhelical turns further decreased the reactivities of both adenine and guanine as can be seen from the comparison of the photoalkylations of the relaxed circular DNA (RFII) and the covalently closed supertwisted DNA (RFI). These differences can be interpreted in terms of the secondary and tertiary structure of DNA. The access of the ketyl free radical to the 8 position of the purine is hindered by the phosphate sugar backbone of the DNA. This hindrance in ssDNA is expected to be lower than in dsDNA because of the less rigid conformation of the ssDNA backbone. In addition, electronic factors may influence the reactivities of the purines since base–base interactions in ssDNA are not identical with those in dsDNA. These differences may alter the properties of the purines as free radical scavengers, thus contributing to the difference in their reactivities. The influence of hydrogen bonding on the reactivity of purines is not likely to be direct, since the atoms involved in base pairing are not directly involved in the alkylation. However, base pairing probably exerts its influence indirectly through contribution to base-stacking interactions (Orenstein & Rein, 1979).

Comparison of the relative reactivities of guanine and adenine reveals variations in the values of R_{Gua}/R_{Ade} of the various forms of DNA (Table II). Although the reactivities of both adenine and guanine are suppressed in the dsDNA as compared to the ssDNA, the suppression is stronger for guanine (Tables I and II). The relatively higher reactivity of adenine, as compared to guanine, in dsDNA can be anticipated

Table III: Relative Rates of Photoalkylation of Purines in Deoxyribopolynucleotides

[^{14}C]A polynucleotide	conversion to 8-hpAde	[^{14}C]G polynucleotide	conversion to 8-hpGua
poly[d(A-T)]·poly[d(A-T)]	0.15	poly[d(G-C)]·poly[d(G-C)]	0.40
poly(dA)·poly(T)	0.05	poly(dG)·poly(dC)	<0.01
poly[d(T-G)]·poly[d(A-C)]	0.08	poly[d(T-G)]·poly[d(A-C)]	0.13

since AT-rich regions in dsDNA (RFII) are temporarily unwound due to the cooperative breathing of the DNA (von Hippel & Wong, 1971). A higher degree of unwinding occurs in RFI DNA, in which the superhelical turns create partial unwinding of AT-rich regions which in extreme cases may appear as stretches of single-stranded DNA (Palecek, 1976), thus exposing more adenines than guanines to the attack of ketyl free radicals.

An additional effect which may contribute to the variations observed in the relative reactivities of adenines and guanines in the various forms of ϕX174 DNA may reside in the sequence dependence of the reactions. If this is the case, then the occurrence in a particular DNA molecule of a larger number of more reactive sequences incorporating a certain purine may explain its higher reactivity relative to the other purine. Examining a series of ribotrinucleoside diphosphates, we have already shown that the reactivity of pyrimidines depends on the type of their flanking bases [Livneh (Noy) et al., 1978]. Here, we demonstrate that the reactivity of purines in deoxyribonucleotides is also sequence dependent. As can be seen from Figure 7, the reactivity of the 3′-terminal adenines in deoxyribodinucleoside monophosphates depends on their 5′ neighbors whereas the reactivity of 5′-terminal adenines does not depend on their 3′ neighbors. Thus, the sequences Py-A are more reactive than the sequences Pu-A. This observation has been substantiated by using synthetic double-stranded deoxyribopolynucleotides (Table III). It can be seen that purines flanked by pyrimidines, such as those in the alternating self-complementary deoxyribopolynucleotides and in poly[d(T-G)]·poly[d(A-C)], are more reactive than purines flanked by purines, such as those in the homopolymers poly(dA)·poly(dT) and poly(dG)·poly(dC). It can, therefore, be concluded that the middle purines in the sequences Py-*Pu*-Py or Py-*Pu*-Pu are more reactive than the respective purines in the sequences Pu-*Pu*-Py or Pu-*Pu*-Pu.

We, thus, compared the frequency of occurrence of all possible trinucleotide sequences in ϕX174 DNAs in which adenine or guanine occupy the middle position. Figure 8 shows the ratios of the number of guanine-containing triplets to the number of the respective adenine-containing triplets in the single-stranded and in the double-stranded forms of ϕX174 DNA, as computed from their known sequences (Sanger et al., 1978). Concerning the reactive sequences, it can be seen that the sequences Py-G-Py and Py-G-Pu are more abundant in the ssDNA than the sequences Py-A-Py and Py-A-Pu. However, in the dsDNA, the relative abundance of guanine-containing reactive sequences diminishes. This is consistent with our results (Table II) which indicate that the reactivity of guanine relative to that of adenine in double-stranded forms (RFI and RFII) of ϕX174 DNA is lower than that of the respective bases in the single-stranded form. We can thus conclude that both the preferential unwinding of AT-rich regions in superhelical DNA and the sequence dependence of the photoalkylation reactions contribute to the variations found in the reactivities of the purines in the various forms of ϕX174 DNA.

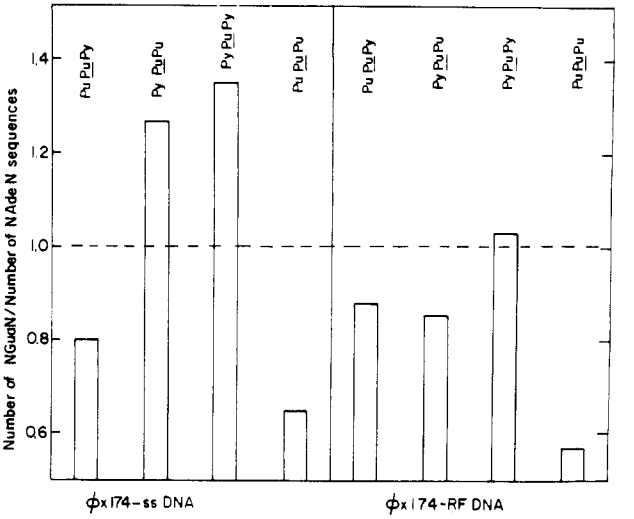

FIGURE 8: Relative frequency of appearance of adenine- and guanine-containing trinucleotide sequences in DNA from phage ϕX174.

