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Photosensitized reduction and DNA covalent binding of aziridinylquinones

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Abstract

Photolysis of anaerobic aqueous mixtures (at wavelength maxima above 600 nm and at pH 7.4) containing either aluminum phthalocyanine tetrasulfonate ($AlPcS_4$), chlorin e6 (CHLORIN), pheophorbide-a (PHEO) or a novel tetracationic phthalocyanine derivative (TETCHLORIN) in the presence of the quinones diaziquone (AZQ), carboquone (CARBOQ) or 2,5-dicloro-diaziridinyl-1,4-benzoquinone (AZDClQ) produces the corresponding semiquinones. Photolysis of these mixtures under the conditions stated above, but in the presence of DNA and at pH 5.5 produces quinone–DNA covalent adducts. Absorption bands seen in irradiated solutions suggest binding of these quinones to DNA through the open aziridine ring. In general, the quinone CARBOQ yielded the largest amounts of adducts photosensitized by the dyes studied here. No quinone–DNA adducts were detected if samples were irradiated at pH 7.4.Thus, both photoreduction of these quinones and an acidic environment are needed for these quinones to bind DNA. These results suggest a potential mode of therapy with special applications to hypoxic regions in solid tumors which are characterized by an acidic environment.

Keywords: Aziridinylquinones; DNA covalent binding; Photosensitization; Phthalocyanine; Chlorin; Pheophorbide; Carboquone; Diaziquone

1. Introduction

Photodynamic therapy (PDT) is a cancer treatment that uses a combination of red laser light, a photosensitizing agent, and molecular oxygen to produce the therapeutic effect [1,2]. Porphyrins (POR), phthalocyanines (PC), chlorins (CHL), and other dyes are currently being used in photodynamic treatment of tumors due to their large absorption coefficients in the 500–800 nm range [1,2]. In the presence of air these photosensitize the production of singlet oxygen and superoxide [1,2]. Singlet oxygen production, the so-called Type II pathway, is claimed to be the most important process that kills tumor cells. However, Type I pathways, those involving photoreduction or photooxidation of substrates, have also been proposed as photocytotoxic events in PDT, especially in hypoxic environments [3].

Solid tumors are often hypoxic. Therefore, direct killing of these hypoxic cells by singlet oxygen is very limited [1,4]. However, since red-light-absorbing dyes also photoreduce oxygen [5–7], these should also photoreduce molecules having nearly equal or more positive redox potentials than oxygen in anoxic/hypoxic cells. If this reducible substrate is a DNA-alkylating quinone, which is activated by reduction, DNA alkylation should be expected, with the consequent cell death.

In this work we demonstrate that aziridinyl-containing quinones are photoreduced by red-light-absorbing dyes under anoxic conditions, and that both photoreduction and acidic pH conditions are needed for quinone–DNA covalent adduct formation.

2. Experimental

2.1. Chemicals

The dyes aluminum phthalocyanine tetrasulfonate ($AlPcS_4$), chlorin e6 (CHLORIN), pheophorbide-a (PHEO), and a novel

Abbreviations: $AlPcS_4$, aluminum phthalocyanine tetrasulfonate; AZDClQ, 2,5-dicloro-diaziridinyl-1,4-benzoquinone; AZQ, diaziquone; CARBOQ, carboquone; CHL, chlorins; CHLORIN, chlorin e6; PC, phthalocyanines; PDT, photodynamic therapy; PHEO, pheophorbide-a; POR, porphyrins; TETCHLORIN, chlorin e6 tris(*N*,*N*,*N*-trimethylethylenediamine amide)*N*,*N*,*N*-trimethylethylenediamine vinyl adduct tetrachloride

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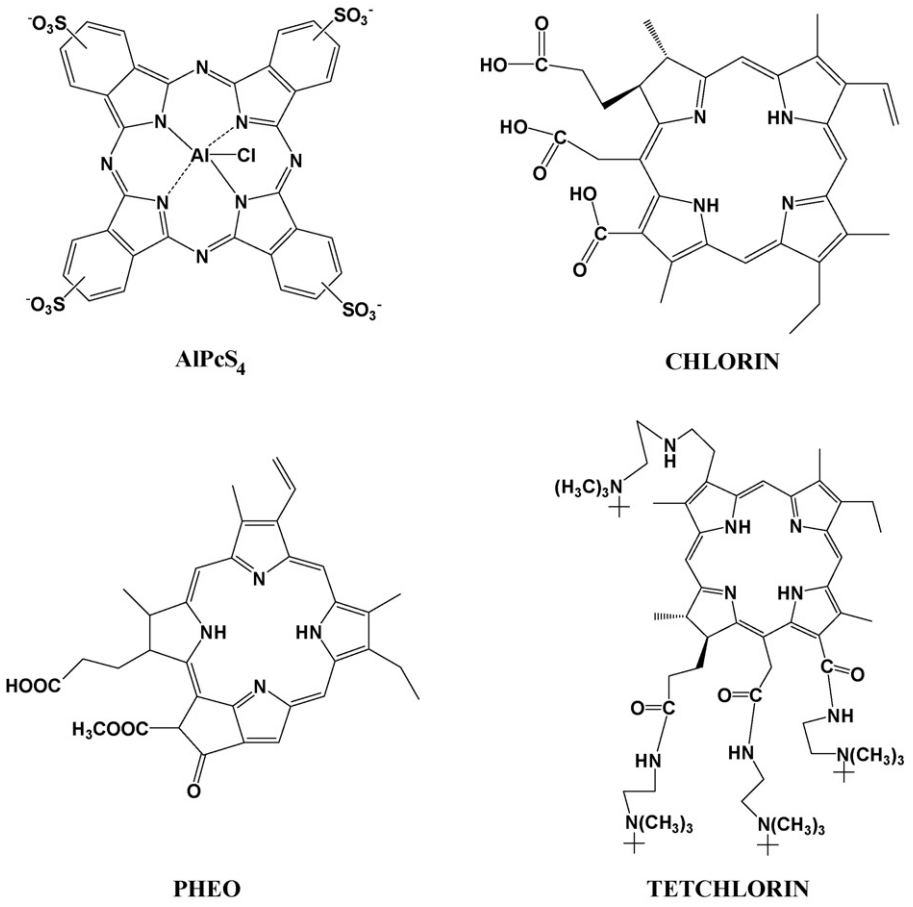

