Inhibition of Mammalian DNA Polymerases by Hematoporphyrin Derivative and Photoradiation

James J. Crute, Alan F. Wahl, Robert A. Bambara, Richard S. Murant, Scott L. Gibson, and Russell Hilf

Department of Biochemistry [J. J. C., A. F. W., R. A. B., R. S. M., S. L. G., R. H.] and The University of Rochester Cancer Center [R. A. B., S. L. G., R. H.], The University of Rochester, School of Medicine and Dentistry, Rochester, New York 14642

ABSTRACT

Hematoporphyrin derivative (HPD) plus photoradiation caused the inactivation of DNA polymerases from calf thymus and R3230AC rat mammary tumor. Photosensitization of purified DNA polymerase-α as well as two forms of DNA polymerase-δ (I and II) from calf thymus were evaluated. Although all polymerase enzyme forms were inactivated at 70 µg HPD/ml, DNA polymerase-δ II was the most sensitive, displaying a 90% inactivation under conditions that did not cause significant inactivation of the other polymerase forms. Unlike DNA polymerase-α, the δ-forms have an associated 3′- to 5′-exonuclease activity. The exonuclease associated with DNA polymerase-δ II was uniquely sensitive to a low level of HPD and light exposure. DNA polymerase-δ II can be distinguished from other polymerase forms in cell extracts by its relative insensitivity to the polymerase inhibitor N2-(p-n-butylphenyl)deoxyadenosine 5'-triphosphate. In cytosols prepared from calf thymus and R3230AC rat mammary tumors, DNA polymerase-δ II was preferentially inhibited by HPD plus light. Furthermore, in experiments in which tumor-bearing rats were administered HPD prior to preparation of tumor cytosols, DNA polymerase-δ II was specifically inactivated by exposure to light. These results are discussed in view of their possible role in cancer therapy, and the potential use of HPD as a specific inhibitory agent of DNA polymerase-δ II is suggested.

INTRODUCTION

Investigation of the efficacy of photodynamic therapy of cancer, involving the administration of hematoporphyrin derivative followed by photoradiation of malignant lesions, has been underway for approximately 10 years. Recent clinical reports utilizing this treatment modality have been encouraging (1–5). Tumor regression induced by HPD4 photosensitization is generally believed to be mediated by the singlet oxygen radical produced by the exposure of HPD to visible light (6–10). The intracellular site or sites that are affected by HPD photosensitization which may ultimately lead to cell cytotoxicity have yet to be determined. Reports in the literature indicate that damage to the plasma membrane (11, 12), mitochondria (15–18), and DNA in the nucleus (19–22) may each be involved in the process of cytotoxicity. Damage to purified DNA has been reported to involve photodegradation of the guanine residue exclusively (21, 23).

Intracellular uptake of porphyrins in vitro has been investigated. Moan et al. (19) reported that the uptake of hematoporphyrin into the nuclei of NIH3T3 cells displayed the same kinetics as was observed for total intracellular uptake. However, Cozzani et al. (24) found increasingly higher concentrations of hematoporphyrin in the nuclei of ascites cells for up to 6 h after injection of the dye into the host. These observations, along with studies in vitro showing porphyrin-induced damage to DNA in the form of single strand breaks and sister chromatid exchanges (19, 20, 25), indicate that perturbations of the nucleus may ultimately produce tumor cell cytotoxicity. On the other hand, it was reported that repair of photosensitization-induced damage to DNA occurred rapidly, within 15 to 30 min for completion (19, 20, 26, 27). Gomer (20) reported that DNA damage remained unrepaired after exposure of Chinese hamster ovary cells in culture to HPD (50 µg/ml) and 1.2 J/cm2 of red light; however, with lower total energy densities, the cells were able to repair the DNA damage. It was concluded that the repair process was compromised, i.e., polymerases or other repair enzymes had sustained functional damage, under certain conditions of photosensitization. Such observations are in accord with those of others, who studied the effects of porphyrin photosensitization on virus associated DNA and RNA polymerases (28–30) and found that these polymerases were photoinactivated when exposed to hematoporphyrin and light. The purpose of our current work is to establish whether the major cellular DNA polymerases are photoinactivated by exposure to HPD and light. In this report, we examined three types of polymerases: DNA polymerase-α, which is recognized as an enzyme primarily responsible for chromosomal replication but is perhaps also involved in DNA repair (31, 32); and two additional α-like polymerases, DNA polymerase-δ I and DNA polymerase-δ II (33, 36). The sensitivity of these polymerases to aphidicolin, an inhibitor of eukaryotic chromosomal replication, and their presence in rapidly growing tissue suggests that these enzymes play a role in DNA replication (37). Additional similarities among these three enzymes are their molecular weights (greater than 200,000) and their sensitivity to the α-polymerase specific inhibitor, aphidicolin. These features distinguish DNA polymerases-α and -δ from DNA polymerases-β and -γ, also found in mammalian tissue (34–37). The unique feature of the δ-polymerases is their association 3′- to 5′-exonuclease activity, the presence of which suggests that the δ-polymerase may participate in proofreading repair during replication (37).

