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Interaction of 9,10-anthraquinone with adenine and 2′-deoxyadenosine

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ABSTRACT

Laser flash photolysis has been used for the study of the interaction of 9,10-anthraquinone (AQ) with the DNA base, adenine (A) and its corresponding nucleoside, 2′-deoxyadenosine (dA). This study has provided two very important observations. AQ has been found to support electron transfer in different categories of media, acetonitrile/water on one hand and SDS micelles on other. While in our earlier work 2-methyl 1,4-naphthoquinone was found to undergo a switchover in reactivity (J. Am. Chem. Soc. 126 (2004) 10589–10593). Again A and dA are found to behave differently on account of an extra sugar unit, which not only affects the rate of reaction but the reaction pathway has been found to be modified too.

1. Introduction

9,10-anthraquinone (AQ) derivatives represent one of the most important classes of anticancer agents. For example, mitoxantrone (1, 4-dihydroxy-5, 8-bis [[2-[(2-hydroxy-ethyl) amino] ethyl] amino]-9, 10-anthraquinone) is now licensed for clinical use in a number of countries against acute leukemia [1]. Anthraquinone compounds are good electron transfer (ET) agents due to the presence of the electron-accepting quinone group. Breslin et al. have shown that photo excited AQ (^{3}AQ*) is responsible for DNA damage through ET from DNA base [2] and Bergeron et al. have observed UV-induced cross-links in anthraquinone–DNA duplexes [3]. DNA is well known to be sensitive to UV radiations, which cause the formation of pyrimidine dimers by direct photoexcitation, or indirectly, various effects by the interaction of DNA in the ground state with another excited molecule. Anthraquinone embedded in DNA has been extensively used as a photosensitizer to study a variety of photoinduced damages, as alkaline labile breaks, interstrand cross-links, damages at all four DNA bases and relaxation of supercoiling circular DNA, which demonstrate the reactivity of base radicals imbedded in DNA duplexes. So there are some reports on direct interaction of AQ with the DNA molecule. There are also some reports on interaction of AQ derivatives with individual DNA bases using laser flash photolysis [4–8] and thus ET is an established reaction between these AQ derivatives and the bases. In connection to these photochemical effects it is relevant to find out the role of electron transfer in drug–DNA interactions and of the H atom transfer in antioxidant–DNA interaction. We are currently interested in the study of 9,10-anthraquinone (AQ) and 2-methyl 1,4-naphthoquinone (menadione, MQ) molecules with all the DNA bases and their nucleosides [9–11] in an effort to understand their individual behavior pertaining to 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. Although there have been several reports on quinone–DNA interaction using laser flash photolysis [2,12–16] but till now to the best of our knowledge, there has been no report on the effect of medium on the interaction of these molecules with quinones. In our earlier work we have shown this effect on MQ. A study of the medium dependence is important in understanding the effect of environment on the action of a potential drug with biological molecules. This work aims in unraveling the change in reactivity of the bare AQ molecule in its interaction with the DNA base adenine (A) and its nucleoside 2′-deoxyadenosine (dA) on changing media from homogeneous one (ACN/H_2O, 4:1, v/v) to a heterogeneous (SDS micellar) one. Micelles formed by surfactant molecules serve as simple membrane mimetic systems that allow a controlled study of the effect of confined medium on the interaction of different molecules. In this work we have attempted to provide the molecular details of the reactions of the quinone molecules with the bases right from the initiation of their interaction. We have explained the course of their reaction, which has been undergoing alteration on changing reaction media. In this connection we have applied an external magnetic field (MF) in conjunction with laser flash photolysis for the proper identification of the exact nature of reactions, in the two contrasting media. In this work we have focused on two very different reactions, ET and hydrogen (H) abstraction, between reactant molecules which

