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Solvent effects on the reactivity of fluorenyl nitrenium ion with DNA-like probes

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Abstract

Laser Flash Photolysis (LFP) experiments carried out on 2-azidofluorene in aqueous systems generate 2-fluorenyl nitrenium ion ($\lambda_{\max}=450$ nm) which decays with first order rate constant and is quenched with 2′ -deoxyguanosine originating an intermediate, namely the C8 adduct of 2-fluorenyl nitrenium ion, with bimolecular rate constant in the order of 1.3×10^9 M^{-1} s^{-1} in pure water. This intermediate very likely mimics the intermediate formed from carcinogens (i.e.: arylnitrenium ions formed through metabolic activation pathways from aminoaromatic substrates) and DNA rests in vivo. Solvent effects demonstrate and support the further stabilization of this intermediate (with respect to fluorenyl nitrenium ion) through hydrogen bonding as compared to other probe systems, and accounts for the enhanced metabolic carcinogenecity observed for this type of compounds. Diverse solvent systems, such as mixtures of water with acetonitrile, 1,1,1-trifluoroethanol, and 1,1,1,3,3,3-hexafluoroisopropanol, are used to interpret solvent–complex interactions.

Keywords: Fluorenyl nitrenium ion; Laser flash photolysis; Photochemistry

1. Introduction

Arylnitrenium ions are the nitrogen analogs of arylcarbenium ions. Unlike the carbon counterpart, the nitrenium ion can adopt either a singlet or triplet electronic configuration. While the parent NH_2^+ is a ground state triplet, phenyl substitution stabilizes the singlet relative to the triplet, and phenylnitrenium ion is calculated to be a ground state singlet, by ~20 kcal/mol [1]. Until recently, nitrenium ions had been studied experimentally as steady-state intermediates of ground state reactions, usually SN1 solvolysis reactions [2].

Knowledge of the reactivity of nitrenium ions is relevant since these electrophiles are proposed to be the DNA-binding intermediates responsible for the carcinogenicity of aromatic amines such as 4-aminobiphenyl and 2-aminofluorene [3]. 4-Aminobiphenyl and 2-aminofluorene are generally known to undergo a 2-fold metabolic activation to *O*-acetate or *O*-sulfate esters, followed by N–O heterolysis to an arylnitrenium ion **2** and subsequent DNA-tagging, **3**, Scheme 1 [4].

2-Aminofluorene and its *N*-acetyl derivative have received comprehensive investigation since they were found to be potent carcinogens over 50 years ago. These two compounds are representative of a diverse class of carcinogenic aromatic and heteroaromatic amines present in the environment in tobacco smoke, automobile exhaust, broiled meats and fish, and as side products of industrial processing [5]. A common feature of the class is the transfer of the arylamine group to DNA. The predominant site of attachment on DNA is guanine.

Due to the relevance of arylamine carcinogenicity a number of studies have been geared at probing the chemistry of arylnitrenium ions when formed as intermediates of solvolysis and related reactions [6].

A nitrene is also the conjugate base of a nitrenium ion, and therefore the trapping of this species by protonation can be regarded as a route for effecting photochemical entry into these cations (the two intermediates can in principle be linked by protonation/deprotonation) [4].

We found that 2-fluorenylazide photolyzed in aqueous solutions produces high yields of the corresponding nitrenium

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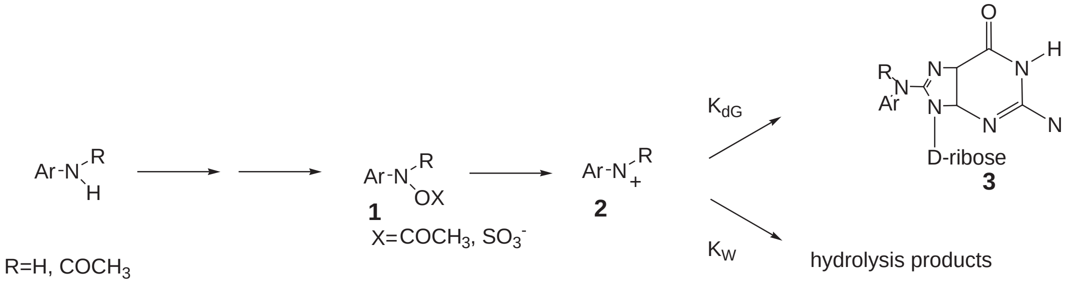


