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Furanochromone radical cations: generation, characterization and interaction with DNA

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Abstract

The radical cations of naturally occurring furanochromones visnagin (VI) and khellin (KH) have been generated and identified for the first time by use of laser flash photolysis and pulse radiolysis techniques. The lifetimes of $VI^{\cdot+}$ and $KH^{\cdot+}$ are determined as ~ 6 and ~ 35 μs under these conditions, respectively. Direct 308-nm excitation of VI in aqueous buffer at physiological pH results in monophotonic photoionization to generate $VI^{\cdot+}$, with a quantum yield of 0.075, which is much higher than that of 8-methoxypsoralen and KH under identical conditions. Though $VI^{\cdot+}$ is a more powerful oxidant than $KH^{\cdot+}$, both of them react with guanosine mononucleotide ($k = 1.2 \times 10^9$ and 3.8×10^7 $dm^3\ mol^{-1}\ s^{-1}$, respectively) via electron transfer to give the guanine radical cation. Furthermore, selective oxidation of guanine in single and double strand DNA by $VI^{\cdot+}$ was also observed. These novel findings suggest that electron transfer reactions involving furanochromone radical cations may be of considerable importance in furanochromone photochemotherapy.

Keywords: Furanochromones; Photoionization; Radical cation; Electron transfer; DNA; Laser flash photolysis

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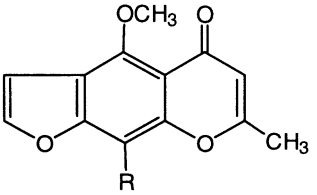
1. Introduction

Visnagin (VI) and khellin (KH) are two naturally occurring furanochromones in various parts of *Ammi visnaga* L. (Apiaceae) [1] that present similarly molecular structure with the well-known photosensitizers 5-methoxypsoralen and 5,8-dimethoxypsoralen. They have long been known to be spasmolytic and vasodilatory agents and are used in photochemotherapy (in conjunction with UVA radiation) to treat dermatological disorders such as vitiligo. Indeed, KH has been proved to be at least as effective as psoralen derivatives in the photochemotherapy of vitiligo [2,3]. The low genotoxity and lack of long-term side effects and phototoxic skin erythema response makes KH a valuable alternative to the use of psoralen derivatives [3–5]. Furthermore, both VI and KH have been referred to as being photoactive to cells and microorganisms [3,4,6].

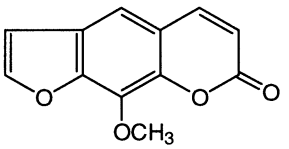
In view of the advantage of KH in photochemotherapy, it is of interest to obtain information on the mode of the photodynamic action of furanochromones. As furanocoumarins, the therapeutic activities of such compounds are often associated with DNA. Furanochromones do undergo intercalation with DNA in the dark, but their photobinding is much less than that of psoralens [7,8]. In addition, VI and KH have been reported to be both type I and type II photosensitizers [4], and activated oxygen species, singlet oxygen ($^1O_2$) and superoxide radical anion (O_2^-), are formed in the interaction of VI (and KH to a lower extent) with DNA upon UV irradiation [9]. Borges et al. [10] attributed the larger photobiological activity of VI vs. KH to its relatively high quantum yield of triplet formation (Φ_T) in solvents with different polarity. However, Φ_T strongly decreases as the solvent polarity increases, it is lower than 0.05 in water for both compounds [10] and in methanol Φ_T is too low to be determined [11]. Though Trabalzini et al. [8] reported that no photosensitization of DNA was observed, Chen and Kagan [12] gave the results that extensive DNA cleavage was photo-induced by VI and KH in both the presence and absence of oxygen. This gives the possibility that electron transfer reactions involving their radical cations may be involved in the process of furanochromone photosensitization.

Recently, it has been reported that significant photoionization yields (Φ_I) were observed for psoralen and coumarin derivatives [13]. In particular, the radical cation of 8-methoxypsoralen (8-MOP), a widely used photomedicine, was generated in aqueous solution by 355- and 308-nm excitation via a monophotonic process, and a bimolecular rate constant of 2.5×10^9 dm^3 mol^{-1} s^{-1} was determined for the reaction of 8-MOP$^{\cdot+}$ with guanosine mononucleotide, which means that the radical cations of psoralens are of considerable importance in psoralen/UVA therapy [14]. Considering the structure similarity of furanochromone to furanocoumarin, in this paper, we studied the photochemistry properties of VI and KH by use of laser flash photolysis techniques, and the results suggest that their radical cations can be produced by photoionization and may be involved in furanochromone photochemotherapy.