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produces radical pairs (RPs) and radical ion pairs (RIPs) respectively. Micelles also provide a cage environment for these RPs/RIPs where random encounter is reduced on one hand and a suitable distance is maintained between them so that exchange interaction (J) becomes negligible but spin correlation is maintained. This makes micelles an ideal media for observing magnetic field effect (MFE). MFE can be utilized only in those reactions where RPs/RIPs are formed. Now if either of the reaction occurred when the photosensitizer (AQ) is in the triplet state the initially formed RPs/RIPs will be in the triplet state too. Similar situation prevails for singlet state. 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 split 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 results in an increase of the initial spin state population of RPs/RIPs. So an enhancement in absorbance value on application of external magnetic field coupled with laser flash photolysis suggests an ET or H abstraction from a triplet precursor generating a triplet RP/RIP. Thus MF helps in the identification of an existence of RP/RIP on one hand and the initial spin state of the transients on the other [17–21]. We have thus utilized MFE as an effective tool in the proper identification of intermediates out of several possibilities. This is then utilized in the elucidation of reaction mechanisms between reactants of our choice. A change in the behavior of molecules on changing the media has a direct bearing on the structure of the interacting molecules. An addition of sugar moiety has been found to have a significant effect on the reaction course too. We have attempted to provide suitable explanations of all these behaviors.

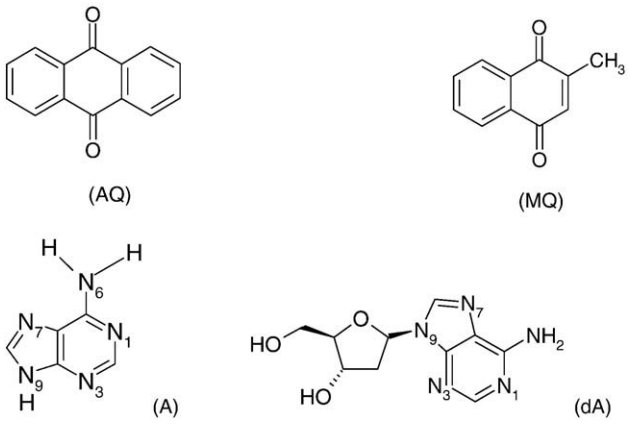
2. Experimental

2.1. Materials

Adenine (A), 2′-deoxyadenosine (dA), 2′-deoxyribose (Rb) and sodium dodecyl sulphate (SDS) were purchased from Sigma. 9,10-anthraquinone (AQ) was obtained from Aldrich and was recrystallised from ethanol. UV spectroscopy grade acetonitrile (ACN) 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.

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 8ns. 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 was 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 T) on the transient spectra has been studied by passing direct current through a pair of electromagnetic coils placed inside the sample chamber.



Scheme 1. Structures of the molecules.

3. Results and discussion

3.1. Triplet and radical absorption spectra

Fig. 1 displays transient optical absorption spectra upon irradiation of a 0.4 mM AQ solution separately and in presence of 5 mM A and dA after 1 μs of laser flash in ACN/H_2O (4:1, v/v). AQ alone presents strong maxima around 360 nm, which is due to triplet absorption of ^{3}AQ [22]. Addition of DNA bases leads to an increase in absorption around 360–380 nm regions with a hump at 500 nm. We have reported earlier AQ radical anion ($AQ^{\cdot -}$) absorbs around 390–400 nm with a small hump around 540 nm in pure ACN media [22]. So the 500 nm hump can be well associated to the second peak of $AQ^{\cdot -}$. Confirmatory 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 [23,24]. Hence an occurrence of ET between A, dA and AQ is confirmed in ACN/H_2O medium. A closer analysis of the peaks of AQ–A and AQ–dA reveals a shift in the λ_{max}. H abstraction from A/dA will produce $AQH^{\cdot}$, which is reported to absorb around 370 nm [22]. Since $AQ^{\cdot -}$ absorbs very close to it, so a coexistence of $AQ^{\cdot -}$ and $AQH^{\cdot}$ is marked by a broad spectrum around 370–400 nm [10]. But here such broad peak is absent so we conclude, a simultaneous H abstraction is not encouraged with ET.