Scheme 1.

ion and this intermediate was detectable with LFP [5]. 2-Fluorenylnitrene is a relatively strong base capable of being intercepted by protonation by H^+ and even water to give nitrenium ions in competition with other reaction channels such as intersystem crossing and ring expansion.

Both biphenylyl- and fluorenylnitrenium ions react with guanine derivatives such as 2′ -deoxyguanosine (2′ -dG) with rate constants that are close to or at the diffusion limit (2×10^9 M^{-1} s^{-1}).

With imidazoles, nitrenium ions show reactivity trends that parallel the nitrenium–2′ -dG reaction, with rate constants leveling at the 2×10^9 M^{-1} s^{-1} limit for the more reactive cations. Imidazole itself and 1-methylimidazole are generally less reactive than 2′ -dG, while 2-methylimidazole and 1,2-dimethylimidazole are very similar.

Low concentrations of 2′ -deoxyguanosine (2′ -dG) result in the formation of the C8-adduct at the expense of the water products (Scheme 2), with no change in rate constant [2].

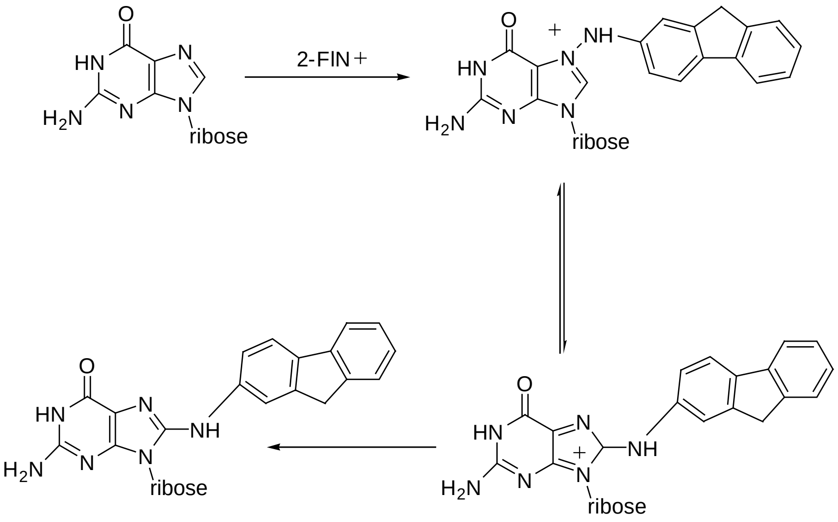
The C8 adduct is the only product from reaction of 2-fluorenyl azide with 2′ -dG in water; on the other hand, carbon electrophiles show no instance of reaction at C8.

Very recent flash photolysis experiments in our group have shown that the decay of a nitrenium ion in the presence of 2′ -dG is accompanied by the formation of an intermediate absorbing in the range 300–400 nm. Several pieces of evidence suggest that the species is the C8 adduct **4** (Scheme 2). In particular the decay of that intermediate exhibits a very large primary isotope effect when comparing guanine C8-H and C8-D (Scheme 2). In addition to the direct observation of these electrophiles, the absolute rate constants k_w and k_{dG} were measured, the former representing the decay of the cation in the solvent alone and the latter, the reaction with added 2′ -dG. Thus, it was concluded that the transients observed by LFP are ground-state arylnitrenium ions that these same species are formed in the ground-state hydrolysis of ester precursors, and that these electrophiles do react with guanine derivatives to form the C8 adduct (**4** in Scheme 2).

In our LFP experiments with 2′ -dG present, we observed a growth of absorbance at 300–370 nm occurring with the same rate as the decay of the nitrenium ion. This species is attributed to an intermediate, moreover, one that forms in the reaction of the nitrenium ion and 2′ -dG.