Fig. 1. Dyes used in this work.

tetracationic phthalocyanine derivative chlorin e6 tris(*N*,*N*,*N*-timethylethylenediamine amide)-*N*,*N*,*N*-trimethylethylenediamine vinyl adduct tetrachloride (TETCHLORIN) were obtained from Frontier Scientific and used as received (Fig. 1). The antitumor quinones diaziquone (AZQ) and carboquone (CARBOQ) were kindly provided by the Drug Synthesis and Chemistry Branch of NIH (USA) (Fig. 2). The quinone 2,5-dicloro-diaziridinyl-1,4-benzoquinone (AZDClQ) was purchased from Aldrich Chemicals and used as received. Calf thymus DNA and Sephadex G-25 (superfine, DNA grade), were purchased from Sigma Chemicals. All other chemicals were of the highest purity commercially available and were used without further purification. Aqueous DNA stock solutions were freshly prepared in water each day and their concentration determined from the absorbance at 260 nm and using a molar absorption coefficient of $6.6 \times 10^3\ M^{-1}\ cm^{-1}$. Deionized and Chelex-treated water was used in the preparation of all stock and sample solutions. All reagent stock solutions were deareated by flushing with nitrogen previous to mixing with other reagents. Oxygen was then excluded from samples by keeping a positive pressure of N_2 gas in the sample.

2.2. Sample photolysis

Nitrogen-saturated samples containing 875 μM quinone, 1 mg/mL CT-DNA, and a dye concentration to produce an absorbance of 1 at the dye maximum wavelength above 600 nm in 20 mM phosphate buffer at either pH 5.5 or 7.4 were photolyzed in quartz cuvettes of 1.00 cm light path, under continuous stirring, for periods of time ranging from 0 to 60 min. Samples were protected from external light during and after preparation. Irradiations were performed using a 1000 W xenon arc lamp coupled to a Schoeffel grating monochromator with a bandwidth of ±20 nm. Quantum yields of covalent adduct formation were estimated from the amount of adduct formed during a given irradiation period, and the number of incident photons during that irradiation period. The latter was calculated from the relative light energy flux, determined by using an International Light radiometer model IL-1700 with an SED-033 probe, coupled to actinometry at 653 nm based on the photosensitized generation of singlet oxygen by methylene blue in air saturated methanol, for which the quantum yield for singlet oxygen production is reported to be 0.50 [8]. Singlet oxygen formation was quantified by the consumption of 1,3-diphenylisobenzofuran. The amount of covalently bound quinone to DNA was estimated from the quinone maximum absorbance in the 340–360 nm region assuming the same molar absorption coefficient as that of the parent quinone.

2.3. Photosensitized semiquinone formation

Samples with the same composition as that described above were photolyzed for increasing periods of time followed by