Fig. 2 shows the transient spectra obtained on irradiating a 0.1 mM solution of AQ with and without addition of A in 10% SDS medium after 1 μs of laser flash. Addition of A results in a strong absorption with maxima around 370–390 nm regions with a second peak around 480 nm region. Increase in peak height on application of external magnetic field points towards the formation of a geminate spin-correlated RIP. $AQ^{\cdot -}$ is reported to absorb around 390–400 nm while $AQH^{\cdot}$ absorbs around 370 nm. So we can associate the 370–400 nm broad regions with the existence of $AQ^{\cdot -}$, $AQH^{\cdot}$ and $A^{\cdot +}$. The peak at 480 nm region can be associated to AQH_2 [22] formed by two simultaneous H atom transfer to AQ.

$$^3AQ^* + H^{\cdot} \rightarrow AQH^{\cdot} \quad (1)$$

$$AQH^{\cdot} + H^{\cdot} \rightarrow AQH_2$$

Observation of AQH_2 is a confirmatory evidence of H atom transfer from A to AQ. AQH_2 being a non-radical does not show appreciable MFE. The second peak due to $AQ^{\cdot -}$ is probably eclipsed by the AQH_2 peak in SDS. A simultaneous ET and H atom transfer to AQ by bases is comprehended by a broad absorption spectra around 380–400 nm

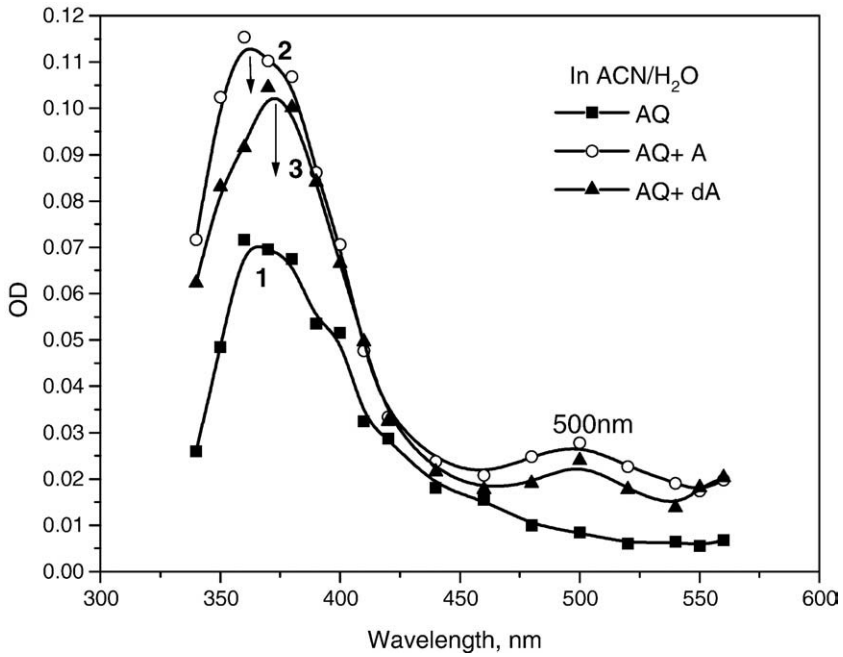


Fig. 1. Transient absorption spectra of (1) 9,10-anthraquinone (AQ) (0.4 mM) (■), (2) AQ (0.4 mM)–adenine (A) (5.0 mM) (○) and (3) AQ (0.4 mM)–2′-deoxyadenosine (dA) (5.0 mM) (▲) at 1.0 μs time delay after laser pulse with excitation wavelength 355 nm in ACN/H_2O (4:1, v/v).

while H atom transfer generates $AQH^{\cdot}$ only, with a sharp peak at 370 nm [10]. Fig. 2 has revealed a broad peak with appreciable MFE around 370–400 nm. Hence we conclude SDS medium has supported H atom transfer along with ET with A. Fig. 3 shows the effect when dA replaces A. Here the nature of the peaks remain same but with a decreased peak height than A. This indicates the possibility of both reactions with dA also. AQ is seen to opt for a dominant ET in both media but in SDS some H atom transfer has also been discerned. Our earlier works with MQ has shown a somewhat different result [11]. With MQ, homogeneous medium has supported only ET while heterogeneous micellar medium (SDS) has shown a dominant H abstraction with almost no ET.