The sequence dependence of the reactivity of the purines can be interestingly correlated to their involvement in base-stacking interactions with neighboring bases. The more reactive sequences, Py-Pu, have been calculated to have the weakest stacking interactions of all possible dinucleotide sequences, while the sequences which we have shown to be less reactive, namely, Pu-*Pu*-Pu and Pu-*Pu*-Py, contain the dinucleotides Pu-Pu and Pu-Py which are strongly stacked (Orenstein & Rein, 1979). A similar sequence dependence has been demonstrated for the binding of intercalating agents to oligonucleotides. Binding to Py-Pu sequences was stronger than to Pu-Py sequences (Krugh et al., 1975; Patel & Canuel, 1975). The effect of sequence on the reactivity can be attributed to both electronic and conformational factors. The electronic interactions of the reacting base with different neighboring bases may cause alterations in its properties as a free-radical scavenger and thus lead to variations in its reactivity. When conformation is considered, both local and overall factors should be taken into account. Thus, certain dinucleotides (e.g., those with poor base–base interactions) may allow greater dynamic variations in the relative orientation of the bases, which may eventually lead to a higher degree of exposure of the 8 position of the reacting purine. Overall conformational changes may play an important role, too, since certain sequences may dictate conformations other than the classic B form of DNA, such as Z-DNA or A-DNA (Wells et al., 1980; Selsing et al., 1979; Wang et al., 1979; Arnott et al., 1980), in which the purines may be more or less reactive than in the B-DNA. For example, purines in Z-DNA are in the syn conformation, and their 8 position is highly exposed to the attack by external agents (Wang et al., 1979).

Reactivity of Thymines. The substitution at the methyl group of thymine is selective for ssDNA (Figure 6). As can be seen from Table I, thymine shows the smallest degree of decrease in reactivity upon passage from ssDNA to dsDNA. This behavior could have been expected since examination of a model of B-form DNA reveals that the access of extraneous agents to the methyl group of the thymine is less hindered than the access to the 8 position of purines. The reactivity of thymine in RFI DNA is 1.9 times higher than that in RFII DNA—a value similar to that observed for adenine (Table I). This may be explained, as for the case of adenine, by the existence of unwound regions in AT-rich sequences in the DNA.

In addition to the substitution reaction at the methyl group of thymine, 2-propanol adds across the 5–6 double bond of thymine forming 6-(2-hydroxy-2-propyl)-5,6-dihydrothymine (Figure 2, IV; Frimer et al., 1976). This addition product appeared in trace amounts in irradiated ssDNA and could not be found at all in dsDNA (RFI and RFII). It is likely that this behavior is due to the involvement of the pyrimidines in stacking interactions which hinder the approach of ketyl free radicals to its 5–6 double bond [Livneh (Noy) et al., 1978].

Reactivity of the Bases in the Intact Phage. Since nucleic acids in nature appear in association with proteins, it was of interest to examine the reactivity of the bases in a native nucleoprotein complex, where both the folding of the DNA and its interaction with proteins may alter the reaction efficiency. The reactivities of the bases in the intact phage were found to be lower than in ssDNA, but still higher than in RF DNA (Table I). This means that the conversion of ssDNA into the double-helical structure caused a higher suppression of the reactivity of the bases than the packaging of the ssDNA into the phage particle. The diminished reactivities of the bases in ssDNA, as compared to the intact phage, can be attributed to partial protection by the protein shell.

Biological Implications of Photoalkylation Reactions. The biological damaging effects of the studied photoproducts have so far been demonstrated at the level of translation. Poly(A) incorporating 8-(2-hydroxy-2-propyl)adenine and poly(U) incorporating 6-(2-hydroxy-2-propyl)-5,6-dihydrouracil have been found to have reduced functional activity as translational templates (Livneh et al., 1980). Of more biological relevance is our finding that 8-(2-hydroxy-2-propyl)purines in dsDNA are specifically recognized by an endonucleolytic activity present in extracts of *M. luteus*, and may be repaired in vitro by nucleotide excision repair by using the *M. luteus* repair system (Livneh et al., 1979). The studied photoalkylation reactions belong to a broad spectrum of reactions which cause damage to DNA via free-radial mechanisms. Some of these reactions produce in the DNA types of damages similar to those obtained in our study. For example, ionizing radiation leads, among other products, to the substitution of purines at the 8 position, to substitution of thymine at the C-5 methyl group, and to addition across the 5–6 double bond of pyrimidines (Scholes, 1976; Cerutti, 1976). Another feature, possibly common to reagents causing substitution at the C-8 position of purines, is the tendency of 8-substituted purines to assume the nonregular syn conformation. Indeed, 8-(2-hydroxy-2-propyl)adenosine has been shown by X-ray analysis to be in the syn conformation (Birnbaum & Shugar, 1978). A similar conformational change is assumed to occur in guanines substituted at their 8 position with *N*-acetoxy-2-(acetylamino)fluorene (Nelson et al., 1971; Lefèvre et al., 1978). Whether or not 8 substitution of purines in DNA causes an anti to syn conformational change is, as yet, unknown. Nonetheless, if this were the case, then local distortions induced in the DNA by such substitutions may conform a common damaging effect of this class of chemical changes.

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