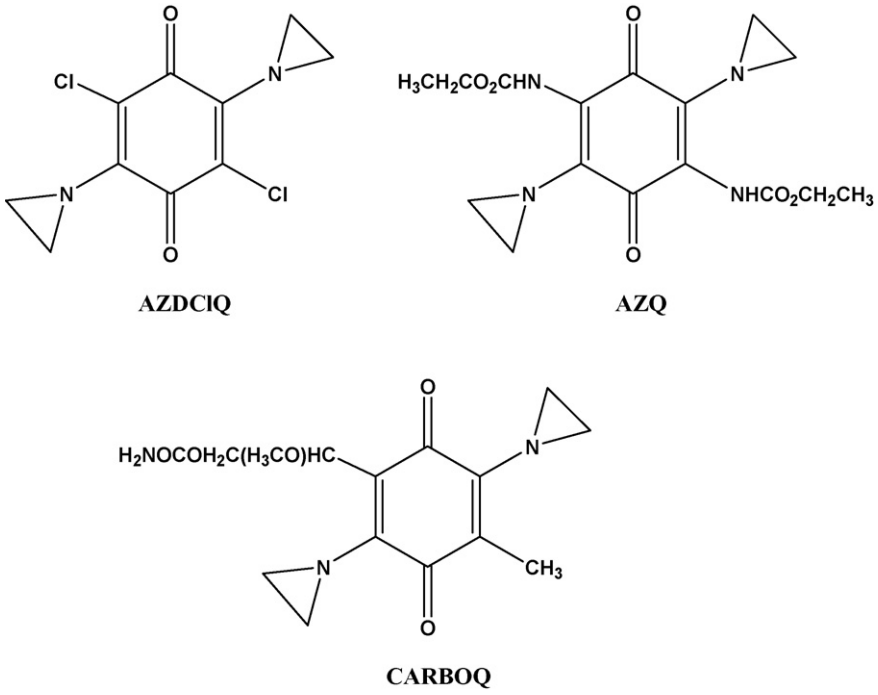

Fig. 2. Quinones used in this work.

transference of these to deareated EPR quartz flat cells. EPR spectra were then recorded on a Bruker ER-200D spectrometer at 100 kHz magnetic field modulation. Semiquinone ($Q^{\bullet -}$) concentration was obtained by comparing the semiquinone over-modulated EPR double-integrated spectral area with that corresponding to a 3-carbamoyl-2,2,5,5-tetramethyl-3-pyrrolin-1-oxide spin standard.

2.4. Semiquinone disproportionation constant

The equilibrium constant for the semiquinone disproportionation reaction (K_1, Eq. (1)) was determined at pH 7.4 by reducing the quinone with $NaBH_4$, as described previously [9];

$$2Q^{\bullet -} + 2H^+ \leftrightarrow Q + QH_2; \quad K_1 = \frac{[QH_2][Q]}{[Q^{\bullet -}]^2[H^+]^2} \tag{1}$$

and determining the concentration of reduced quinone species ($[Q^{\bullet -}] + [QH_2]$) by absorption spectroscopy and the semiquinone concentration by EPR spectrometry. For this purpose, a nitrogen-saturated quinone solution containing 20 mM phosphate at pH 7.4 was reacted with successive aliquots of a $NaBH_4$ solution and both the absorption and EPR spectra of the resulting solutions were then measured. The decrease in absorbance of these solutions was monitored at a wavelength above 300 nm where the hydroquinone species have negligible absorption [9], using an Agilent 8453 diode-array spectrophotometer. From this decrease in absorbance and the corresponding molar absorption coefficient at that wavelength, the concentration of reduced quinone species ($[Q^{\bullet -}] + [QH_2]$) was determined. The equilibrium quinone concentration was determined from the absorbance at this wavelength after reduction. The semiquinone concentration was determined as described above, and this was subtracted from the total reduced species concentration to obtain the hydroquinone concentration.

2.5. Quinone covalent binding to DNA

The procedure used in this determination was adopted from that described by Lusthof et al. [10] and used previously by us [11]. This is based on Sephadex spun exclusion [12] of bound and free DNA from non-bound quinone. After photolysis, samples were left standing (1 or 16 h) at room temperature under a N_2 atmosphere and covered with aluminum foil. This was followed by Sephadex spun exclusion. After this procedure, samples were analyzed by absorption spectroscopy. Absorption bands above 300 nm should correspond to covalently bound quinone (for example, the DNA-2,5-bis(1-aziridinyl)-1,4-benzoquinone adduct has an absorption maximum wavelength at 343 nm [10]), unless a non-photolyzed identical solution (a blank), treated in the same manner as the sample, presented similar absorption bands. The presence of this absorption band in the blank should indicate that non-covalently bound quinone is being excluded with the DNA. In such cases, samples and blanks were submitted again to Sephadex spun exclusion several times until no absorption band over 300 nm was detected in blank samples. This was then followed by extraction with chloroform and with diethyl ether, using a 1:1 (v/v) of aqueous to organic solvent ratio, followed by DNA precipitation and redissolution in water, as described elsewhere [13]. Samples and blanks treated in this manner were then submitted to absorption spectroscopy analysis. Sample pH values were measured before and after photolysis and before Sephadex extraction and were found to be constant.

2.6. DNA cross-linking

The percent of cross-links was determined as described by Lusthof et al. [10,14]. Helium-saturated samples containing 875 μM quinone, 1 mg/mL CT-DNA, and a dye concentration to produce an absorbance of 1 at the dye maximum wavelength above 600 nm in 20 mM phosphate buffer at pH 5.5 were photolyzed for 30 min. The mixture was under a high purity helium atmosphere for 60 min before photolysis under dark. The photolyzed sample was incubated 16 h in an inert atmosphere and under dark conditions. Incubation was followed by chloroform and diethyl ether extractions (1:1, v/v) and ice-cold ethanol DNA precipitation until no dye absorbance band was detected. The DNA precipitate was redissolved in 1 mL deoxygenated deionized water and left overnight at 4 °C. A 500 μL sample of this solution was mixed with 2500 μL of a deoxygenated solution containing 10 μg/mL of ethidium bromide and fluorescence was measured at 580 nm exciting at 520 nm. The sample was then heat-denatured at 95 °C for 5 min, quickly followed by cooling at room temperature for 3 min. Immediately after this, the fluorescence of the sample was measured. The ratio of the fluorescence after and before denaturation $[(F_a/F_b)_s]$ is compared with same ratio for a blank sample $[(F_a/F_b)_b]$. A solution similar to the sample was used as the blank, where the aziridinyl-containing quinone is replaced by ubiquinone-0. This quinone has no aziridinyl groups. Using these ratios, an interstrand cross-link percentage is calculated using Eq. (2). As noted by

$$\%\,\text{Cross-link} = \frac{[(F_a/F_b)_s - (F_a/F_b)_b]}{[1 - (F_a/F_b)_b]} \times 100\% \quad (2)$$

Lusthof et al. [10], this percentage of cross-links is in fact the percentage return of double stranded DNA that causes fluorescence of ethidium bromide.