As depicted in Fig. 1 (vide infra), the 2-fluorenyl nitrenium ion (2-FlN+) is strongly absorbing with a λ_{max} at 450 nm. At the completion of the decay, there is little OD left, other than the band for the other species at 335 nm. The decay is accelerated with 2′ -dG present, by a factor of 200 at the concentration of Fig. 1.

In order to throw more light on the reactivity of 2-FlN+ ions with 2′ -dG, we carried out solvent studies. In particular, we are



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Scheme 2.

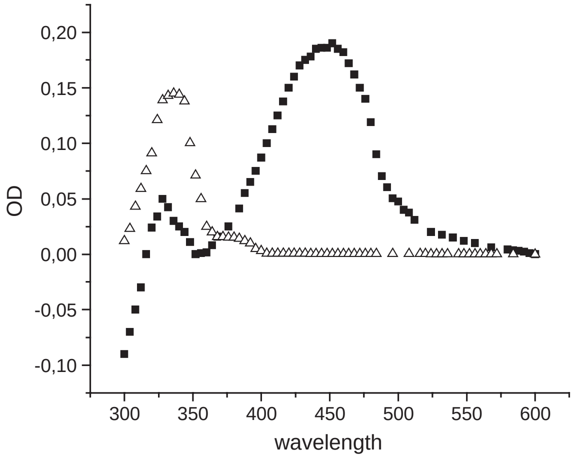

Fig. 1. Spectra obtained on 308 nm laser irradiation of 2-azidofluorene (50 μM) in 20% TFE. (■) Data after the laser pulse. (△) Data at the completion of the first-order process with 0.0012 M 2′ -deoxyguanosine present.

interested in casting some light into the hydrogen bonding interactions present in the 2-FlN–2′ -dG+ complex (**4** in Scheme 2) which would confer the suspected stabilization of the intermediate (with respect to fluorenyl nitrenium ion), and the longer lifetimes observed. We will attempt to demonstrate that the hydrogen bonding interactions arising from the presence of the free amino group (of the guanidine moiety in **4**) and water in the complex 2-FlN–2-dG+ can account for the stabilization observed (illustrated by pronounced solvent effects) as compared to the probes herein utilized, 1,3-dimethoxybenzene (DMB) and 1,3,5-trimethoxybenzene (TMB), where such hydrogen bonding stabilization is not expected.

2. Results and discussion

LFP experiments were carried out with laser irradiation (with ca. 20 ns pulses, ca. 80 mJ per pulse) at 308 nm in aqueous solutions with varying concentrations of a co-solvent, acetonitrile (ACN), 1,1,1- trifluoroethanol (TFE) and 1,1,1,3,3,3-hexafluoroisopropanol (HFIP). The transient behavior is the same as that reported previously [5], and illustrated in Figs. 1 and 2 for the new solvent systems (20% TFE and HFIP–water mixtures). This consists of a relatively intense transient with $\lambda_{\max}$ ca. 450 nm. This decays with first order kinetics. Quenching with 2′ -dG results in bimolecular rate constants as given in Table 1.

Solutions of the 2-fluorenylazide were 50 μM, and the experiments were carried out under air. The rate constants k_{dG} were calculated as the slopes of the plots of k_{decay} versus quencher concentration, for seven concentrations of quencher (2′ -deoxyguanosine, 2′ -dG; and 1,3-dimethoxybenzene, DMB), over the range 0–10 mM. Concentrations for the quencher 1,3,5-trimethoxybenzene (TMB) were in the range 0–1 mM. These were all linear plots, with correlation coefficients (r^2) equal to or above 0.998.

Both DMB and TMB were used as model quenchers of 2-FlN+ ions, in order to mimic the guanosine reactivity and assess the hydrogen bonding interactions. In the present LFP experiments with 2′ -dG in mixtures of water with ACN, TFE, and HFIP, we also observed a growth of absorbance at 300–370 nm occurring with the same rate as the decay of the nitrenium ion (see Figs. 1 and 2). This species is the same as observed in pure water, and attributed to the intermediate that forms in the reaction of the nitrenium ion and 2′ -dG.

