Journal of Photochemistry and Photobiology A: Chemistry 201 (2009) 197–202

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

journal homepage: www.elsevier.com/locate/jphotochem

Role of sugar in controlling reaction pattern: A comparative study with adenine and 2′-deoxyadenosine

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ARTICLE INFO

Article history:
Received 18 June 2008
Received in revised form 22 October 2008
Accepted 29 October 2008
Available online 6 November 2008

Keywords:
2-Methyl-1,4-naphthoquinone
Adenine
2′-Deoxyadenosine
Electron transfer
Hydrogen abstraction

ABSTRACT

Interaction between 2-methyl-1,4-naphthoquinone (MQ) and adenine (A) and 2′-deoxyadenosine (dA) reveals interesting differences with respect to electron transfer (ET) and hydrogen abstraction (HA). In our case A and dA exhibited ET from different sites, which has been associated to the presence of a sugar unit in dA. Sugar unit has also reduced the rate of ET from dA. We have utilized an external magnetic field in conjunction with our laser flash photolysis set-up to establish our data. In a polar MeCN/H_2O medium A and dA are found to promote ET but HA from dA is seen to be insignificant. However shifting to a heterogeneous micellar medium has been found to have a marked effect in increasing the HA rate for dA only. Separate experiments with 2′-deoxyribose sugar have revealed sugar moiety to be more susceptible to HA in SDS medium.

1. Introduction

The quinones are extremely important group of compounds especially in biological systems because when they are attached to proteins can act as prosthetic groups involved in electron and hydrogen transfers [1,2]. In our studies we have chosen 2-methyl-1,4-naphthoquinone (MQ) well known for its anticancer activity. We are currently interested in the study of 9,10-anthraquinone (AQ) and 2-methyl-1,4-naphthoquinone (menadione, MQ) molecules, which are basically model drugs with all the DNA bases and their nucleosides [3,4] in an effort to understand their individual behavior pertaining to electron transfer (ET). We hope these studies will be beneficial in predicting the photochemical behavior of the drugs with DNA bases and ultimately with the DNA molecule as a whole. In this work we have highlighted a detailed investigation on the interaction of a DNA base, adenine (A) and also its corresponding nucleoside, 2′-deoxyadenosine (dA), which serve as electron and hydrogen (H) donors, with MQ, the electron and H acceptor during ET and hydrogen abstraction (HA) respectively. We are mainly interested to find any change in the ET/HA phenomenon between the molecules in different environments. Hence we have utilized two categories of solvents, a homogeneous one composed of acetonitrile/water (MeCN/H_2O, 4:1, v/v) and a heterogeneous SDS micellar medium. The ET and HA reactions produce radical pairs/radical ion pairs (RPs/RIPs). Micelles provide a cage environment for these RPs/RIPs where random encounter gets reduced. In micellar medium we have applied an external magnetic field (MF) for proper detection of RPs/RIPs. Magnetic field effect (MFE) can be utilized only in those reactions where RPs/RIPs are formed. Now if either of the reaction occurred when the photosensitizer (MQ) is in the triplet state the initially formed RPs/RIPs will be in the triplet state too. Similar situation prevails for singlet state. The MFE is basically interplay between spin dynamics and diffusion dynamics. By diffusion the RPs/RIPs can separate to an optimum distance where the exchange interaction $J \approx 0$ but spin correlation is maintained. In this situation, the electron–nuclear hyperfine coupling induces efficient mixing between the triplet ($T_{\pm}$, T_0) and the singlet (S) states. At zero fields, all triplet sublevels undergo electron–nuclear hyperfine-induced intersystem crossing (ISC) to S. In a weak magnetic field where T_+, and T_- are splitted away from each other on account of Zeeman splitting, $T_{\pm}$–S ISC cannot occur. Since T_0 still remains degenerate with S, with increasing magnetic field, the ISC may show a remarkable decrease in the lower field. These result in an increase of the population of RPs/RIPs with the initial spin state. So an enhancement in absorbance value on application of external magnetic field coupled with laser flash photolysis suggests an ET or HA from a triplet precursor generating a triplet RP/RIP. Thus MF

Abbreviations: MQ, 2-methyl-1,4-naphthoquinone; A, adenine; dA, 2′-deoxyadenosine; ISC, intersystem crossing; MF, magnetic field; MFE, magnetic field effect; S, singlet; T, triplet; ET, electron transfer; RP, radical pair; RIP, radical ion pair; SDS, sodium dodecyl sulphate; MeCN, acetonitrile; H_2O, water; R–H, H–$CH_2(CH_2)_{11}OSO_3Na$.