3. Results and discussion

3.1. Semiquinone formation

Photolysis of N_2-saturated samples containing 875 μM of either AZDClQ, AZQ or CARBOQ and $AlPcS_4$ in the presence or absence of DNA at pH 7.4 produces the corresponding semiquinone, as demonstrated from the observed EPR spectra (Fig. 3). Irradiation of the other dyes under similar conditions produces the corresponding EPR spectrum of the semiquinone. A resolved EPR spectrum for the semiquinone of CARBOQ was only detected at relatively high pH (pH 9, Fig. 3) due to its large semiquinone disproportionation constant, although an overmodulated spectrum of this semiquinone was observed at pH 7.4. The semiquinone concentration increases with irradiation time in the absence and presence of DNA (Fig. 4) and its production was also observed if the other dyes PHEO, CHLORIN and TETCHLORIN were used instead. Semiquinone formation was not observed at pH 5.5 most probably due to the shift in the semiquinone disproportionation equilibrium to the right at smaller pH values (Eq. (1)). Photolysis yields of semiquinone were larger for AZDClQ and AZQ than for CARBOQ, due to a much

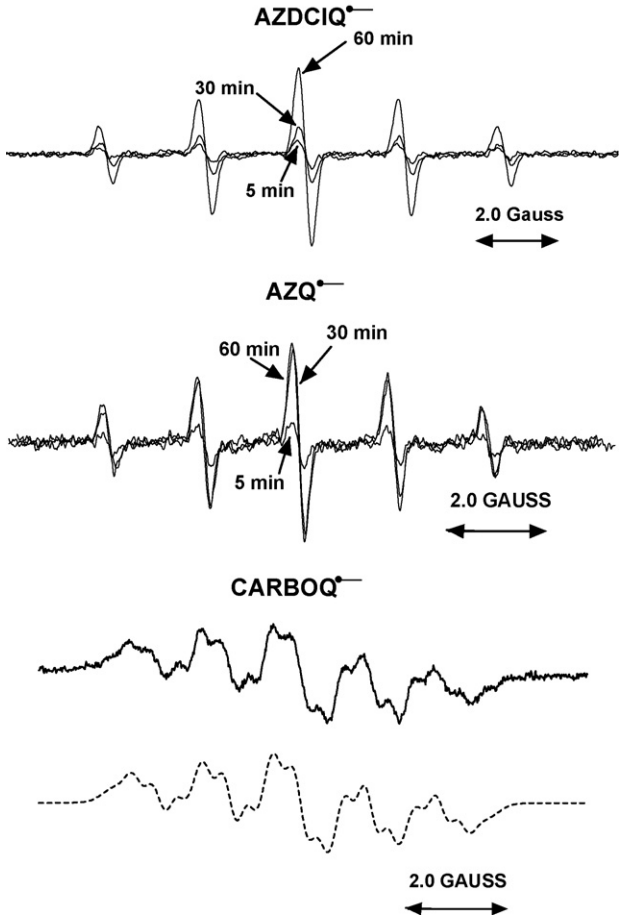

Fig. 3. Semiquinone EPR spectra observed after photolysis at 675 nm of a nitrogen-saturated solution containing 875 μM of either AZDClQ, AZQ or CARBOQ and $AlPcS_4$ (absorbance of 1), 1 mg/mL of CT-DNA and 20 mM phosphate buffer (pH 7.4 except for CARBOQ which was at pH 9). Irradiation time is shown. The dotted line spectrum is a simulation of that corresponding to $CARBOQ^{\bullet-}$ using $A_N(1N) = 1.53$ Gauss, $A_N(1N) = 1.63$ Gauss, $A_H(3Hs) = 0.50$ Gauss and $A_H(1Hs) = 0.38$ Gauss. Hyperfine splitting constants of $AZDClQ^{\bullet-}$ and $AZQ^{\bullet-}$ EPR spectra are in agreement with previously reported values [9,11].