Bimolecular quenching rate constants of 2-FlN+ with DMB show deceleration upon increasing ACN concentrations (Fig. 3, Table 1), consistent with a destabilization of the cationic intermediate proposed in the reaction of 2-FlN+ with DMB , as medium polarity decreases with decreasing water content. This is probably due to an increased polar character in the product (complex between FlN+ and DMB) with respect to reactant (FlN+), due to the presence of two extra methoxy polar groups in the former.

There is, at low ACN concentrations (<8%), a dip in the continuous upward trend observed for the quenching of 2-FlN+ ions with DMB, as water concentrations increase; that is, rate constants decelerating at very low ACN concentrations. This behavior has been noted for some systems, and attributed to changes in viscosity and aggregation at low ACN concentrations. Plots of quenching of 2-FlN+ with 2′ -dG versus volume percentage of ACN show three distinctive regions (Fig. 3); firstly, a decrease of k_{q} with decreasing water content (starts at around 1.3×10^{9} M^{-1} s^{-1} with 0% ACN up to 20% ACN), then a plateau region, with k_{dG} almost constant (ca. 7×10^{8} M^{-1} s^{-1}, 20–50% ACN), and a third, where bimolecular quenching rate constants increase up again with further decreasing water contents (55–80% ACN). This parabolic-like behavior is suggesting of the existence of a molar fraction range of ACN:W system where hydrogen bonding overweighs solvent aggregation. Excess molar properties of the system ACN:W do not show such hyperbolic-like behavior. However, at around 0.3–0.4 ACN molar fractions (in ACN:W mixtures), it is predicted that the percentage of ACN molecules in ACN:W mixtures that are hydrogen-bonded and those that are free levels off at ca. of 50% (lower ACN fractions promote

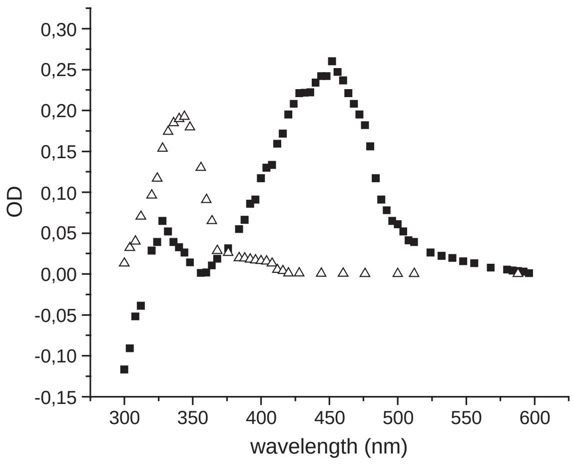

Fig. 2. Spectra obtained on 308 nm laser irradiation of 2-azidofluorene (50 μM) in 5% HFIP. (■) Data after the laser pulse. (△) Data at the completion of the first-order process with 0.0012 M 2′ -deoxyguanosine present.

Table 1
Bimolecular quenching rate constants of FlN+ with 2′ -dG, DMB, and TMB in ACN:Water mixtures

%ACN	$k_{q(2'\text{-dG})}$ ± E10^9 M^{-1} s^{-1}	$k_{q(\text{DMB})}$ ± E10^9 M^{-1} s^{-1}	$k_{q(\text{TMB})}$ ± E10^9 M^{-1} s^{-1}
0	1.31 ± 0.01	–	–
2		0.97 ± 0.03	3.73 ± 0.09
5	–	0.93 ± 0.02	3.65 ± 0.04
10	0.95 ± 0.02	1.00 ± 0.03	3.62 ± 0.03
20	0.736 ± 0.008	0.852 ± 0.005	3.24 ± 0.04
35	0.72 ± 0.02	0.512 ± 0.008	3.07 ± 0.05
50	0.72 ± 0.01	0.369 ± 0.007	3.52 ± 0.05
65	1.05 ± 0.02	0.336 ± 0.003	3.26 ± 0.02
80	1.33 ± 0.03	0.310 ± 0.003	4.15 ± 0.05
90	–	0.37 ± 0.01	–