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doi:10.1016/j.jphotochem.2008.10.017

helps in the identification of an existence of RP/RIP on one hand and the initial spin state of the transients on the other [5–8]. We have thus utilized MFE as an effective tool for the proper identification of intermediates out of several possibilities. This is then utilized in the elucidation of reaction mechanisms between reactants of our choice.

1.1. Reaction scheme and the spin dependent phenomenon

The reaction scheme of the present system is as follows:

$$Q \xrightarrow{h\nu} {}^1Q^* \xrightarrow{ISC} {}^3Q \quad (1)$$

$${}^3Q + RH(SDS) \rightarrow {}^3(QH^{\bullet\bullet}R)(Habstraction) \quad (2)$$

$${}^3(QH^{\bullet\bullet}R) \underset{MF}{\overset{HFI}{\longleftrightarrow}} {}^1(QH^{\bullet\bullet}R) \quad (3)$$

$${}^3(QH^{\bullet\bullet}R)MF \rightarrow QH^{\bullet} + R^{\bullet}(escapeproduct) \quad (4)$$

$${}^1(QH^{\bullet\bullet}R) \rightarrow QH - R(cageproduct) \quad (5)$$

$${}^3Q + D \rightarrow {}^3(Q^{\bullet-}D^{\bullet+})(ET) \quad (6)$$

$${}^3(Q.^-D.^+) \underset{MF}{\overset{HFI}{\longleftrightarrow}} {}^1(Q.^-D.^+) \quad (7)$$

$${}^3(Q^{\bullet-}D^{\bullet+}) \rightarrow Q^{\bullet-} + D^{\bullet+}(escape\ product) \quad (8)$$

Following steps of reactions are for HA in case of bases with quinones:

$${}^3Q^* + D \rightarrow {}^3[QH^{\bullet\bullet}D(-H)](Habstraction) \quad (9)$$

$${}^3[QH \cdot \cdot D(-H)] \underset{MF}{\overset{HFI}{\longleftrightarrow}} {}^1[QH \cdot \cdot D(-H)] \quad (10)$$

The excited triplet state of quinone, ^3Q, is produced via the excited singlet state through ISC. It abstracts a hydrogen atom from a sodium dodecyl sulphate (SDS/RH) molecule (or any suitable H donor) to produce a spin-correlated RP in the triplet state, $^3(QH^{\bullet\bullet}R)$, which consists of a semiquinone and a dehydrogenated SDS radical. The ISC from the triplet pair $^3(QH^{\bullet\bullet}R)$ to the singlet pair $^1(QH^{\bullet\bullet}R)$ and the escape of component radicals into free radicals take place competitively. The singlet geminate RPs disappear due to cage reaction and the triplet RPs decay by diffusion of the radicals out of the micelle. Similar phenomena occur during ET.

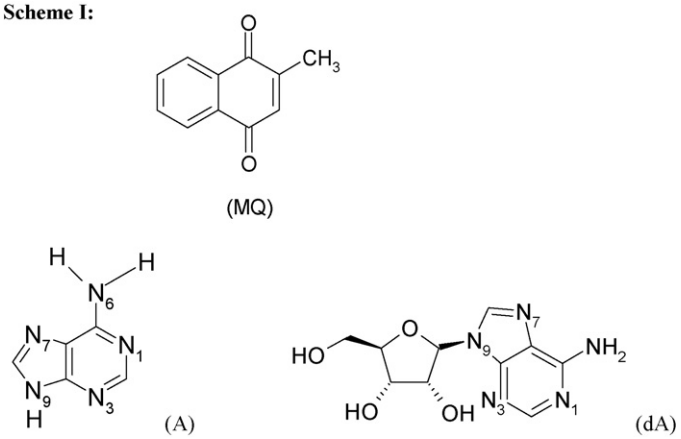
In our earlier work [9] we investigated the medium effect of dA in its interaction with MQ in pure MeCN and SDS micelles. There we showed that in MeCN, ET dominates whereas in SDS, it gets completely eclipsed by HA. In this work, we have utilized these results in understanding the differential behavior of A and dA with MQ in a more polar medium, MeCN/H_2O and SDS again. Here we have found how an additional sugar moiety becomes crucial in altering the reactivity of these molecules in different categories of media.

2. Experimental

2.1. Materials

2-Methyl-1,4-naphthoquinone (MQ), adenine (A), 2′-deoxyadenosine (dA) and sodium dodecyl sulphate (SDS) were purchased from Sigma. UV spectroscopy grade acetonitrile (MeCN) was obtained from Spectrochem and used without further purification. Water used for preparation of solutions was triply distilled. All micellar solutions were made by sonication. Chemical structures of the molecules used in this work are shown in Scheme 1.