3.2. Magnetic field effect

A kinetic analysis of MFE has given a confirmatory proof of the simultaneous existence of $AQ^{\cdot-}$ and $AQH^{\cdot}$ in micelles. Fig. 4 displays the normalized decay traces of AQ in the presence and absence of bases. In the presence of an external magnetic field, the decay of the transient at 370 nm becomes slower accompanied by an enhanced absorption in the

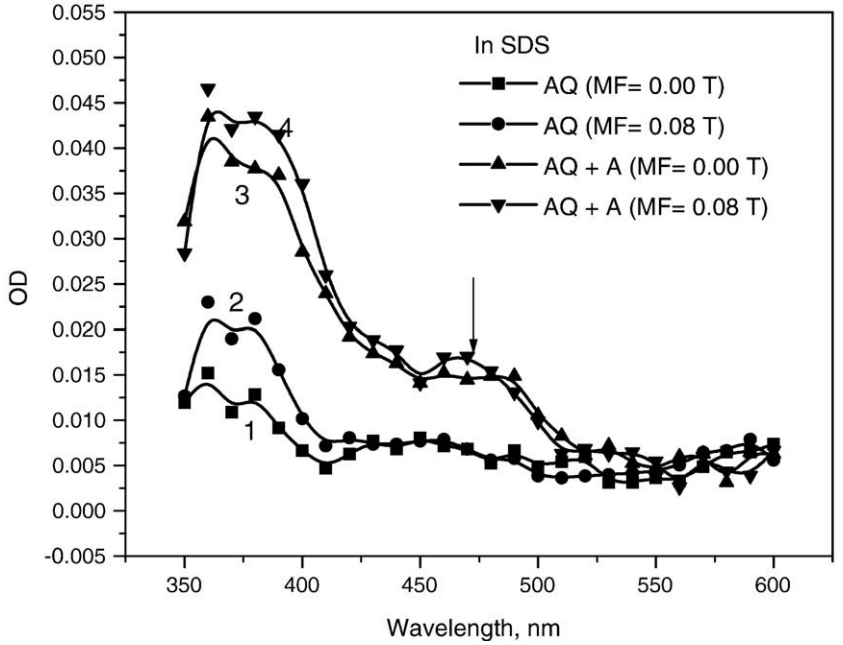


Fig. 2. Transient absorption spectra of AQ (0.1 mM) in (1) the absence (■) and (2) presence of magnetic field (●) and AQ (0.1 mM)–adenine (A) (5.0 mM) in (3) the absence (▲) and (4) presence of magnetic field (▼) at a delay of 1.0 μs in SDS micelles.