more hydrogen-bonded ACN molecules, whereas at higher ACN fractions, far freer ACN molecules prevail, augmenting the hydrogen bonding capability of water, which increases the stabilization of the complex). This is probably a key point, since that is the trend observed for 2′ -dG quenching of 2-FlN+ (Fig. 3), but not for DMB or TMB quenching, where hydrogen-bonded intermediates are not expected. At high water concentrations in ACN:W mixtures, the rate constants for quenching of 2-FlN+ with 2′ -dG are high and start to decrease as water content decreases, up to a point when they start to increase back again when more water molecules (not hydrogen-bonded to ACN) are available to the complex for hydrogen bonding. We attribute the enhanced hydrogen bonding capability of FlN–2′ -dG+ complex to the presence of the extra amino group of the guanosine moiety, which is acting as a hydrogen-bond acceptor. This further stabilization of the complex through hydrogen bonding is translated into enhanced quenching rate constants.

When another probe for 2-FlN+ quenching is used, such as TMB, near diffusion limit bimolecular quenching rate constants are obtained (Table 1). A logarithmic plot, such as that described in Fig. 3, shows that ca. 3.5×10^9 M^{-1} s^{-1} constitutes such limiting values for the quenching of fluorenyl azide with TMB in ACN:W systems.

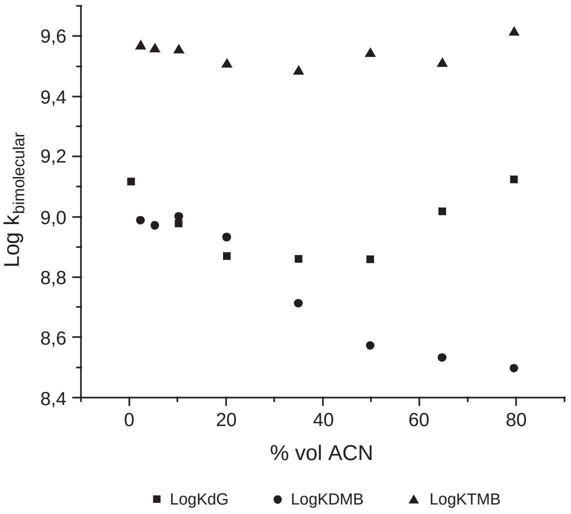

Fig. 3. Plots of the logarithm of bimolecular quenching rate constants (log$k$ in M^{-1} s^{-1}) of 2-FlN+ with 2′ -dG (squares), DMB (circles), and TMB (triangles) versus increasing acetonitrile (%ACN) content.

Table 2
Bimolecular quenching rate constants of FlN+ with different quenchers in different solvent systems

Quencher	Solvent system	k_q ± E10^9 M^{-1} s^{-1}
2′ -dG	100% D_2O	1.18 ± 0.01
2′ -dG	20% ethanol : 80% W	0.75 ± 0.07
DMB	20% ACN : 80% D_2O	0.88 ± 0.07
	20% ethanol : 80% W	0.74 ± 0.02
2-deoxyinosine	60% TFE : 40% W	0.00031 ± 0.00008
	80% TFE : 20% W	0.00008 ± 0.00001

A small deuterium isotope effect of approximately 1.1 (ca. 10%) is observed with 2′ -dG quenching (100% D_2O, Table 2). DMB, instead, (in 80% D_2O : 20% ACN) does not show isotope effect (Table 2). This can be ascribed to the stronger hydrogen bonds of D_2O–water (and D_2O–D_2O) than H_2O–water, with consequence on the stabilization of the complex between 2-FlN+ and 2-dG [7]. These results emphasize the presence of hydrogen-bond interactions between solvent molecules, and also throw light on the role of hydrogen bonding in the stabilization of FlN+2′ -dG complex in relation to the probes used (this further stabilization of the complex can be viewed as being relative to the precursor (2-FlN+) as well as to the other probes utilized (DMB and TMB) where no hydrogen bonding is possible in the complexes that form with 2-FlN+).