Scheme I:



Scheme 1. Structures of molecules.

2.2. Spectral methods

The excitation light was the third harmonic (355 nm) of a Nd:YAG laser (DCR-11, Spectra Physics) with duration of 8 ns. The analyzing light was from a 250 W xenon lamp. The laser and analyzing light beams, crossed at right angles, passed through a quartz cell with 1 cm^2 cross-section. A monochromator equipped with an IP28 photo-multiplier was used to analyze transient absorption (Applied Photophysics). The signals from the photo-multiplier were displayed and recorded as a function of time on a Tektronix 500 MHz (1 Gs/s sampling rate) oscilloscope. Each data point was obtained with multi-times average to improve the signal-to-noise ratio. The transient absorption were obtained from a series of oscilloscope traces measured with the same solution in a point-by-point manner with respect to the wavelength using the Origin 5.0 software. The samples were deaerated by passing pure argon gas for 20 min prior to each experiment. No degradation of the samples was observed during the experiments. The MF effect (0.08 Tesla) on the transient spectra has been studied by passing direct current through a pair of electromagnetic coils placed inside the sample chamber.

3. Results and discussion

3.1. Triplet and radical absorption spectra

3.1.1. MQ with adenine and 2′-deoxyadenosine in acetonitrile/water

The transient optical absorption spectra obtained on irradiating 0.4 mM MQ separately and in presence of purine base A (5 mM) and its corresponding nucleoside dA (5 mM) dissolved in MeCN/H_2O (4:1, v/v) 1 μs after laser flash at 355 nm are displayed in Fig. 1. Irradiation of MQ alone generates a peak around 370 nm, which has been assigned to triplet–triplet absorption of ^{3}MQ [3,9,10]. Addition of DNA bases, A and dA have resulted in a much stronger absorption around 370 nm and a broad peak around 500 nm region. The peak due to A (curve 2) also shows a hump around 410 nm. Now an ET from bases to MQ will result in formation of MQ radical anion, $MQ^{\bullet-}$ which is known to absorb around 380 nm with a small peak around 480–490 nm [3,10]. So the strong absorption maxima at 380 nm and the broad peak at 500 nm can be associated to $MQ^{\bullet-}$, generated on ET from DNA base to the quinone. Further support of ET comes from an observation of a concomitant radical cation from the electron donor, the DNA base. Literature values suggest radical cation from A/dA to absorb at 360 nm [11,12]. So the combined effect of radical cation absorption at 360 nm and radical anion at

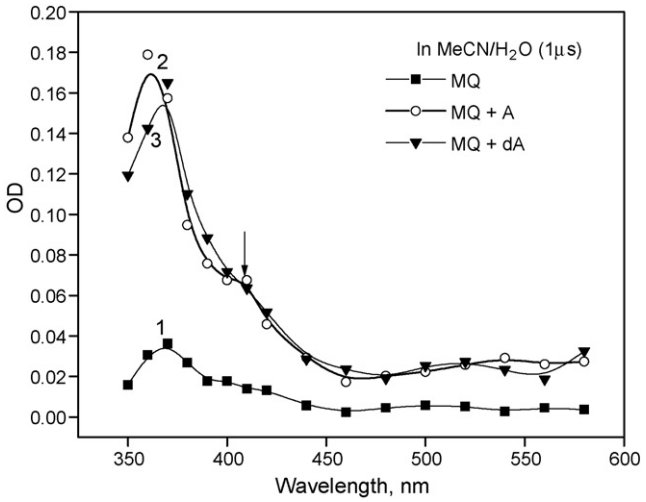


Fig. 1. Transient absorption spectra of (1) MQ (0.4 mM) (■), (2) MQ (0.4 mM)–A (5.0 mM) (○), and (3) MQ (0.4 mM)–dA (5.0 mM) (▼) at 1.0 μs time delay after laser pulse with excitation wavelength 355 nm in MeCN/H_2O (4:1, v/v).