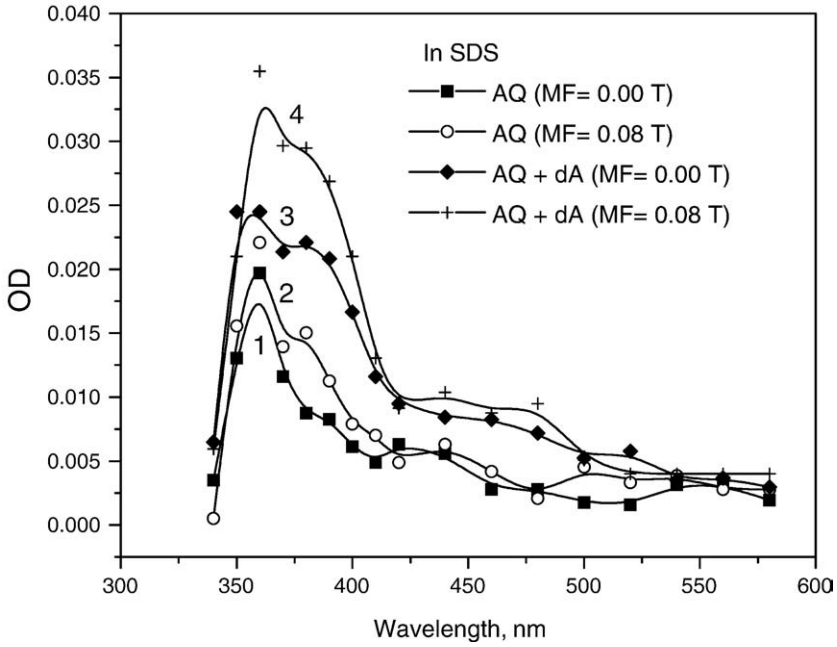


Fig. 3. Transient absorption spectra of AQ (0.1 mM) in (1) the absence (■) and (2) presence of magnetic field (O) and AQ (0.1 mM)–2′-deoxyadenosine (dA) (5.0 mM) in (3) the absence (♦) and (4) presence of magnetic field (+) at a delay of 1.0 μs in SDS micelles.

spectrum (Fig. 2). The formation of a spin spin-correlated radical pair (^{3}AQH$^{\cdot}$R) explains this MFE. It is noteworthy that the nature of the decay profiles (at 370 nm) of AQH$^{\cdot}$ is different in the presence of both A and dA, particularly in presence of a MF. This implies that the transients formed in the presence of A/dA behave differently with MF than with AQ alone. We believe this difference must have arisen due to the presence of some different species. In SDS, AQ alone generates only AQH$^{\cdot}$, which will have its own mode of interaction with the field, so obviously A/dA produces something in addition to AQH$^{\cdot}$. We think these species may be the RIPs formed by ET from bases to AQ. Again a complete absence of H abstraction with A/dA is also not feasible in SDS, where H atom transfer is possible from the SDS molecule itself [25]. Hence we are confirmed of a simultaneous occurrence of ET and H abstraction.

In the presence of an external magnetic field, the decay of the RP is expected to be biexponential [26] i.e., the following equation is obeyed for the change in absorbance $A(t)$

$$A(t) = I_f \exp(-k_f t) + I_s \exp(-k_s t) \quad (2)$$

where k_f and k_s are the respective rate constants for the fast and slow components of the decay profiles. The fast components of this equation correspond to the RP decay in the micellar cage, while the slower one is due to the reaction of the escaped radicals. On giving a biexponential fit to curves 3, 4, 5 and 6 (Fig. 4) the k_f values obtained are 6.5×10^6 s^{-1}, 1.8×10^6 s^{-1} and 3.4×10^6 s^{-1}, 1.9×10^6 s^{-1} respectively as shown in Table 1. It is thus evident an application of magnetic field has resulted in a decrease in the decay rate constant i.e., an increase in