We measured HPD-induced photosensitization on these polymerases, using highly purified polymerase forms prepared from calf thymus, cytosols prepared from calf thymus and R3230AC mammary adenocarcinomas, and cytosols prepared from tumors...
harvested from rats that had been given injections of HPD at various times prior to sacrifice (15). The data indicate that DNA polymerase-δ II was preferentially inhibited by HPD and light exposure.

MATERIALS AND METHODS

Materials. All reagents used were obtained from Sigma Chemical Company (St. Louis, MO) unless otherwise noted. poly(dA)2000-5000, oligo(dT)16, polydeoxynucleotide and nucleotides with an average of 400 nucleotides, and deoxynucleoside triphosphate were purchased from the Midland Reagent Company (Midland, TX). [3H]dTTP (specific activity, 80 Ci/mmol) and Omnifluor were obtained from the New England Nuclear Corp. (Boston, MA). Unlabeled 3'-deoxythymidine 5'-triphosphate was obtained from ICN Pharmaceuticals (Plainview, NY). ddTTP and ddGTP were obtained from Pharmacia-PL Biochemicals (Milwaukee, WI). BuAdATP was a generous gift from Dr. George Wright of the Department of Pharmacology, The University of Massachusetts Medical School (Worcester, MA).

Preparation of HPD. HPD was prepared according to the method of Lipson et al. (38) from hematoporphyrin dihydrochloride. Seven mg of this acetylated porphyrin mixture were dissolved in 1 ml 0.1 N NaOH, stirred in the dark 1 h at room temperature, and then brought to pH 7.0 by a dropwise addition of 0.1 N HCl. This mixture represents the alkalized and subsequently neutralized solution of porphyrins. This solution was then brought to a final volume of 10 ml with 0.9% NaCl solution (700 µg/ml HPD). All experiments were performed using dilutions of this stock solution in 0.9% NaCl solution.

Purification of DNA Polymerases. DNA polymerase-α was purified from calf thymus by the immunoadfinity method of Wahl and coworkers (39) through step 2. DNA polymerases-δ I and δ II were purified from calf thymus according to the method of Crute et al. (fraction III) (33). The specific activity of these enzyme preparations ranged from 200-800 units/mg protein and, on this basis, represented 2–8% purity with no evidence of the presence of other contaminating polymerases. One unit of polymerase activity is defined as the amount that catalyzes the incorporation of 1 mmol of nucleotide in the DNA substrate.

Preparation of Cytosols. Cytosols were prepared from calf thymus and R3230AC tumors according to the method of Wahl et al. (39). Several calf thymus glands as well as tumors from several different rats were used in each preparation. Each sample was then dialyzed against a solution containing 20 mM Tris-HCl (pH 7.5), 20% glycerol (v/v), 20 mM KCl, 5 mM dithiothreitol, 4 mM ATP, 4.0 mM MgCl2, 100 µM EDTA, 100 µM EGTA, leupeptin (0.5 µg/ml), and pepstatin (0.5 µg/ml) in order to achieve standardized conditions for subsequent HPD photosensitization.