VI : R = H

KH : R = OCH_3



8 - MOP

2. Materials and methods

2.1. Biological and chemical materials

VI and KH (all Aldrich products) were purified by recrystallization from *n*-hexane-methanol mixtures. dGMP, dAMP, dCMP, TMP and calf thy-

mus DNA were all obtained from Sigma and used as received. Single strand DNA (ssDNA) was prepared by heating calf thymus DNA in neutral aqueous solution at 90°C for 10 min following by chilling in an ice-salt bath. Benzophenone (BP) and sodium persulfate ($K_2S_2O_8$) (all analytic grade) were recrystallized from water–ethanol mixtures and triply distilled water, respectively. *Tert*-Butyl alcohol (*t*-BuOH) was distilled before use. All solvents were of the highest available commercial grade except the water, which was triply distilled. The samples were prepared in sodium phosphate buffer (pH 7.0) or in its mixture with acetonitrile, and purged with nitrogen or oxygen or nitrous oxide for 20 min in different experimental situation. All experiments were carried out at room temperature.

2.2. Methods

Laser flash photolysis experiments were performed using a home-made excimer laser which provided 308 nm (XeCl) pulses with a duration of 20 ns. The maximum energy was 40 mJ per pulse. The source of analyzing light was a 500-W xenon lamp, its intensity was increased approximately 100 times during the detection of transient absorption. The laser and analyzing light beam passed perpendicularly through a 10×10-mm quartz cell. The transmitted light entered an auto-monochromator equipped with a R955 photomultiplier. The signals were collected using a HP54510B 300-MHz transient recorder and then processed with a PC-486 personal computer. When the sample was prepared with 0.1-mM photosensitizer, the corresponding absorbance at 308 nm was approximately 0.39 for VI and 0.35 for KH. In the quantum yield experiments, the absorbance at 308 nm for VI, KH, 8-MOP and BP were all adjusted to 0.32.

Pulse radiolytic experiments were conducted using a linear accelerator providing 8 MeV, 8 ns electron pulse. Detailed descriptions of the equipment and experimental conditions have been described elsewhere [15]. In this work, the dose per electron pulse was approximately 10 Gy.

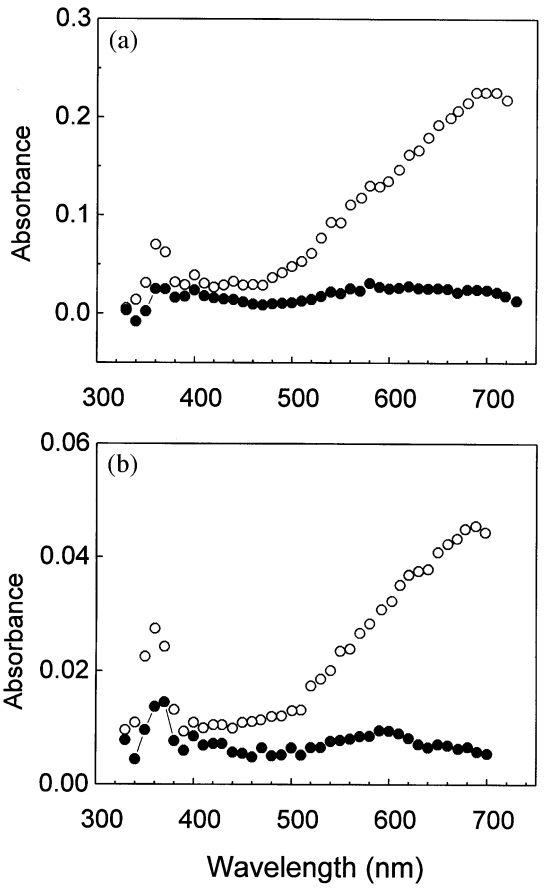


Fig. 1. Transient absorption spectra from 308-nm excitation of 0.1 mM photosensitizers in N_2-saturated aqueous solution at pH 7.0, (a) VI at delays of 0.1 μs (○) and 3 μs (●), (b) KH at delays of 0.1 μs (○) and 3 μs (●).