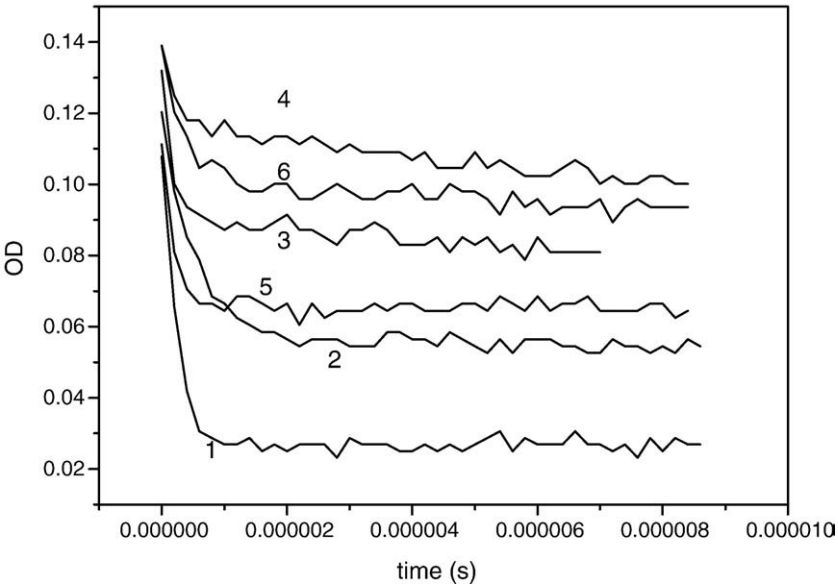


Fig. 4. Normalized OD traces at 370 nm obtained by laser flash photolysis (λ = 355 nm) of AQ (0.1 mM) in SDS in (1) absence and (2) presence of magnetic field, AQ (0.1 mM) and adenine (A) (5.0 mM) in (3) the absence and (4) presence of magnetic field, AQ (0.1 mM) and 2′-deoxyadenosine (dA) (5.0 mM) in (5) the absence and (6) presence of magnetic field.

Table 1
Variation of decay rate constant (k_f) with magnetic field for aqueous micellar solution (SDS) of AQ and the bases

Base	Magnetic field (Tesla)	Decay rate constant (k_f) $(s)^{-1}$
No base	0.00	3.0×10^6 (±0.07)
	0.08	1.5×10^6 (±0.01)
A	0.00	6.5×10^6 (±0.02)
	0.08	1.8×10^6 (±0.01)
dA	0.00	3.4×10^6 (±0.01)
	0.08	1.9×10^6 (±0.02)

lifetime of the RPs/RIPs for both A and dA. This has been accounted to the triplet geminate RP/RIP precursor involved in these reactions. This indicates ET and H abstraction reactions to occur in triplet state in case of these bases.

3.3. Mechanism of action

Closer inspection of Fig. 1 reveals a slight shifting in the λ_{max} of the A–AQ and dA–AQ peak. They are generated by superposition of radical anion and radical cation peaks. Since the radical anion ($AQ^{\cdot-}$) will be the same for both the bases so the shifting has been associated to two different radical cation formations from A and dA. Song et al. in their work with guanine, guanosine has observed analogous phenomenon. They have associated the difference to stem from different sites of deprotonation of the same base [16]. 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 is probably not involved in ET with quinones. 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 [27]. In a theoretical work Rodgers et al. 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 [28]. Hence on similar grounds we think a facile ET will occur from N3 of A to AQ. But in dA, the neighboring larger sugar moiety probably 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 [27] 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 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 intensity. 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 is greater than pyrimidines, so the non-involvement of π electrons of purines during ET can be logically associated to its higher aromaticity [29]. Therefore we can conclude that the two pyrimidine nitrogen (N1 and N3) are involved in the donation of electron density to quinones during ET.

AQ is seen to favor ET in both media but seems to encourage a better H abstraction in SDS medium than in homogeneous one. 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 [30]. We have reported earlier [9,10,31] that SDS medium promotes H atom transfer on account of close sequestering of participant molecules which leads to an initial 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 distribution, close proximity between reactants at a particular time is rare. Thus ET will be the dominating pathway here with or without H abstraction depending on nature of the reacting species. Earlier works in our laboratory [11] has pointed to a dominance of H atom transfer in SDS medium using A and dA with MQ owing to greater H bonding in hydrophobic micellar medium with almost negligible ET. But here with AQ we have also noticed an ET to occur and it seems both reactions become competitive in micellar environment. Tanimoto et al. has suggested a complete micellization of AQ based on its hydrophobic character [32,33]. Closeness among the reactants has favored H abstraction from bases in SDS while it is almost absent in ACN/H_2O. So although the ET channel exists in SDS, the H abstraction