380–390 nm will give a resultant peak near 370 nm, which is indeed our case. Confirmatory support of RIP formation is obtained from observation of growth curves at corresponding wavelengths. Fig. 2 displays the growth profile obtained at 390 nm on addition of A while Fig. 3 gives the growth curve at 410 nm. This growth profiles at 390 and 410 nm followed by a slow decay indicates the formation of a stable species with a long lifetime [13]. Observation of such growth curves is the most convincing proof of formation of new species at the specified wavelengths. Inset to Fig. 3, shows the decay curve at 370 nm. Absence of growth at 370 nm points towards the non-existence of RIPs at that wavelength. So ET is found to be a dominant reaction pathway between the purine base A and its corresponding nucleoside dA with MQ. The hump at 410 nm from A (curve 2) is associated with a semiquinone formation, as no radical cation/anion is known to absorb there [3,10]. Semiquinone formation of MQ ($MQH^{\bullet}$) is possible on H atom abstraction from a suitable donor by quinone molecules after an initial H bonding between them. Now H_2O in medium and DNA base can serve as potent H donors. Absence of similar 410 nm peak in case of dA points to a weaker HA from dA in MeCN/H_2O medium. Absence of $MQH^{\bullet}$ peak in case of MQ alone eliminates the possibility of H_2O serving as H donor. So we conclude that A can participate in both ET and HA in

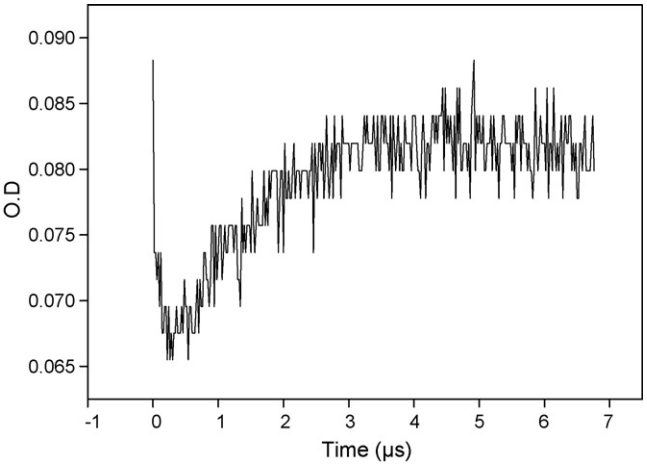


Fig. 2. Normalized OD traces at 390 nm obtained by laser flash photolysis (λ = 355 nm) of MQ (0.4 mM) and A (5.0 mM).

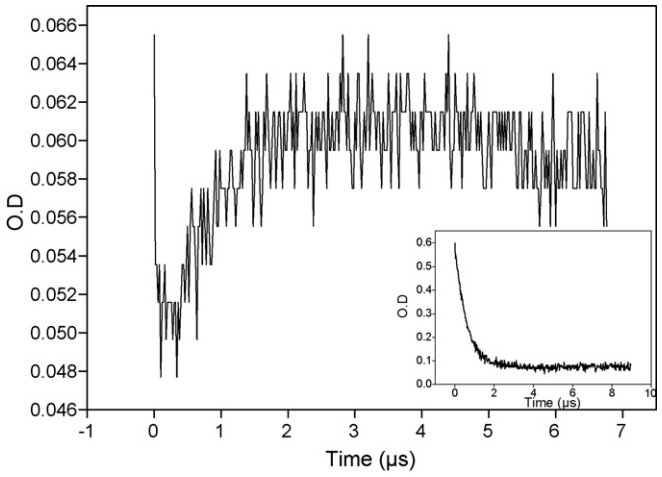


Fig. 3. Normalized OD traces at 410 nm obtained by laser flash photolysis (λ = 355 nm) of MQ (0.4 mM) and A (5.0 mM). Inset: normalized OD traces at 370 nm obtained by laser flash photolysis of MQ (0.4 mM) and A (5.0 mM).

MeCN/H_2O while dA, the nucleoside is more prone towards ET than HA in this medium. A mere glance at curves 2 and 3 shows dA to have a lower intensity peak than A. This can be attributed to a lower rate of reaction of dA than A.

3.1.2. *MQ with adenine and 2′-deoxyadenosine in micelles*

Fig. 4 displays the transient absorption spectra of 0.4 mM MQ in absence and presence of 5 mM of A in 5% SDS medium after 1 μs of laser flash. Broad absorption peaks around 380–390 nm and small humps around 500 nm are obtained. Curve 1 reveals features pertaining to behavior of MQ alone in SDS. Curve 2 depicts the effect on application of an external MF. An enhancement at 370 and 410 nm is a clear indication of a radical which has been earlier assigned to be $MQH^{\bullet}$ [9]. $MQH^{\bullet}$ is formed upon HA by MQ from a nearby SDS molecule. Addition of A has resulted in a further increment of peak intensity at 380 nm with a second peak at 500 nm. Both these regions have exhibited appreciable field effect. So existence of RIPs, formed through a possible ET between A and MQ, is justified. Fig. 5 displays the transient absorption spectra of 0.4 mM MQ in absence and presence of 5 mM dA in 5% SDS medium after