Preparation of DNA Substrates. poly(dA)2000-5000-oligo(dT)16 primer-template was prepared by combining appropriate amounts of each to obtain an average of 150 nucleotides between each primer. Both template and primer were diluted in 10 mM Tris-HCl (pH 7.5) containing a final concentration of poly(dA)2000-5000 of 1.0 mM (in nucleotide). This solution was subsequently heated to 37°C in a water bath followed by a slow cooling period of several hours. Polydeoxynucleotide and deoxynucleoside triphosphate were purchased from the Midland Reagent Company (Midland, TX) by addition of approximately one nucleotide per polymer yielding [3H]dTTP at 0.1 Ci/mmol of total nucleotide. The resulting product was depurinated and isolated free of monomeric nucleotides according to the method of La Duca et al. (42).

Measurement of DNA Polymerase Activities. Activities of DNA polymerases were determined in a total volume of 25 µl containing 20 mM Tris-HCl (pH 7.5), 20% glycerol (v/v), 10 mM dithiothreitol, bovine serum albumin (200 µg/ml), 15 mM MgCl2, 2.0 mM ATP, 80 µM poly(dA)2000-5000-oligo(dT)16 (see above), 25 µM [3H]dTTP (4.0 Ci/mmol), and 2.0 µl of the purified polymerase sample. All reactions were allowed to proceed for 20 min at 30°C in the dark and were then halted by the addition of 10 µl of 0.5 M EDTA (pH 7.5). To remove the unlabeled substrate from the extended DNA polymer, the reaction mixtures were centrifuged through Sephadex G-50 fine resin desalting columns according to the method of Penefsky (43). Material eluted from the column was counted in an aqueous scintillation fluor containing toluene (2 liters), Triton X100 (1 liter), and Omnifluor (12.0 g/liter). To determine the individual activities of the polymerases, specific inhibitors were added. A concentration of ddTTP (100 µM), a level which inhibits DNA polymerase-β and -γ by greater than 95%, was added (44). When it was necessary to inhibit α I and δ DNA polymerases, BuAdATP was added at a concentration of 100 µM; this level of drug is 100 times the Kd for DNA polymerase-α and 10 times the Kd for δ DNA polymerase (data not shown) and it inhibits these two polymerases by 95% and 80%, respectively (33). Under these same conditions, δ polymerase II is inhibited by only 20%.

Measurement of Exonuclease Activities. The determinations of exonuclease activity were performed under the same assay conditions described above for DNA polymerase activity. However ATP, [3H]dTTP, and the poly(dA)2000-5000-oligo(dT)16 template were omitted. Instead, as the DNA substrate, 60 pmol (in nucleotide) of polydeoxynucleotide with an average of 400 nucleotides, which was labeled at the 3' -end with [3H]TMP, was added. The volume of the polymerase samples was increased to 4.0 µl. The samples were incubated as above (20 min in the dark) and assayed as for polymerase to determine the loss of radioactivity, and rates were calculated by extrapolation to time 0 of the amount of loss of radioactivity, assuming a simple exponential decay.

Photoradiation of Polymerase Containing Preparations. Ninety µl of either the purified polymerase preparation or cytosol prepared from calf thymus or the R3230AC tumor were mixed with 10 µl of an HPD solution in final a concentration of HPD (0.7, 7.0, or 70.0 µg/ml). Ten µl of the final mixtures were transferred to 1.5 ml Eppendorf centrifuge tubes and kept at 0–4°C on ice. These tubes were then exposed to photoradiation from a 15 W fluorescent light placed 6 cm from the open top of the tubes [energy intensity 0.1 mw/cm2, measured using a power radiometer (RK 5200, Laser Precision Corp., Utica, NY) equipped with a RK545 radiometer probe], and tubes were removed at selected times for determination of polymerase activities (see above). It should be noted that enzyme activity data are reported relative to control samples (=100%) to which we added 10 µl of 0.9% NaCl solution as for the HPD samples and which were then exposed to light for the duration of the experiment. The zero energy density samples are those that contained HPD but remained in the dark throughout the duration of the experiment. These samples were monitored to determine whether HPD had any effect on DNA polymerase or nuclease activity in the dark under the same assay conditions as those samples that contained HPD but were exposed to light.