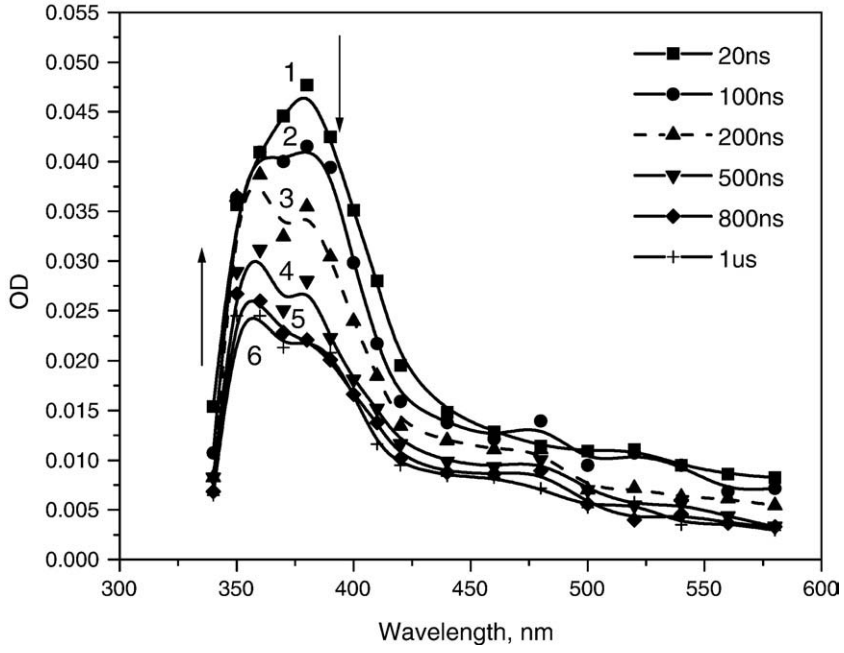


Fig. 5. Time-resolved spectra of AQ (0.1 mM) and dA (5.0 mM) in SDS (1) 20 ns (■), (2) 100 ns (●), (3) 200 ns (▲), (4) 500 ns (▼), (5) 800 ns (♦) and (6) 1 μs (+).

pathway gets preference here, which introduces a competition between the two.

Presence of an extra sugar unit in dA has been found to have a significant effect on its chemistry. Addition of sugar moiety to A is seen to decrease the rate of reactions for AQ in both media. An investigation of lifetime at 370 nm in ACN/H_2O for AQ gives 2.83 μs for A and 1.59 μs for dA. S. Steenken has reported a drop in reaction rate on going from A to dA by electrophilic SO_4^- [34] which he considers to be due to a fall in electron density due to replacement of H at N9 by electron-withdrawing ribose unit. This is quite plausible since by rotation of N9-sugar bond, the electronegative oxygen atoms can exert sufficient field effect [35] to pull away electrons from the base unit. Moreover absence of N9 hydrogen in dA leads to H abstraction from some other center, viz., amino hydrogen of A moiety, which is energetically costlier [23]. So addition of sugar unit to the A moiety will decrease both the ET and H abstraction rate, which is reflected in the lifetime data.

Fig. 5 shows the effect of time on ET and H atom transfer rate in case of AQ–dA in SDS. We notice a steady decrease in the 390 nm peak with a concomitant increase in the 370 nm one pointing towards a steady decrease in ET and increase in H abstraction rate with time. Similar effects were not shown by A (graph not shown). Can this be also due to the sugar unit? In order to answer this question we have performed separate laser flash photolysis experiments using a 2′-deoxyribose (Rb) sugar with AQ in both media.

Fig. 6 reveals the effect of Rb on AQ in SDS medium. We find almost no change around 370 nm but a new peak is generated around 460 nm on addition of Rb to AQ. Characteristic peaks of RIPs are not detected. Earlier we have assigned this 460 nm peak with AQH_2, a non-radical species.

Inset to Fig. 6 reveals the same in ACN/H_2O medium where the 460 nm peak is totally absent. Thus we can infer, AQH_2 formation is favored in SDS medium only. This may be due to a favorable H atom transfer from the sugar unit to AQ [36–38], which becomes more facile on a closer approach between the two entities, which is possible only in SDS. Thus the time dependent increase in extent of H abstraction in case of AQ in presence of dA can be well associated to involvement of the sugar unit (in dA) in SDS medium. Except H atom transfer sugar unit possibly does in no other way interact with AQ.