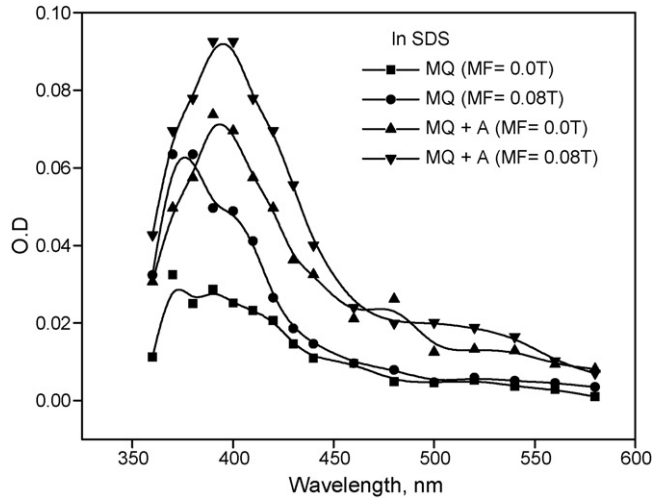


Fig. 4. Transient absorption spectra of MQ (0.4 mM) (1) in absence (■) and (2) presence of magnetic field (●), MQ (0.4 mM)–A (5.0 mM) in (3) absence (▲) and (4) presence of magnetic field (▼) at a delay of 1.0 μs in SDS micelles.

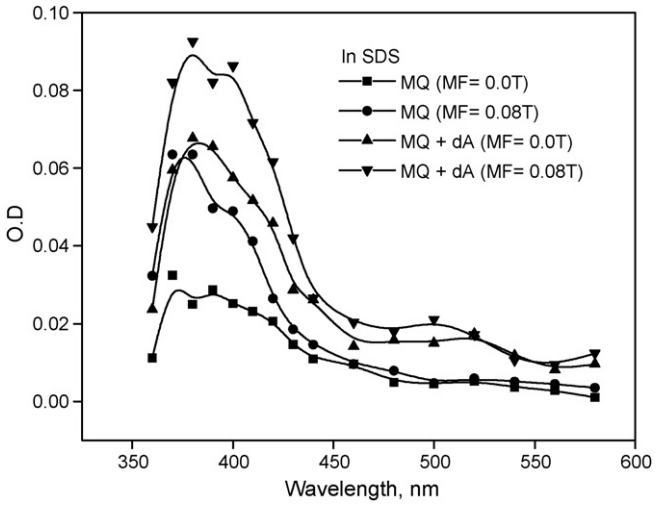


Fig. 5. Transient absorption spectra of MQ (0.4 mM) (1) in absence (■) and (2) presence of magnetic field (●), MQ (0.4 mM)–dA (5.0 mM) in (3) absence (▲) and (4) presence of magnetic field (▼) at a delay of 1.0 μs in SDS micelles.

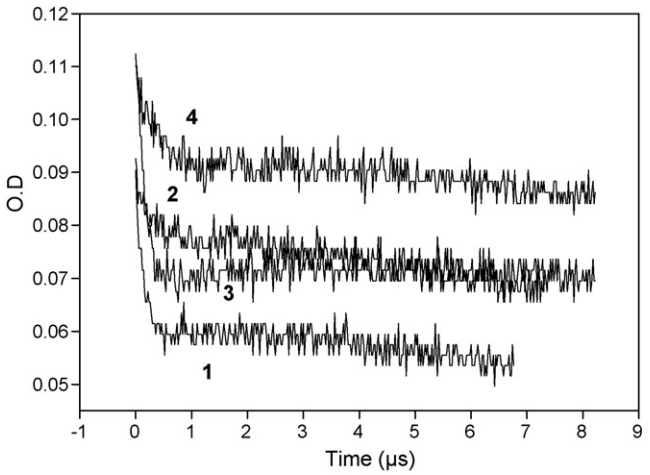


Fig. 7. Normalized OD traces at 380 nm obtained by laser flash photolysis (λ = 355 nm) of MQ (0.4 mM) and A (5.0 mM) in SDS in (1) absence and (2) presence of magnetic field, MQ (0.4 mM) and dA (5.0 mM) in (3) absence and (4) presence of magnetic field.

1 μs of laser flash. Here addition of dA is seen to result in a broader peak ranging from 370 to 400 nm regions. A second peak around 500 nm is also present. The peak due to A is sharper with λ_{max} at 380 nm while the one due to dA is found to be much broad. This is attributed to a higher concentration of $MQH^{\bullet}$ with λ_{max} at 370 nm, in case of dA. So dA seems to generate $MQH^{\bullet}$ more than A in SDS. So we find, change in medium from MeCN/H_2O to SDS has induced more HA from dA. Fig. 6 depicts the transient absorption spectrum of MQ in presence of A and dA separately in SDS. Similar to that in MeCN/H_2O, dA is again found to have a lower peak intensity than A. Now we have already confirmed dA to exhibit a higher HA in SDS. So the lower reactivity of dA than A irrespective of medium is associated with a reduced ET.