3. Results and discussion

3.1. Direct excitation of VI and KH

Direct 308-nm excitation of an N_2-saturated VI aqueous phosphate buffer solution (pH 7.0) gave rise to a transient absorption spectrum with maxima at 700 and 360 nm (Fig. 1a). The transient species with maximum absorption at 700 nm decayed fast with a rate constant of 4.3×10^6 s^{-1}, and was efficiently removed by N_2O or O_2 saturated in the solution, thus should be assigned to the solvated electron. The absorption spectra that remained after removal of the solvated electron

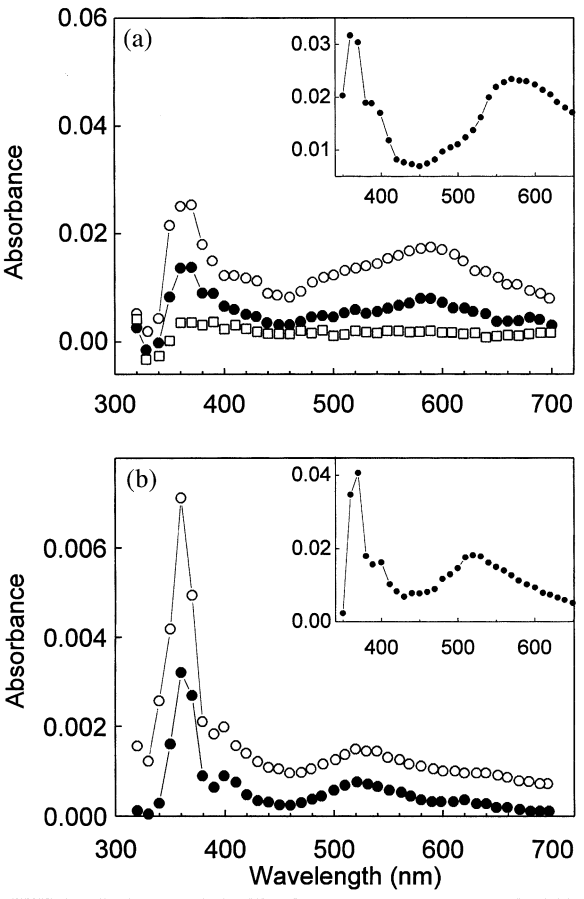


Fig. 2. Transient absorption spectra from 308-nm excitation of 0.1 mM photosensitizers in O_2-saturated acetonitrile/aqueous buffer mixtures, (a) VI at delays of 0.2 μs (○), 3 μs (●) and 12 μs ( ), (b) KH at delays of 0.2 μs (○) and 40 μs (●). Insets: characteristic absorption spectra of $VI^{\cdot+}$ and $KH^{\cdot+}$ obtained by pulse radiolysis (at 5 μs after the electron pulse) of 0.1 mM VI (a) and 0.1 mM KH (b) in air-saturated aqueous solution containing 0.1 M *t*-BuOH, 20 mM $K_2S_2O_8$.

were shown in Fig. 2 with absorption maxima at 580 and 360 nm and a shoulder at 390 nm. However, negligible triplet VI with low Φ_T in water reported in the literature [10] was involved since N_2O cannot quench triplet VI as oxygen does. In fact, similar spectra and decay kinetics were observed in O_2-saturated buffer/acetonitrile (4:1) mixtures (Fig. 2a). Under this condition, only $VI^{\cdot+}$ could be detected, because the triplet VI (if it existed) would be quenched by oxygen and the electron should be trapped by acetonitrile to give a dimer radical anion that did not absorb appreciably below 750 nm [16].

The VI radical anion ($VI^{\cdot-}$), whose absorption band is very similar to that of $VI^{\cdot+}$ confirmed by pulse radiolysis in ethanol (not shown), must be formed in N_2-saturated aqueous buffer, so the residual absorption after the solvated electron decay shown in Fig. 1a should be ascribed to the mixture of $VI^{\cdot+}$ and $VI^{\cdot-}$.