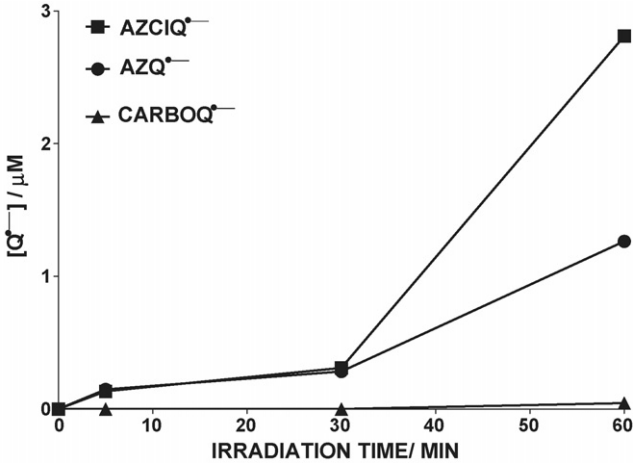

Fig. 4. Dependence of the semiquinone production on the irradiation time at 675 nm. Samples contained 875 μM of the corresponding quinone, $AlPcS_4$ (absorbance of 1), and 20 mM phosphate buffer (pH 7.4).

Table 1
Semiquinone disproportionation constants

Quinone	K_1 (10^{20})
CARBOQ	160 ± 10
AZQ	1.2 ± 0.4
AZDClQ	0.0015 ± 0.004

Table 2
Dye molar absorption coefficients in water

Dye	λ (nm)	ε (10^4 M^{-1} cm^{-1})
PHEO	688	3.0 ± 0.4[a]
$AlPcS_4$	678	16.6 ± 0.1
CHLORIN	655	3.98 ± 0.01
TETCHLORIN	649	1.42 ± 0.01

[a] From Ref. [25].

larger semiquinone disproportionation constant of the latter (Table 1).

3.2. Quinone covalent binding of DNA

Nitrogen-saturated solutions containing 875 μM quinone, 1 mg/mL CT-DNA, and dye with an absorbance close to 1 at the dye maximum wavelength above 600 nm in 20 mM phosphate buffer, at pH 5.5, were photolyzed, as indicated above. The molar absorption coefficients used for these dyes in water are shown in Table 2. Irradiation was followed by an incubation period of 1 or 16 h under argon. Samples were then submitted to Sephadex spun filtration, organic solvent extraction and DNA precipitation/redissolution. After this procedure, absorption spectra of these samples revealed a band with maxima ca. 350 nm for AZQ-, CARBOQ- and AZDClQ-containing samples (Fig. 5). These wavelength maxima are larger than those corresponding to the unreduced parent quinones, i.e. 339 for AZQ, 331 for CARBOQ and 345 nm for AZDClQ. A similar increase in absorption wavelength maxima has been reported for 2,5-bis(1-aziridinyl)-1,4-benzoquinone upon reduction and alkylation of calf thymus DNA [10]. The latter was ascribed to the formation of products with open aziridine rings upon quinone reduction [10,15]. Non-irradiated samples of identical composition as those photolyzed and undergoing same DNA isolation procedures, however, did not reveal these absorption bands (Fig. 5). The observation of a band assigned to DNA covalent adducts and the fact that the DNA-bound quinone is only observed at pH 5.5 and not at pH 7.4, strongly suggests that the bound species is the aziridinyl quinone bound through its opened aziridinyl group to DNA. The increased alkylation of DNA by reduced aziridinylquinones at lower pH values indicates that protonation of the aziridine ring is an essential step for alkylation, in agreement with a high reactivity of protonated aziridines towards nucleophilic species [16]. Furthermore, irradiation of samples not containing quinone with 875 μM of 2-methyl-1,4-benzoquinone or ubiquinone-0 and submitted to the same DNA isolation procedure did not produce an absorption band above 300 nm (Fig. 5), indicating that the DNA-bound quinone is produced only when the aziridinyl group is present at the quinone.

No reports, to our best knowledge, have been published regarding the DNA base-aziridinylquinone adduct isolation and identification by spectroscopic techniques and, in fact, one unsuccessful attempt to do so has been reported [17]. Additional evidence for DNA covalent binding is DNA cross-links