3.1.3. Magnetic field effect

In the presence of an external magnetic field, the decay of the transients at 380 nm becomes slower (Fig. 7) accompanied by an enhanced absorption in the spectrum (Figs. 4 and 5). The formation of a spin correlated radical pair ($^3MQH^{\bullet\bullet}R$) explains this MFE.

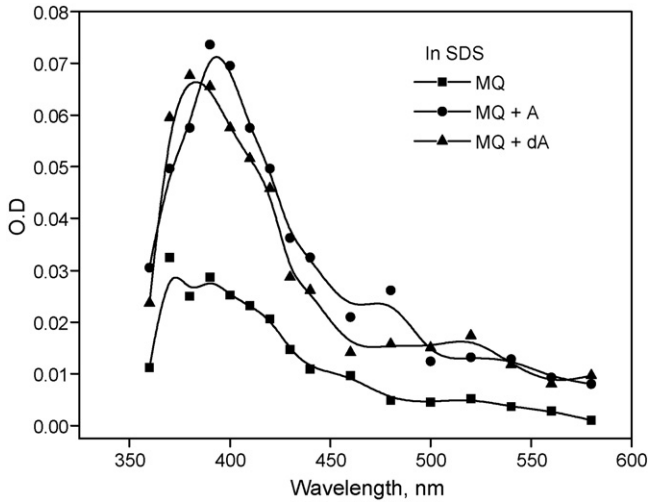


Fig. 6. Transient absorption spectra of (1) MQ (0.4 mM) (■), (2) MQ (0.4 mM)–A (5.0 mM) (●), (3) MQ (0.4 mM)–dA (5.0 mM) (▲) at 1.0 μs time delay after laser pulse with excitation wavelength 355 nm in SDS micelles.

It is noteworthy that the nature of the decay profiles (at 380 nm) is different in presence of both A and dA. This implies that the transients formed in the presence of A and dA behave differently with MF. We believe this difference must have arisen due to presence of different species from A and dA. Although both A and dA produce similar species $MQH^{\bullet}$, $MQ^{\bullet-}$ and $A/dA^{\bullet+}$, yet the yield of RPs/RIPs, specially $MQH^{\bullet}$ and $MQ^{\bullet-}$, are different in SDS. In SDS, dA produces more $MQH^{\bullet}$ than A. This is reflected in the decay curves. Hence MFE also provides indirect evidence for the differential reactivity of A and dA.

3.2. Mechanism of action

Closer inspection of Fig. 1 reveals a slight shifting in the λ_{max} of the MQ-A and MQ-dA peak around 380 nm. They are generated by superposition of radical anion and radical cation peaks. Since the radical anion ($MQ^{\bullet-}$) will be the same for both the bases A and dA so the shifting has been associated to two different radical cation formations from dA and A. For further evidence, we have investigated the independent time-resolved transient absorption spectra in different time delays as shown in Figs. 8 and 9. In every case, a shifting is evident. Song et al. in their work with guanine, guanosine had observed similar phenomenon. They had associated the shifting to stem from different sites of deprotonation of the same base unit [14]. We think our system also behaved in a similar way. Electrons of the nitrogen of the five-member imidazole ring (N7, N9) are involved in maintaining ring aromaticity so are probably not involved in ET with MQ. While nitrogen of the six member pyrimidine ring (N1, N3) can donate their electrons during ET, as these electrons are not involved in maintaining ring aromaticity [15]. Rodgers and Armentrout have compared the electron donating potential of N1 and N3 in adenine (A). Their studies have revealed a better stabilization of metal-adenine complex when it is N3 centered rather than N1. They have associated it to the umbrella motion of the NH_2 (amino) moiety [16]. Hence in our case we think a facile ET will occur from N3 of A to MQ. But in dA, the neighboring larger sugar moiety hinders a smooth ET from N3. Thus the only possible alternative is ET from N1 in dA. Moreover N6 electrons are delocalised with the ring, which results in a decrease in their basicity [15] so their involvement in ET should be insignificant. Thus we believe A produces a N3 centered radical cation while dA produces an N1 centered one. Again the purine ring also possesses π