Photoionization was also observed by direct 308-nm excitation of KH in aqueous buffer and buffer/acetonitrile (4:1) mixtures and the results were similar to that of VI. In N_2-saturated aqueous phosphate buffer at pH 7.0, the transient absorption of solvated electron is visible at 690 nm with apparently smaller yield than that of VI under identical conditions (Fig. 1b). The residual absorption at approximately 600 nm after the solvated electron decay should be assigned to $KH^{\cdot-}$, which was also confirmed by pulse radiolysis. In O_2-saturated buffer/acetonitrile (4:1) mixtures, the transient absorption resulted from direct excitation should only be attributed to $KH^{\cdot+}$, for the similar reason as mentioned above.

3.2. Generation of radical cations by pulse radiolysis

The insets of Fig. 2 shows the transient absorption spectra after pulse radiolysis of the air-saturated aqueous solution containing 0.1 M *tert*-Butyl alcohol (*t*-BuOH), 20 mM $K_2S_2O_8$ and furanochromones. The composition of the aqueous solution ensures scavenge of OH radicals by *t*-BuOH and selective generation of strongly oxidizing radical $SO_4^{\cdot-}$ ($E = 2.5$–3.1 V vs. NHE) [17] via the following reaction:

$$e_{aq}^- + S_2O_8^{2-} \rightarrow SO_4^{\cdot-} + SO_4^{2-}$$

The $SO_4^{\cdot-}$ further single electron oxidizes furanochromones to give their radical cations. The spectra of $VI^{\cdot+}$ and $KH^{\cdot+}$ obtained by this method were identical to those obtained by laser flash photolysis in O_2-saturated buffer/acetonitrile (4:1) mixtures, which further demonstrated that VI and KH were photoionized by direct 308-nm excitation.

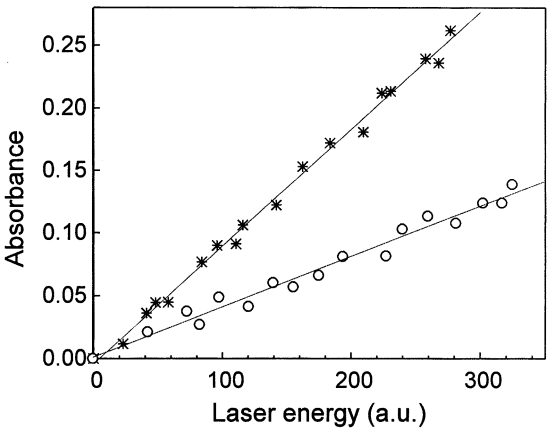


Fig. 3. Dependence on laser intensity of the absorbance at 700 nm 0.08 μs after the laser pulse: VI (*); 8-MOP (○).

3.3. Laser energy dependence and quantum yields of photoionization

Under the excitation of VI in N_2-saturated solutions with $\lambda_{ex} = 308$-nm laser, the absorption of solvated electron at 700 nm was determined by subtracting the decay profile obtained for an N_2O-saturated solution ($VI^{\cdot+}$ alone) from that obtained for an N_2-saturated solution ($VI^{\cdot+}$ and solvated electron) at the identical laser energy. The resulting profiles corresponded to the solvated electron alone. A plot of the relative laser energy vs. the amplitude of absorption (at 0.08 μs after the pulse) was found to be linear and to pass through zero (Fig. 3), which was an indication of monophotonic process.

The quantum yield of VI photoionization process was determined in a comparative experiment using benzophenone in benzene as the reference [$\Phi_T = 1$, ε_T (525 nm) = 7870 [18]]. Before laser excitation, the absorbance of benzophenone in benzene was adjusted to be identical ($A = 0.32$) to that of VI in phosphate buffer solution. The laser energy dependence of the benzophenone triplet absorption at 525 nm was compared to that of the solvated electron at 700 nm for VI. When the slopes of the two laser energy dependence plots and a value of 18 500 for ε (e^-_{aq}) [19] were substituted in Eq. (1), a value of 0.075 for Φ (e^-_{aq}) and hence, the quantum yield of photoionization (Φ_I), was obtained. For KH, the quantum yield of photoionization was too low to be accurately determined, and an upper limit of < 0.01 was estimated.

$$\Phi_I = \Phi\,(e^-_{aq}) = \left\{\text{slope}\,(e^-_{aq})/\text{slope(BP)} \times \left[\varepsilon_T^{BP}/\varepsilon\,(e^-_{aq})\right]\right\} \Phi_T^{BP} \quad (1)$$

Here Φ (e^-_{aq}) is the quantum yield of solvated electron, slope (e^-_{aq}) and slope (BP) are the laser energy dependence of the absorption of solvated electron and benzophenone triplet, respectively, ε_T^{BP} and ε (e^-_{aq}) are the extinction coefficients of benzophenone triplet and solvated electron, Φ_T^{BP} is the quantum yield of triplet benzophenone.