In Vivo-in Vitro Protocol. The effects of HPD photosensitization on polymerase activities were also studied using an in vivo-in vitro protocol (15). Briefly, animals were given injections i.p. of HPD (80 µg/kg), and sacrificed at selected times; tumors were removed, and cytosols were prepared in the dark. The samples were then photoradiated, and polymerase activities were measured. This experimental procedure differed from that described above for calf thymus and tumor tissue in that the cytosols obtained from tumors from injected animals were not dialyzed prior to assay (see above). It was reasoned that the dialysis procedure might elute some of the HPD bound to the macromolecules in the supernatant solutions. The omission of this step did not allow the polymerase activity.

RESULTS

Effects of Light on the Activities of DNA Polymerases

Purified from Calf Thymus. To ascertain the effects of photo-

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radiation on the activities of DNA polymerases, in the absence of HPD, DNA polymerases purified from calf thymus were exposed to light under the standard conditions to be used for photosensitization with HPD. The data are summarized in Table 1. Compared to the zero energy density sample (representing 100% enzyme activity), the data indicate that there was a modest decrease in DNA polymerase activity during the period of light exposure. However, this loss in enzyme activity was relatively small (<20%) when compared to the samples photoradiated in the presence of various concentrations of HPD (see below).

Effects of HPD Photosensitization on the Activities of DNA Polymerases Purified from Calf Thymus. Chart 1 depicts the results obtained when the activities of purified calf thymus DNA polymerases were assayed during exposure to HPD and light. At a dose level of HPD (0.7 \( \mu \)g/ml) (Chart 1A), DNA polymerases-\( \alpha \) and -\( \delta \) II activities were actually stimulated above control levels (exposure to light in the absence of HPD), an effect particularly evident over the range of 0.06 to 0.18 J/cm\(^2\). In contrast, under the same conditions, the activity of DNA polymerase-\( \delta \) II was dramatically reduced compared to control samples. Also illustrated are the activities for the zero energy density samples (y axis), samples incubated for 1 h in the dark on ice in the presence of HPD (see "Materials and Methods"). The reduced activity seen with the polymerase samples kept in the dark may result from a direct interaction between the HPD and the DNA polymerase.

Table 1

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<th>Energy density (J/cm(^2))</th>
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The results presented in Chart 1B (7.0 \( \mu \)g HPD/ml) also depict a reduction in purified calf thymus DNA polymerase activity at zero energy density. Photosensitization of HPD at this intermediate dose produced a sharp increase in the activity of \( \delta \)-polymerase \( \delta \) II, and no apparent stimulation of the activity of DNA \( \alpha \)-polymerase. Under the same conditions, the activity of DNA polymerase-\( \delta \) II was reduced about 1.5 times more rapidly than that observed at the lower dose of HPD (0.7 \( \mu \)g/ml; Chart 1A). The results obtained at the highest dose of HPD (70 \( \mu \)g/ml; Chart 1C) indicate that there was considerable sensitivity of all the DNA polymerases to HPD in the dark. At zero total energy density, under the conditions used, a loss of 50, 75 and 95% of enzyme activity occurred with DNA \( \delta \) I, \( \delta \) II, and \( \alpha \)-polymerase, respectively, when compared to control samples not exposed to HPD. Curiously, a slight stimulation of DNA polymerase-\( \delta \) I occurred after exposure to 0.015 J/cm\(^2\) total energy density. However, enzyme activity was reduced to nearly zero at energy densities of 0.14–0.27 J/cm\(^2\). By comparison, the activities of DNA polymerase-\( \alpha \) and \( \delta \) II were reduced to nearly zero after exposure to as little as 0.015 J/cm\(^2\) total energy density in the presence of HPD (70 \( \mu \)g/ml).