2.1. 1,1,1-Triflluoroethanol–water system

For a more acidic-less nuclephilic solvent such as 1,1,1-trifluoroethanol, TFE, a pronounced descent in bimolecular quenching rate constants is observed as TFE concentrations are enhanced in TFE:W mixtures (Fig. 4). According to Table 3, at 80% TFE:W, there is a decrease of two orders of magnitude in the rate constant of 2-FlN+ with 2′ -dG as compared to the corresponding value in 80% ACN:W given in Table 1

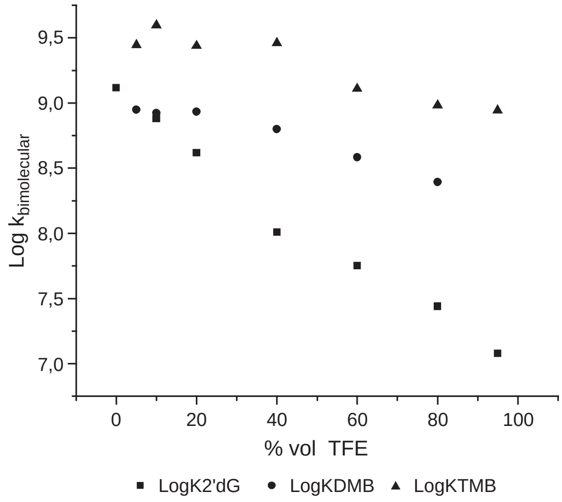

Fig. 4. Plots of the logarithm of bimolecular quenching rate constants (log$k$ in M^{-1} s^{-1}) of 2-FlN+ with 2′ -dG (squares), DMB (circles), and TMB (triangles) versus increasing TFE (%TFE) content.

Table 3
Bimolecular quenching rate constants of FlN+ with 2′ -dG, DMB, and TMB in TFE:Water mixtures

%TFE	$k_{q(2'\text{-dG})}$ ± E10^9 M^{-1} s^{-1}	$k_{q(DMB)}$ ± E10^9 M^{-1} s^{-1}	$k_{q(TMB)}$ ± E10^9 M^{-1} s^{-1}
5	–	0.88 ± 0.02	2.79 ± 0.08
10	0.76 ± 0.01	0.83 ± 0.01	3.9 ± 0.5
20	0.42 ± 0.01	0.85 ± 0.02	2.75 ± 0.03
40	0.102 ± 0.001	0.631 ± 0.007	2.89 ± 0.03
60	0.056 ± 0.001	0.384 ± 0.005	1.29 ± 0.02
80	0.0275 ± 0.0004	0.246 ± 0.003	0.96 ± 0.01
95	0.0196 ± 0.0003	–	0.880 ± 0.07

(2.7×10^7 and 1.3×10^9 M^{-1} s^{-1}, respectively).

Surprisingly, there is no hyperbolic-like behavior of the quenching of 2-FlN+ with 2′ -dG in the range of TFE concentrations used, as observed for ACN:W system in Fig. 3. Fig. 4 shows a monotonic descent of bimolecular rate constants as TFE concentrations are increased, both for the quenching of 2-FlN+ with 2′ -dG and with DMB as well.

DMB shows a similar trend (Fig. 4) in bimolecular quenching rate constants at low TFE concentrations as that observed in Fig. 3 for low ACN concentrations, that is, a shallow dip in the monotonous increase in bimolecular rate constants as TFE concentrations are increased. Gente and La Mesa [8] showed that the occurrence of solute–solvent interactions can be put in evidence, in particular, by excess molal quantities. For TFE, at mole fractions close to 0.2, a significant maximum, or minimum, in excess functions is found. In this context, the TFE–water system is less stabilized by hydrogen bonds than EtOH–water. This can be observed from the values of the rate constants of quenching of FlN+ with 2′ -dG, both in Ethanol:W and TFE:W mixtures (from Table 2, 7.5×10^8 M^{-1} s^{-1}, and from Table 3, 4.2×10^8 M^{-1} s^{-1}, respectively). For instance, the partial molar volume V_2 of TFE–W mixtures passes through a minimum at very low TFE concentrations (0.07 molar fraction) and increases therefrom The partial molar volume of water, calculated from the Gibbs–Duhem relation, behaves in the reverse way and indicates the occurrence of significant hydrophobic interactions at moderate alcohol mole fractions.