In order to make a comparison between VI and the widely used photo-medicine 8-MOP on photoionization, the laser energy dependence of the solvated electron absorption for an optically matched (at 308 nm) solution of 8-MOP in phosphate buffer was determined and shown in Fig. 3. From Eq. (1), Φ_I was calculated to be 0.03, much lower than that for VI. These results indicated that VI is much less stable than 8-MOP under light irradiation while KH showed reverse properties.

3.4. Interaction of furanochromone radical cations with DNA bases

Since DNA bases are the most important substrates in biological systems, the mononucleotides of the four bases were added as potential quenchers of $VI^{\cdot+}$ and $KH^{\cdot+}$. Experiments were carried out in O_2-saturated buffer/acetonitrile (4:1) mixtures, and the $VI^{\cdot+}$ and $KH^{\cdot+}$ were all generated by direct 308-nm photoionization. Enhancement of the $VI^{\cdot+}$ and $KH^{\cdot+}$ decay was observed in the presence of purine mononucleotides while pyrimidine mononucleotides had negligible influence on it.

Fig. 4a shows the transient absorption spectra after laser photolysis of the VI (0.1 mM) solution containing dGMP. On the basis of the concentrations and extinction coefficient of the reactants in the solution, the laser energy was absorbed pre-

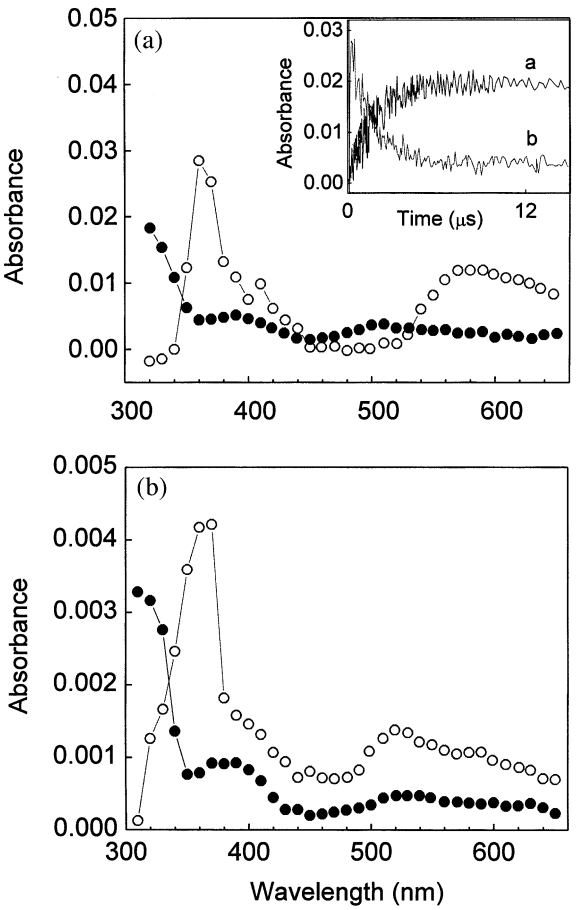


Fig. 4. Transient absorption spectra from 308-nm excitation of 0.1 mM photosensitizers in O_2-saturated acetonitrile/aqueous buffer mixtures containing dGMP, (a) VI and 0.2 mM dGMP at delays of 0.1 μs (○), 10 μs (●), (b) KH and 3 mM dGMP at delays of 0.1 μs (○) and 20 μs (●). Inset: growth and decay trace at 320 nm (a); and 580 nm (b).