In our earlier works we have observed MQ to undergo a dominant ET with the same bases in homogeneous medium but in SDS there has been a dominant H abstraction [11]. But in this case, with AQ, ET was found to be the dominant mode of reaction in both media. So with change in the medium type, an alteration in the mode of reaction has been achieved by changing the quinone size. This demands an explanation.

For H abstraction to occur we assume, there must be an initial H bonding type interaction between the H donor and acceptor molecules. In AQ the quinone moieties being flanked on both sides by bulky phenyl groups, probability of H bonding with base molecules decreases. But in smaller MQ a bulky phenyl group is replaced by a small methyl group so closer approach of A/dA is not hindered. This phenomenon becomes more important in a micellar cage, as the molecules are closely sequestered in it with minimum movement. So probability of H bonding with MQ is much more increased than in a homogeneous medium, where the motions of the molecules are chaotic. So a dominant H abstraction with MQ is evident in SDS. In AQ, the bulky phenyl groups pose a barrier to a closer approach of base molecules thus reducing the possibility of sufficient H bonding hence ET remains the dominant reaction in SDS too.

4. Conclusions

We have demonstrated the dependence of model anticancer drug–DNA base interaction (AQ–A/dA) on environmental conditions. Again, we have also observed differences in the behavior of adenine and 2′-deoxyadenosine on account of the sugar unit. We have shown that AQ has a preference for ET with A and dA in both homogeneous and confined heterogeneous media. But the smaller analogue, MQ has been found to favor H abstraction in SDS. This has been attributed to size effect and medium characteristics. All these results suggest a possibility of switchover of reaction by manipulating the size of reactants in different media.

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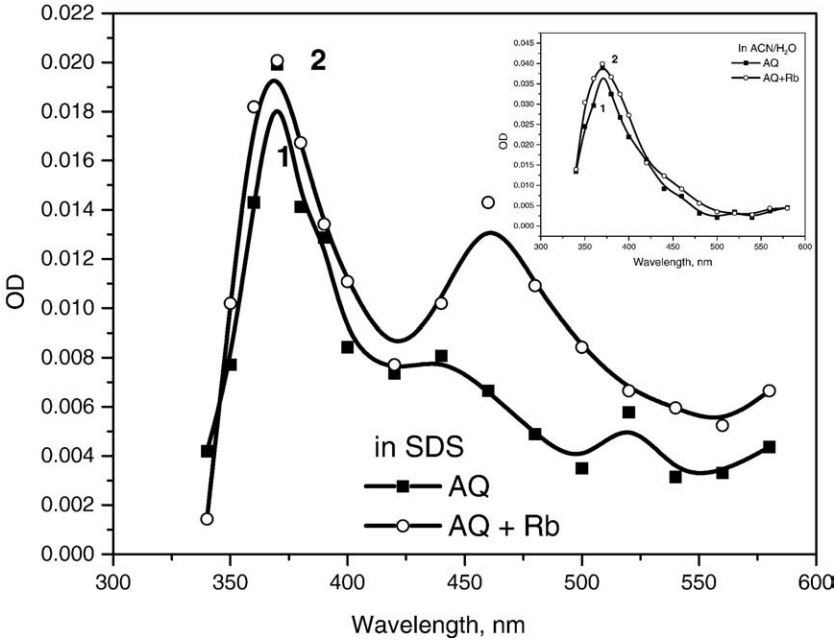

Fig. 6. Transient absorption spectra of (1) AQ (0.1 mM) (■) and (2) AQ (0.1 mM)–2′-deoxyribose (Rb) (5.0 mM) (O) at a delay of 1.0 s in SDS micelles in absence of any magnetic field. Inset: Transient absorption spectra of (1) AQ (0.1 mM) (■) and (2) AQ (0.1 mM)–2′-deoxyribose (Rb) (5.0 mM) (O) in ACN/H_2O (4:1, v/v).

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