As of viscosity of TFE–W mixtures [9], a distinctive minimum (in plots of excess fluidity versus TFE molar fraction in TFE–W mixtures) has been reported at about 0.2 TFE mole fraction, in agreement with volumetric findings. The effect can be partly ascribed to the bulkiness of CF3 groups in the molecule. In addition, van der Waals interactions between the above-mentioned group and water molecules may be weaker than between water and CH3 (in ACN). The breaking of intramolecular hydrogen bonds (as compared to ethanol, for example), in fact, is affected by replacing H with F atoms; changes in the molecular polarizability may also occur. As is well known, fluorine plays a significant role in dispersion interactions, leading to positive contributions to excess quantities.

Fig. 4 shows logarithmic plots of $k_{\text{bimolecular}}$ versus varying TFE:W mixtures for 2′ -dG, DMB, and TMB quenchers of 2-FlN+. The quenching of 2-FlN+ with 2′ -dG is a rather straight plot, denoting a monotonic decrease in bimolecular rate constants as TFE content is increased in TFE:W mixtures. Quenching of 2-FlN+ by DMB is almost a straight plot, except for mixtures of low TFE content. As for TMB quencher, a diffusion limit rate is observed in TFE:W, and a value of ca. 2.8×10^9 M^{-1} s^{-1} is ascribed. As of note, a slight decrease in rate is observed for TMB quenching (Fig. 4) in TFE:W mixtures, as opposed to the behavior of this same quencher in ACN:W mixtures, probably due to further stabilization of TMB–FlN cationic complex in the latter system.

From the slopes of the plots in Fig. 4, it can be inferred that TFE:W mixtures exert a more profound effect on 2′ -dG quenching of 2-FlN+ than on quenching with DMB or TMB, which is in agreement with a system (2′ -dG–FlN) of enhanced hydrogen bonding capability.

When a quencher such as 2′ -deoxyinosine (which lacks the NH_2 group on position 3 of the guanosine moiety) is used, the bimolecular quenching rate constants in TFE:W mixtures drop significantly (Table 2). At 60% TFE, there is ca. a decrease of two orders of magnitude when compared to 2′ -dG quenching (3.1×10^5, and 5.6×10^7 M^{-1} s^{-1}, respectively). At 80% TFE, there is a decrease of almost three orders of magnitude in quenching rate constants with respect to 2′ -dG (Table 2, ca. 8×10^4 M^{-1} s^{-1} and from Table 3, 2.8×10^7 M^{-1} s^{-1}, respectively). The enhanced quenching observed of FlN+ with 2′ -dG (as opposed to the quenching of 2-FlN with 2′ -deoxyinosine) can be explained in terms of the stability of the intermediate (**4** in Scheme 2, $R = H$), where the extra amino group contributes both by accepting hydrogen donation from the solvent and by resonance stabilization.

2.2. Mixtures of hexafluoroisopropanol–water

The acidity and poor nucleophilicity of this solvent system containing hexafluoroisopropanol alcohol (HFIP) are enhanced with respect to TFE:W mixtures.