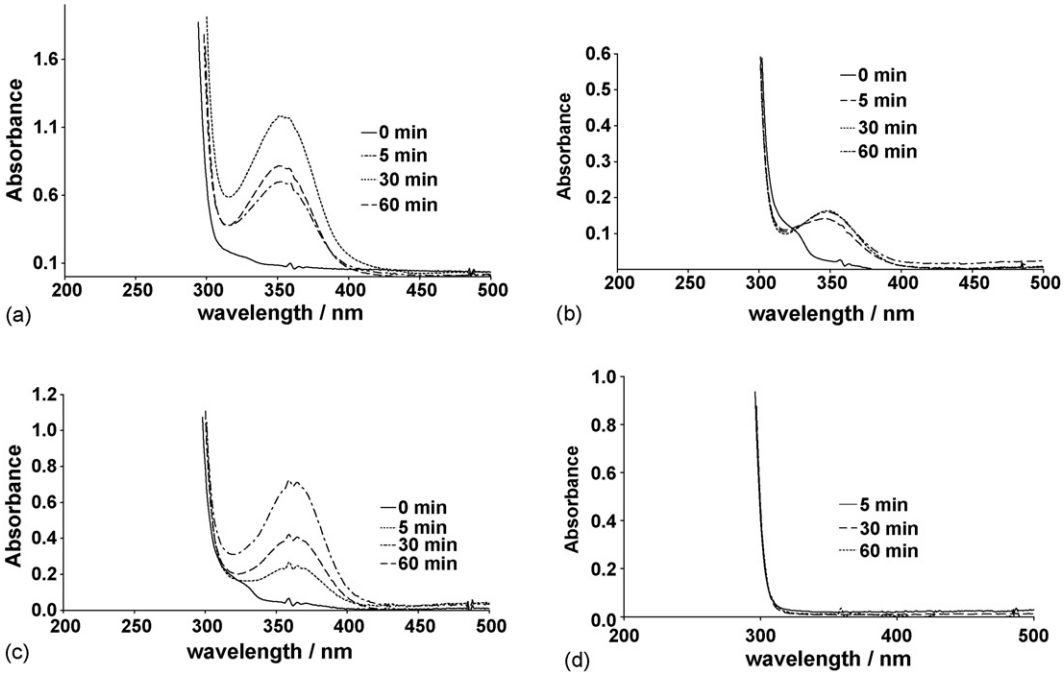

Fig. 5. Absorption spectra after 16 h of incubation under an inert atmosphere of nitrogen-saturated samples containing 1.0 mg DNA/mL, 20 mM phosphate buffer at pH 5.5 and (a) 875 μM CARBOQ + PHEO, (b) AZQ + $AlPcS_4$, (c) AZDClQ + $AlPcS_4$ or (d) ubiquinone-0 + $AlPcS_4$ which were photolyzed at the red light maximum wavelength of the corresponding dye. Similar spectra as that in (d) were observed if 2-methyl-1,4-benzoquinone was used or no quinone was present.

formation by these photoreduced quinones. Photolyses of the quinone–dye couples AZDCIQ–$AlPcS_4$ and CARBOQ–PHEO produced 22 ± 2 and 40 ± 3 cross-links percentages, respectively. Several studies have previously reported DNA alkylation and cross-linking by bis-aziridinylquinones, especially at low pH values, after their activation by reduction [9–11,18–20]. Thus, since these diaziridinylquinones are being photoreduced, DNA alkylation is expected. In fact, both the semiquinone [21] and the hydroquinone [17] have been proposed as the DNA-alkylating species.

The measured quinone absorbances at a wavelength near 350 nm per unit absorbance of DNA at 260 nm (A_Q), obtained after photolysis and incubation under dark, are shown in Fig. 6. A_Q values were larger after 16 h of post-irradiation incubation under dark and anoxia as compared to 1 h incubation. For example, A_Q values after 16 h of post-irradiation incubation for the couple PHEO + CARBOQ were about twice that obtained after 1 h incubation, while 1 h incubation of photolyzed samples of couples AZDClQ + TETRACHLORIN, CARBOQ + TETRACHLORIN, AZQ + PHEO and AZQ + $AlPcS_4$ did not produce detectable amounts of covalent adducts. Thus, quinone–DNA binding takes place slowly after quinone photosensitized activation. Red light irradiations of quinones in the presence of DNA but in the absence of dye did not produce covalent adducts. Photolysis of AZDClQ + $AlPcS_4$ in the absence of DNA followed by addition of DNA yielded about half of the amount of quinone–DNA adducts. Photolysis of CARBOQ + $AlPcS_4$ in the absence of DNA followed by addition of DNA yielded similar amounts of DNA adducts as when photolysis occurred in the presence of DNA. Thus, the DNA presence enhances the photosensitized formation of the activated quinone in the case of AZDClQ + $AlPcS_4$, although no enhancement was observed, within experimental reproducibility, in the case of CARBOQ + $AlPcS_4$. Photolysis of AZDClQ + $AlPcS_4$