dominantly by VI while dGMP had little absorption. Therefore, the transient species produced at 0.1 μs after the laser pulse should be assigned to $VI^{\cdot+}$, which were similar to that in the solution containing only VI. Accompanying the decay of $VI^{\cdot+}$, a new intermediate with absorption peaks at approximately 320 and 390 nm appeared. Owing to its similarity to the literature [20], it was assigned to the dGMP neutral radical, which was produced through one-electron oxidation of dGMP by $VI^{\cdot+}$ followed by deprotonation. As shown in the inset of Fig. 4a, the growth of the trace at 320 nm occurs exactly in the same time interval as the trace at 580-nm decay, which also indicates that $VI^{\cdot+}$ is the precursor of dGMP neutral radical. Through decay kinetic analysis of $VI^{\cdot+}$ at 580 nm at variations of dGMP concentrations (0.1–0.5 mM), the rate constant for the reaction was measured to be 1.2×10^9 dm^3 mol^{-1} s^{-1}, a value significantly higher than that for dAMP (Table 11.0×10^7 dm^3 mol^{-1} s^{-1}). The lower reactivity of dAMP toward oxidation by $VI^{\cdot+}$ is in good agreement with the reported reduction potentials of the neutral purine radicals of 1.29 and 1.42 V (vs. NHE) in aqueous solution [21]. The absorption of dAMP-derived radical after the quenching of $VI^{\cdot+}$ was very weak (spectra not shown), consistent with the lower absorption coefficient for purine radicals and the slower quenching rate constant for dAMP.

In the case of KH, quenching effect of dGMP on $KH^{\cdot+}$ was observed only at high concentrations of dGMP (> 1 mM), and at these conditions the laser energy would be partly absorbed by dGMP. However, nucleic acids and constituent nucleotides are adept at dispersing their absorbed photon energy through non-radiative processes (internal conversion), and this results in very low quantum yield of observable photophysical processes such as intersystem crossing [22,23]. On the other hand, monophotonic ionization of DNA bases by 308-nm excitation is thermodynamically unfeasible [23,24]. All these results indicated that the transient intermediates generated at 0.1 μs on photolysis of the solution containing KH and dGMP should be assigned to $KH^{\cdot+}$ (Fig. 4b). As time increases, the characteristic absorption spectra of deprotonated dGMP radical cation appeared, which resulted from one-electron oxidation of dGMP by $KH^{\cdot+}$. The rate constant for the

Table 1
Rate constants (10^8 dm^3 mol^{-1} s^{-1}) for reaction between $VI^{\cdot+}$ and purine mononucleotides and DNA

Substrate	dGMP	dAMP	ssDNA[a]	dsDNA[a]
k	12	0.1	2.3	1.1

[a]Rate constant per nucleotide unit.

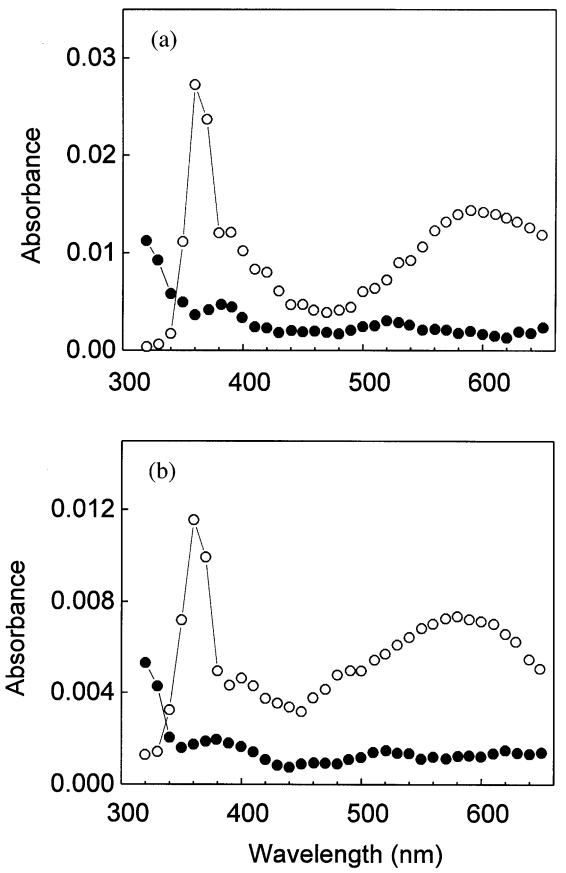


Fig. 5. Transient absorption spectra from 308-nm excitation of 0.1 mM VI in air-saturated acetonitrile/aqueous buffer mixtures containing (a) 2 mM ssDNA at delays of 0.1 μs (○), 8 μs (●), (b) 2 mM dsDNA at delays of 0.1 μs (○) and 10 μs (●).

electron transfer reaction was measured to be 3.8×10^7 dm^3 mol^{-1} s^{-1}.