Effects of HPD Photosensitization on the Activities of Purified Calf Thymus DNA Polymerase-associated Exonucleases. The data presented in Chart 2 were obtained when measurements of DNA polymerase-\( \delta \)-associated exonuclease activities were performed during photoradiation in the presence of various concentrations of HPD. These exonuclease activities were assayed using the same purified calf thymus polymerases that were measured for polymerization activity. In Chart 2A, DNA polymerase-\( \delta \)-associated exonuclease activity showed minimal photoinactivation in the presence of HPD, except at the highest drug dose level [HPD (70 \( \mu \)g/ml)]. At this high concentration of HPD, complete photoinactivation of the exonuclease activity of DNA polymerase-\( \delta \) I occurred at 0.18 J/cm\(^2\) total energy density; however, there was an 85% inhibition in enzyme activity at zero total energy density. The effects of HPD and light on the associated exonuclease activity of DNA polymerase-\( \delta \) II (Chart 2B) demonstrate both a drug dose and energy density dependent photoinactivation. DNA polymerase-\( \delta \) II exonuclease photoinactivation was minimal at HPD (0.7 \( \mu \)g/ml) and increased progressively at HPD (7.0 and 70 \( \mu \)g/ml), respectively. These results are
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Chart 2. Effects of HPD photosensitization on the activities of DNA polymerase-associated exonuclease activities purified from calf thymus (A, δ I exonuclease; B, δ II exonuclease). Various concentrations of HPD were used (○, no HPD; ●, 0.7 μg/ml; Δ, 7.0 μg/ml; and △, 70.0 μg/ml). Each point represents the rate of loss of radioactivity from the substrate and was calculated as a percentage of control activity (see "Materials and Methods"). Each point represents the value obtained from one experiment.

Charts 3 and 4. Effects of HPD photosensitization on DNA polymerase activities measured in cytosol preparations prepared from a pooled sample of calf thymus glands. A depicts the results obtained for the combined activities of DNA polymerases-α, -δ I, and -δ II; B shows the individual activity of DNA polymerase-δ II. Various concentrations of HPD were used (○, no HPD; ●, 0.7 μg/ml; Δ, 7.0 μg/ml; and △, 70.0 μg/ml). Each experimental point represents the calculated percentage of enzyme activity when compared to the control activity (samples exposed to light without HPD) at each energy density level. Each point is the value obtained from one experiment. The standard deviation of each point was found to be less than 5% when calculated by the method of Walpole (48).

Effects of HPD Photosensitization on the Activities of DNA Polymerases Measured in Cytosols from Calf Thymus. Chart 3 depicts the results obtained when the activities of the DNA polymerases were measured in cytosols prepared from calf thymus and subsequently exposed to HPD and light. When the combined activities of DNA polymerases-α, δ I, and δ II were examined under the conditions used, very little sensitivity to photoradiation in the presence of HPD was observed, even at the highest drug dose (Chart 3A). However, when specific measurement of DNA polymerase-δ II was performed (Chart 3B) in an assay conducted in the presence of BuAdATP to inhibit DNA polymerases-α and δ I, a drug dose and energy density dependent relationship was obtained. These data indicate that δ II was preferentially inactivated.

Effects of HPD Photosensitization on DNA Polymerase Activities Measured in Cytosol Preparations from R3230AC Tumors. To ascertain whether the effects observed on polymerase activities were manifest in other tissues, experiments were performed with cytosol preparations made from the R3230AC mammary adenocarcinoma of the Fischer rat. These results (Chart 4A) indicated that the combined activities of DNA polymerases-α, -δ I, and -δ II demonstrated modest photoinactivation, except at the highest HPD dose used (70 μg/ml). The same pattern of inhibition as with calf thymus cytosol was seen when DNA polymerases-α and -δ I were inhibited in the tumor cytosol by the presence of BuAdATP to enable the measurement of DNA polymerase-δ II. Although we observed a greater preference for inhibition of DNA polymerase-δ II by HPD-induced photosensitization with calf thymus cytosol, in general both the calf thymus and tumor tissue cytosols demonstrated this preference.