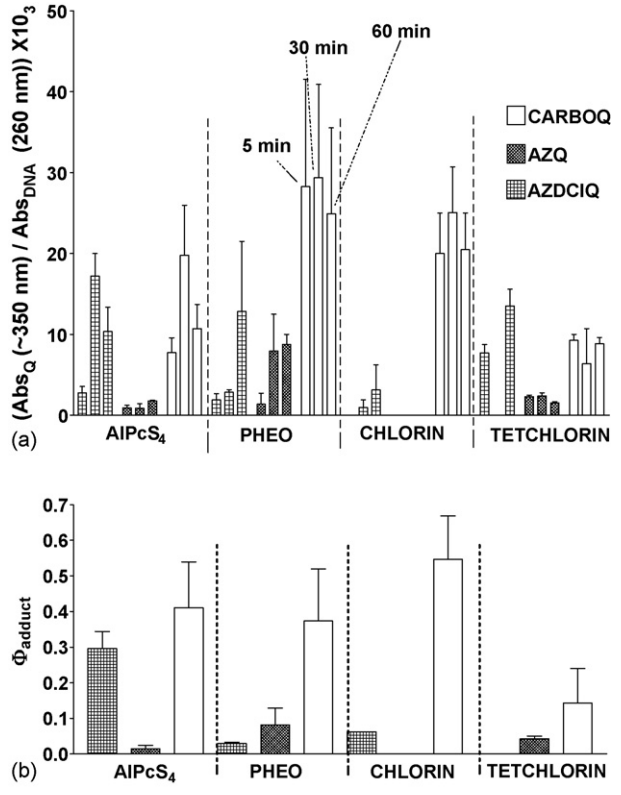

Fig. 6. Photosensitized covalent binding of the aziridinylquinones to CT-DNA. Samples were saturated in N_2 and initially contained 875 μM quinone, 1 mg/mL CT-DNA, and enough dye to produce an absorbance of 1 at the dye maximum wavelength above 600 nm in 20 mM phosphate buffer at pH 5.5. Samples were irradiated at the dye red light maximum wavelength and incubated under N_2 and dark, after photolysis for 16 h followed by DNA isolation to determine A_Q. Values of A_Q are shown in panel (a) and estimated quantum yields for the formation of DNA covalent adducts corresponding to 30 min of photolysis are shown in panel (b). Bars from left to right in (a) correspond to 5, 30 and 60 min of irradiation, respectively.

Photolysis in the presence of DNA

$$\text{Dye} \xrightarrow{h\nu} \text{Dye}^*$$

$$\text{Dye}^* + \text{DNA} \longrightarrow \text{DNA}^{\bullet+} + \text{Dye}^{\bullet-}$$

$$\text{DNA}^{\bullet+} \longrightarrow \text{DNA strand breaks}$$

$$\text{Dye}^{\bullet-} + \text{DNA}^{\bullet+} \longrightarrow \text{Dye} + \text{DNA}$$

$$\text{Dye}^{\bullet-} + \text{Q} \longrightarrow \text{Dye} + \text{Q}^{\bullet-}$$

$$\text{Q}^{\bullet-} + \text{DNA} \longrightarrow \text{Q-DNA}^{\bullet-}$$

$$2\text{Q}^{\bullet-} + 2\text{H}^+ \longrightarrow \text{Q} + \text{QH}_2$$

$$\text{QH}_2 + \text{DNA} \longrightarrow \text{QH}_2\text{-DNA}$$

Photolysis in the absence of DNA followed by DNA addition

$$\text{Dye} \xrightarrow{h\nu} \text{Dye}^*$$

$$\text{Dye}^* + \text{Dye} \longrightarrow \text{Dye}^{\bullet+} + \text{Dye}^{\bullet-}$$

$$\text{Dye}^* + \text{Q} \longrightarrow \text{Q}^{\bullet-} + \text{Dye}^{\bullet+}$$

$$\text{Dye}^{\bullet+} + \text{Dye}^{\bullet-} \longrightarrow 2\,\text{Dye}$$

$$\text{Dye}^{\bullet+} \longrightarrow \text{decomposition product(s)}$$

$$\text{Q}^{\bullet-} + \text{DNA} \longrightarrow \text{Q-DNA}^{\bullet-}$$

$$2\text{Q}^{\bullet-} + 2\text{H}^+ \longrightarrow \text{Q} + \text{QH}_2$$

$$\text{QH}_2 + \text{DNA} \longrightarrow \text{QH}_2\text{-DNA}$$

Scheme 1. Possible mechanisms occurring during the photosensitized reduction of aziridinylquinones and DNA alkylation.

in the presence of DNA, but having both the stock solutions and sample saturated with air, produced only 10–20% of the quinone DNA adducts obtained under nitrogen-saturated conditions. Thus, quinone–DNA adduct formation is partially inhibited by oxygen, as expected, due to a competition of oxygen for the dye triplet state or semiquinone and hydroquinone oxidation to the quinone. However, complete inhibition by oxygen is not observed in this case indicating that quinone–DNA adduct formation could also occur in normoxic environments.

Assuming that the molar absorption coefficient of the quinone bound to DNA is similar to that of the parent quinone, i.e. ca. $1 \times 10^4\ M^{-1}\ cm^{-1}$, quantum yields for the quinone–DNA adduct formation, Φ_{adduct}, were estimated. Those corresponding to 30 min of irradiation are shown in Fig. 6(b). A prominent feature in Fig. 6 is the large efficiency of CARBOQ to form DNA covalent adducts under photosensitization by the dyes used in this study.

As indicated above, both the hydroquinone and the semiquinone of aziridinyl quinones have been proposed as the DNA-alkylating species. Larger Φ_{adduct} values are obtained for CARBOQ than for the other quinones, although less CARBOQ semiquinone concentration is detected than for AZQ or AZDClQ (Fig. 6). Therefore, it seems that hydroquinone is more important to produce DNA covalent binding than semiquinone. However, the semiquinone species could also be the alkylating species while at the same time the hydroquinone is consumed by the shift to the left in the semiquinone disproportionation equilibrium (Eq. (1)) after the semiquinone binds DNA.

An important concern is the initial source of reducing equivalents. Previous reports have shown that dyes are able to photooxidize DNA under anoxia using visible light absorption [22,23]. In fact, one of these reports show that PHEO photooxidizes DNA under anoxia [23], an event which was ascribed to guanine photooxidation by the PHEO singlet state due to the exergonic character of this process ($\Delta G^0 = -0.21$ V versus NHE). When samples are photolyzed in the absence of DNA, electrons could be provided by the dye itself, in which case, dye photodestruction should be detected. The observation stated above that photolysis of AZDClQ + $AlPcS_4$ in the absence of DNA, followed by addition of DNA, yielded about half of the amount of quinone–DNA adducts could then be explained by the additional source of reducing equivalents which could be provided by DNA photooxidation. Photooxidation of DNA is detected using plasmid DNA electrophoresis analysis of strand breaks after photolysis [23]. No change in the dye absorption band is detected if dearated samples containing quinone and dye are photolyzed in the presence of DNA (Fig. 7(a and b)), thus indicating that electrons are not being provided by the dyes during photolysis. However, if photolysis occurs in the absence of DNA, dye photodestruction is observed (Fig. 7(c and d)), thus, suggesting that reducing equivalents are being provided by the dye.