3.5. Interaction of furanochromone radical cations with DNA

In order to make an investigation under conditions as in biological systems, experiments were carried out on the interaction of furanochromone radical cations with DNA. Our results indicate that ssDNA and dsDNA had no significant influence on the decay of $KH^{\cdot+}$, up to concentrations of 3 mM. However, the decay of $VI^{\cdot+}$ was enhanced by addition of both of them.

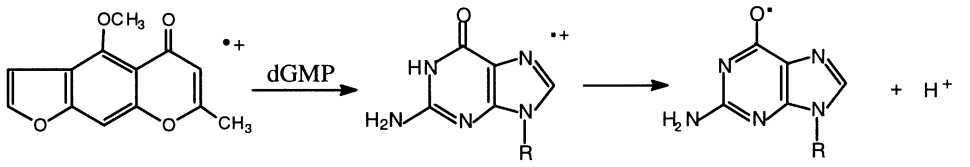
As shown in Fig. 5, the absorption spectra produced immediately after the laser pulse (0.1 μs) were ascribed to $VI^{\cdot+}$, subsequently, the transient species appeared after $VI^{\cdot+}$ decay in Fig. 5a,b should be from ssDNA and dsDNA, respectively. On the basis of the similarity of the transient absorption spectra of the radical species produced from the interaction of $VI^{\cdot+}$ with ssDNA and dsDNA to that of dGMP, and the fact that guanine is more powerful in quenching $VI^{\cdot+}$ than other bases, it is concluded that the predominant species produced in ssDNA and dsDNA are guanine radicals via electron transfer reaction.

Though furanochromones undergo intercalation (weak) with DNA [8], the dependence of the apparent decay rate constants (k_{obs}) of $VI^{\cdot+}$ on concentrations of ssDNA and dsDNA was linear, which indicated that $VI^{\cdot+}$ were mainly free in solution, not in complex with DNA. The bimolecular rate constants of the electron transfer reactions were also determined (see Table 1).

4. Conclusions

The present work provides the first detailed characterization of the radical cations of VI and KH. Of particular importance, direct excitation of VI in aqueous solution results in photoionization to give $VI^{\cdot+}$ via monophotonic process ($\Phi_I = 0.075$), a likely occurrence under clinical conditions. On the basis of comparison experiments under identical conditions, the order of stability of the photosensitizers VI, KH and 8-MOP under light irradiation is determined as VI < 8-MOP < KH. This finding is of considerable significance since the relatively stable 8-MOP (compared with VI) undergoes monophotonic ionization under UVA irradiation [14].

By use of laser flash photolysis techniques, time-resolved spectroscopic and kinetic evidence is presented for the reaction of $VI^{\cdot+}$ and $KH^{\cdot+}$ with purine bases. The reaction with dGMP proceeds via an electron transfer mechanism with rate constants of 1.2×10^9 and 3.8×10^7 dm^3 mol^{-1} s^{-1}, respectively, much faster than with other DNA bases. The subsequently produced



dGMP radical cation ($dGMP^{+}$) will deprotonate rapidly to give dGMP neutral radical, which has a pK_a of 3.9 [25]. The mechanism can be described as follows ($VI^{\cdot+}$ is used as an example):

In the interaction of $VI^{\cdot+}$ with ssDNA and dsDNA, selective oxidation of guanine to generate a guanine-derived radical is observed, consistent with the fact that guanine is the most easily oxidized of the nucleobases [21,26]. These results indicate that photo-irradiation of furanochromones, especially VI, can lead to generation of DNA radicals that results in strand breaks, consistent with previous observations of extensive strand breaks upon irradiation of VI and KH in DNA solution [12], and have obvious implications for understanding the mode of furanochromone photodynamic action. Further experiments on the interaction of furanochromone radical cations with amino acids and model proteins are in progress.

Acknowledgements

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