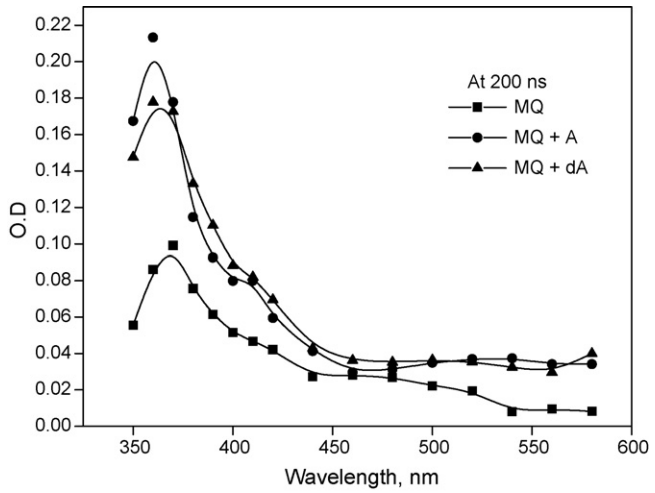


Fig. 8. Transient absorption spectra of (1) MQ (0.4 mM) (■), (2) MQ (0.4 mM)-A (5.0 mM) (●), and (3) MQ (0.4 mM)–dA (5.0 mM) (▲) at 200 ns time delay after laser pulse with excitation wavelength 355 nm in MeCN/H_2O (4:1, v/v).

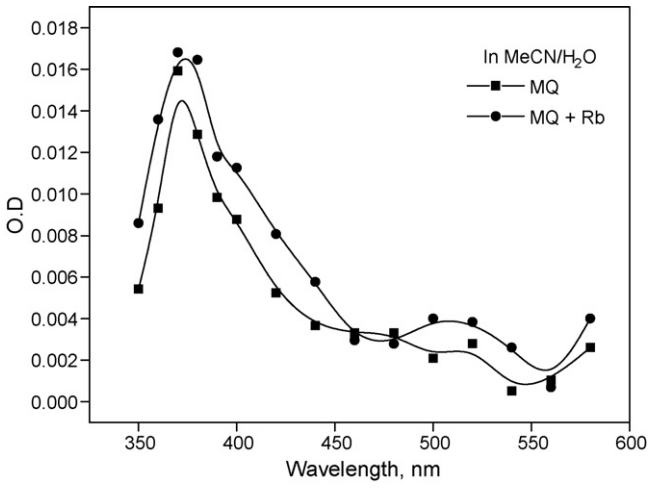


Fig. 10. Transient absorption spectra of (1) MQ (0.4 mM) (■), (2) MQ (0.4 mM)-Rb (5.0 mM) (●) at 1.0 μs time delay after laser pulse with excitation wavelength 355 nm in MeCN/H_2O (4:1, v/v).

electrons, which are responsible for its aromaticity. Now these electrons might be transferred during ET. If ET has been due to these π electrons then both A and dA would have shown exactly similar spectra (excepting the peak height). However this has not been so. Therefore π electrons of purine are probably not involved in ET. Cysewski et al. have reported that aromaticity of purines are greater than pyrimidines, so the non-involvement of π electrons of purines during ET can be logically associated to its higher aromaticity [17]. Therefore we can conclude that the two pyrimidine nitrogen (N1 and N3) are involved in the donation of electron density to MQ during ET. Thus presence of sugar moiety in dA alters the ET site. Addition of sugar moiety to A is seen to decrease the rate of reaction for MQ in both media. Steenken has reported a drop in reaction rate on going from A to dA by electrophilic $SO_4^{\bullet-}$ [18] which he has considered to be due to a fall in electron density due to replacement of H at N9 by electron-withdrawing ribose unit. Hence we find addition of sugar unit is responsible for reducing ET from a nucleoside by pulling away electrons from the base ring.

We have noticed a weaker HA in case of dA with MQ in MeCN/H_2O, which is surprisingly found to become quite significant in SDS. On the other hand, behavior of A has found to remain almost unperturbed on medium change. In order to understand the reason of such differences, we have performed separate laser flash photolysis experiments with 2′-deoxyribose (Rb) with MQ in similar media. Fig. 10 displays the effect of Rb on MQ in MeCN/H_2O while Fig. 11 displays the behavior of same pair of molecules in SDS. From these figures it is very much evident that, introduction of molecules in SDS results in a higher production of $MQH^{\bullet}$ with peak at 370 nm and a shoulder at 410 nm. Thus we find that Rb undergoes H atom transfer to MQ, which is reported to be possible by earlier workers also [19–21], and it becomes the dominant mode in SDS. Hence, dA favors HA more in SDS than MeCN/H_2O. Now the question arises as to why the micellar medium promotes HA better than MeCN/H_2O medium. Nowick et al. emphasizes that introduction of molecules within micelles in aqueous solution results in better hydrogen bonding between them as these molecules get shielded from hydrogen bonds from water [22]. We have earlier reported [3,10] that SDS medium promotes H atom transfer on account of close sequestering of participant molecules which leads to an initial