The main objective of this work is to report the photosensitized reduction of alkylating quinones and the consequent covalent binding of DNA, and is not intended to define unambiguously a mechanism for these photoreactions. However, we propose possible mechanisms involved in the photosensitized reductions of the quinones studied here and consequent DNA alkylation in view of our observations depicted in Fig. 7, and the reported behavior of PHEO (Scheme 1). The excited state of the dye in the mechanism corresponding to photolyses in

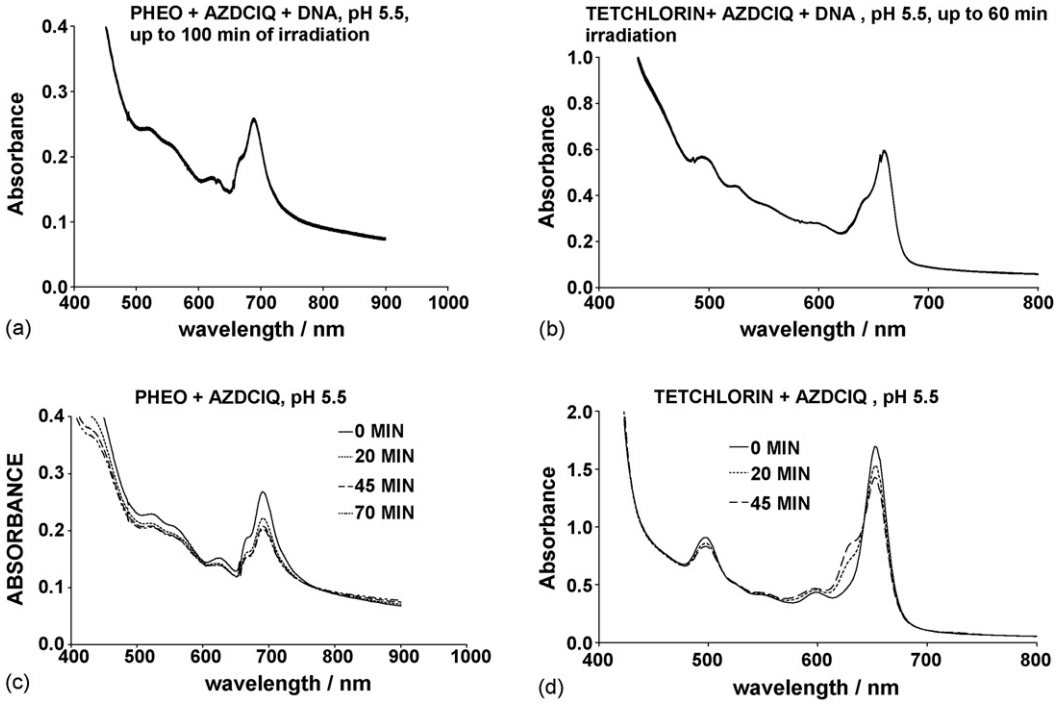

Fig. 7. Absorption spectra of samples after photolysis under anoxia, at pH 5.5, containing (a) 263 μM AZDClQ, 10 μM PHEO and 0.3 mg/mL DNA (sample was photolyzed for up to 100 min), (b) 563 μM AZDClQ, 45.4 μM TETCHLORIN and 0.644 mg/mL DNA (sample was photolyzed for up to 60 min), (c) 263 μM AZDClQ and 10 μM PHEO without DNA, (d) 1485 μM AZDClQ and 119.7 μM TETCHLORIN without DNA.

the presence of DNA could be the singlet state [22,23] while that proposed in the mechanism in the absence of DNA should be the triplet state, due to the diffusion-controlled character of the electron transfer reaction in that mechanism, and to the much longer lifetime of the triplet states of PDT dyes, in general, as compared to that of the singlet state [24]. The fate of the proposed oxidized dye, if formed, is unknown at this moment to our best knowledge. The reduced quinone–DNA adducts (Q–DNA$^{\bullet -}$ and QH_2–DNA) will be oxidized to the parent quinone–DNA adducts upon exposure to air. Ongoing experiments are in progress to detect unambiguosly if DNA photooxidation under anoxia is occurring and to determine the intermediates of the dye, quinone and DNA involved in these photoreactions.

In summary, photolysis of red-light-absorbing dyes in N_2-saturated samples containing calf thymus DNA and diaziridinylquinones produced quinone reduced species and quinone–DNA covalent adducts. These observations suggest the possibility that these dyes could induce cell death in hypoxic regions of tumors if aziridinylquinones are able to react with the excited states of these dyes under hypoxia.

Acknowledgments

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