Effects of HPD Photosensitization on the Activities of DNA Polymerases in Cytosol Preparations from Tumors following Injection of HPD. Chart 5 depicts the results obtained with cytosol preparations made from R3230AC tumors following injection of tumor-bearing hosts with HPD (80 mg/kg) and their sacrifice at indicated times. All cytosol preparations were exposed to 0.18 J/cm² total energy density, and then the activities of DNA polymerases were measured. The data are presented as a percentage of control (the zero energy density sample representing 100%). The data demonstrate that the combined activities of the DNA polymerases were not significantly altered over the time course studied. However, when DNA polymerase-α and -δ I were preferentially inhibited with BuAdATP to allow the measurement of -δ II, a different pattern was observed. The activity of DNA polymerase-δ II displayed reduction in activity at 24 h post-injection. Subsequent to this time, the activity of -δ II increased and returned to approximately the control values by 96 h. These results suggest that photosensitization of DNA polymerase-δ II by HPD can be accomplished in intact tumor tissue.

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Chart 4. Effects of HPD photosensitization on the activities of DNA polymerases measured in a cytosol preparation from a pooled sample of R3230AC tumors. Data in A represent the values obtained for the combined activities of DNA polymerases-α, -β I, and -β II and B the individual activity of DNA-β I. Various concentrations of HPD were used (O, no HPD; •, 0.7 μg/ml; △, 7.0 μg/ml; and Δ, 70.0 μg/ml). Each experimental point represents the calculated percentage of enzyme activity when compared to the control activities (samples exposed to light without HPD) at each level of total energy density. Each point is the value obtained from one experiment. The standard deviation of each point was found to be less than 5% when calculated by the method of Walpole (48). An identical result was found when cytosol was prepared from only one tumor.

DISCUSSION

Photodynamic therapy offers promise as a new modality for the treatment of cancer. The mechanism by which this therapy causes tumor cytotoxicity remains undefined, with reports in the literature indicating that various intracellular sites are damaged by photosensitization with porphyrins. Damage to nuclear DNA in the form of single strand breaks and sister chromatid exchanges (19, 20) are consistent with impairment of the tumor cells' ability to divide and multiply, leading to tumor necrosis and regression. Although these investigators demonstrated structural damage to nuclear DNA, they also observed that, in most cases, the cells were able to repair the damage in vitro within 15 to 30 in following porphyrin photosensitization. Under certain conditions, photosensitization-induced damage to DNA was not repaired, implicating a functional impairment of those enzymes involved in DNA repair and chromosomal replication, e.g., nucleases and DNA polymerases.

To gain further insight into the biological effects of HPD, we examined the effects of HPD photosensitization on purified mammalian DNA polymerase-α and two α-like DNA polymerases, -β I and -β II. The δ-class of DNA polymerase has properties similar to DNA polymerase-α but also has an associated 3'→5' exonuclease activity. Enzymes of the δ-class have been purified and their catalytic mechanisms have been studied (34–37). We took advantage of our recently developed method that provides two forms of this enzyme from calf thymus, designated DNA polymerase-δ I and DNA-δ II (33). Their activities are distinguishable in cell extracts and in the purified state by the compound BuAdATP, a reagent that preferentially inhibits DNA polymerases-α and -δ I. Through use of this compound, it has been possible to quantitate the effects of HPD photosensitization on DNA polymerase-δ II in the presence of the other polymerases in cytosol.