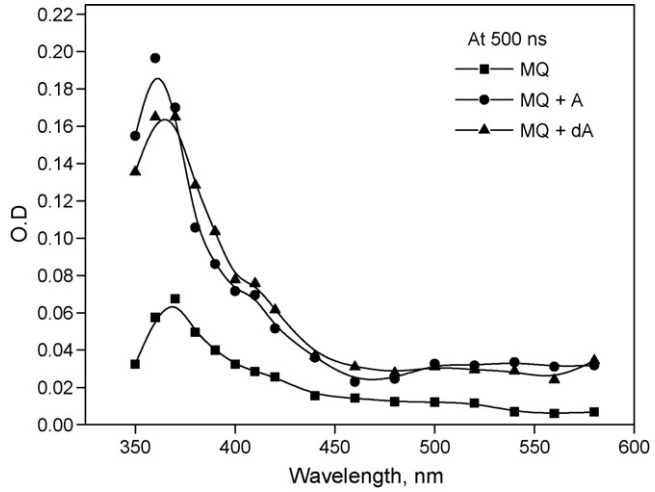


Fig. 9. Transient absorption spectra of (1) MQ (0.4 mM) (■), (2) MQ (0.4 mM)-A (5.0 mM) (●), and (3) MQ (0.4 mM)–dA (5.0 mM) (▲) at 500 ns time delay after laser pulse with excitation wavelength 355 nm in MeCN/H_2O (4:1, v/v).

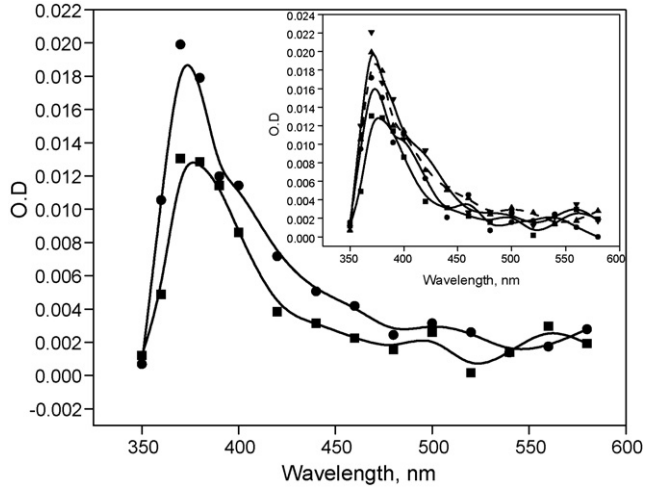


Fig. 11. Transient absorption spectra of (1) MQ (0.4 mM) (■), (2) MQ (0.4 mM)-Rb (5.0 mM) (●) at 1.0 μs time delay after laser pulse with excitation wavelength 355 nm in 5% SDS. Inset: transient absorption spectra of MQ (0.4 mM) (1) in absence (■) and (2) presence of magnetic field (●), MQ (0.4 mM)–Rb (5.0 mM) in (3) absence (▲) and (4) presence of magnetic field (▼) at a delay of 1.0 μs in SDS micelles.

hydrogen bonding type interaction and a resultant H atom transfer from donor to acceptor molecules. This type of interaction is weaker in homogeneous organic medium where due to random motion, close proximity between reactants at a particular time seldom happens. Thus ET will be the dominating pathway here with or without HA depending on nature of the reacting species. Closeness among the reactants has favored HA from dA in SDS while it is almost absent in MeCN/H_2O.

4. Conclusion

We have illustrated a detailed analysis of the reaction between MQ and A as well as dA in both homogeneous and heterogeneous media. Additional sugar unit in dA alters its chemical properties and rate of reaction in comparison to A in both the media. In MeCN/H_2O, A is found to favor both ET and HA while dA predominantly supports ET. Here A and dA are found to have different reaction rates and also different sites of ET. But in SDS, entrapment of molecules in a cage environment is responsible for a dominant HA from the sugar moiety of dA, which is responsible for a competitive HA and ET from dA in SDS. Thus we have shown that mere presence of an extra sugar moiety in dA can change its rate and reactivity with a quinone, MQ, in comparison to that of the bare nucleobase, A.

Acknowledgement

We sincerely thank Mrs. Chitra Raha for her kind assistance and technical support.

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