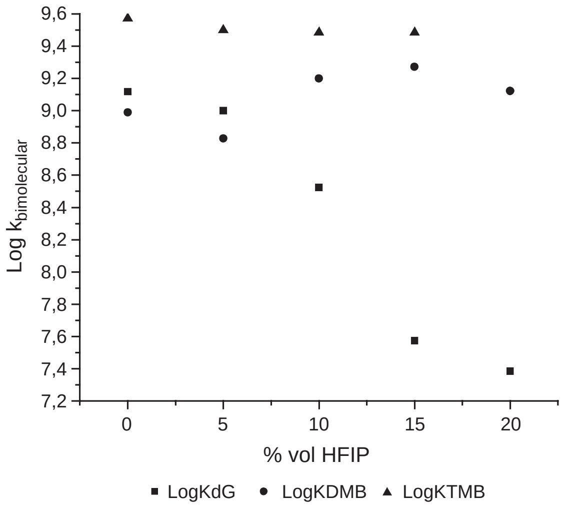

Fig. 5. Plots of the logarithm of bimolecular quenching rate constants (log$k$ in M^{-1} s^{-1}) of 2-FlN+ with 2′ -dG (squares), DMB (circles), and TMB (triangles) versus increasing HFIP (%HFIP) content.

Table 4
Bimolecular quenching rate constants of FlN+ with 2′ -dG, DMB, and TMB in HFIP:Water mixtures

%HFIP	$k_{q(2'\text{-dG})} \pm E10^9$ $M^{-1}\ s^{-1}$	$k_{q(DMB)} \pm E10^9$ $M^{-1}\ s^{-1}$	$k_{q(TMB)} \pm E10^9$ $M^{-1}\ s^{-1}$
5	1.00 ± 0.02	0.67 ± 0.03	3.1 ± 0.2
10	0.333 ± 0.009	1.58 ± 0.02	3.05 ± 0.06
15	0.03 ± 70.001	1.86 ± 0.03	3.05 ± 0.04
20	0.024 ± 0.002	1.32 ± 0.07	1.8 ± 0.2

TMB quenching shows very little change in rate constant with varying HFIP concentration, purporting to a diffusion limit rate of ca. $3.0 \times 10^9\ M^{-1}\ s^{-1}$ (Fig. 5, Table 4).

Regarding DMB quenching, a similar profile to that observed in TFE:W mixtures is observed. At about 5% HFIP content, a minimum is observed, before the actual drop in rate constant is noticed.

The large effect of HFIP:W mixtures on the bimolecular quenching rate constants of 2-FlN+ with 2′ -dG compared with the probe quenchers DMB and TMB is remarkable. The decays of FlN+ in the presence of DMB and TMB remain almost constant throughout the whole range of HFIP:W mixtures (Fig. 5). While up to 20% HFIP, both DMB and TMB quenching proceed at or near diffusion limit rates, 2′ -dG undergoes a two-order magnitude descent in rate constants going from pure water to 20% HFIP (1.3×10^9 and $2.4 \times 10^7\ M^{-1}\ s^{-1}$ respectively, Table 4).

3. Conclusions

This solvent study shows the scope of hydrogen bonding interactions in 2-FlN+ quenching with 2′ -dG, as can be inferred from Figs. 3–5, where the water containing solvents act as hydrogen-bond donors to **4.** The hydrogen bond stabilization argument is evidenced when comparing the stability of the complex FlN-2′ –dG+ in relation to the probe complexes FlN-DMB+ and FlN-TMB+, where the presence of the extra amino group on the guanine moiety of the complex Fl-2′ –dG+ confers the intermediate further stabilization (through hydrogen bonding capability) with respect to reactant (Fl+). Conversely, the lack of hydrogen bonding capability in the probe systems renders these probes less sensitive to variations in water content of the solvent mixtures. Further stabilization by resonance in the complex FlN-2′ –dG+ can be accounted for when comparing the slower rate constants observed of 2- deoxyinosine quenching with those of 2′ -dG by 2-FlN+. In the fluorinated solvent mixtures, where the fluorenyl nitrenium ion is a longer-lived species (the natural lifetimes of fluorenyl nitrenium ion in 20% ACN, 20% TFE, and 20% HFIP are 3.0×10^{-6}; 5.1×10^{-5}; and 1.5×10^{-4} s respectively) as compared to non-fluorinated mixtures, the rates of decay are highly accelerated as a consequence of the faster formation of the complex FlN-2′ -dG+ (**4**). We in fact observed that in fluorinated solvents (20%), the rates of decay match well the rates of formation (growth) of an intermediate (structure **4**).

Acknowledgment

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi: doi:10.1016/j.bpc.2005.09.014.

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