The most interesting finding was the unique sensitivity of highly purified DNA polymerase-δ II to HPD and light (Chart 1). This sensitivity was also observed in cytosols prepared from calf thymus and R3230AC mammary tumors (Charts 3 and 4, respectively). The exonuclease activity associated with DNA polymerase-δ II also demonstrated greater sensitivity to photosensitization by HPD than did the exonuclease activity associated with DNA polymerase-δ I (Chart 2). This differential sensitivity of DNA polymerase-δ II was likewise seen in the tumor cytosols prepared from rats that had been earlier injected with HPD (80 mg/kg) and the cytosols subsequently exposed to light. In this in vivo-in vitro protocol, we observed a 35% reduction in DNA polymerase-δ II at 24 h post-injection, followed by a gradual increase in enzyme activity to approximately the control levels by 96 h. This pattern of inhibition was not detected when the combined polymerase activities (α, δ I, and δ II) were assayed. It should be noted that DNA polymerase-δ II represents approximately 25% of the combined polymerase activity, and as such, changes only in δ II levels would not be detectable when all...
enzymes were assayed together. The observations made here, taken together with earlier reports, suggest a number of conclusions. The inhibitory effects of HPD in the dark [observed at HPD 7.0 μg/ml (1 × 10⁻⁶ M) and higher concentrations] are in close agreement with reports by others (29, 30, 45), indicating that various porphyrins at concentrations of 1 × 10⁻⁶ M (in the dark) inhibit both DNA and RNA polymerases. We suggest that this inhibition may be due to either the binding of HPD to the active site of the enzyme or the DNA template. Since these effects are manifested in the dark, inhibition of enzyme activity cannot be attributable to a photochemical-mediated reaction. Curiously, we also observed stimulation of the activities of DNA polymerases-α (Chart 1A) and -δ (Charts 1) when the purified preparations were exposed to HPD and light. This phenomenon may result from a porphyrin-mediated conformational change in the enzyme structure leading to a more rapid catalysis or more efficient binding to DNA.

The effects of HPD and light on DNA δ-polymerase II may partially account for the cytotoxic effects of photodynamic treatment. Cytotoxicity may result from polymerase damage, even though effects on all three polymerases assayed together were minor in the in vivo-in vitro assay. Since the exact role of each enzyme in DNA replication and repair is not known, specific damage to the δ II form might have preferentially impaired the capacity of the cells to repair DNA damage. Obviously, the δ II enzyme form requires more detailed study to establish its metabolic function. We propose that HPD, which specifically inhibits this enzyme form, could provide an important reagent probe for this purpose. Furthermore, the mechanism of the enzymatic inhibition needs to be elucidated. Since the α- and δ-polymerases are structurally and functionally similar (33), the molecular basis for the specificity of inhibition would be of significant interest.

The separation of the two forms of δ-polymerase observed on Bio Rex-70 resin (33) can be attributed to differences in hydrophobic properties, the δ-polymerase II being the more hydrophobic species. It may be fortuitous that the most effective photosensitizing component in HPD, particularly in vivo or in cultured cells in vitro, is the most hydrophobic porphyrin species, the proposed hematoporphyrin diether (46). The selective inhibition of the more hydrophobic δ-polymerase II may result from its preferential association with HPD. This would allow a greater environmental concentration of HPD to occur around amino acids susceptible to light-induced O₂. Alternatively, since O₂ has a longer half-life in hydrophobic environments (47), the more hydrophobic δ-polymerase would present a microenvironment more conducive to damage by O₂. Related possibilities are that certain amino acids, i.e., histidine, tryptophan, and methionine, known to be oxidized by O₂, reside at or near the active site in DNA polymerase-δ II but not in the other polymerases. In view of the specificity of the inhibition, the use of HPD and light as a reagent probe to study DNA polymerase-δ II may prove to be of considerable value for the elucidation of the structure and the mechanism of catalysis of this α-like DNA polymerase.

Finally, the observations delineated in Chart 5 for the in vivo-in vitro protocol are of potential therapeutic importance. These results indicate that the HPD administered to host rats enters the tumor and is retained in the nuclei of the tumor cells (as indicated by the preferential inhibition of DNA polymerase-δ II). The data obtained for the inhibition of activity of DNA polymerase-δ II in the in vivo-in vitro protocol closely follow the data we reported earlier (16) for the inhibition of the cytosolic enzyme pyruvate kinase assayed under approximately the same conditions. Although direct evidence is lacking, these data together imply that the pharmacokinetics of HPD, or its “active” component(s), in vivo are similar for both the nucleus and the cytosol.

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