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Journal of Photochemistry and Photobiology A: Chemistry 103 (1997) 57–62

Journal of
PHOTOCHEMISTRY
AND
PHOTOBIOLOGY
A:CHEMISTRY

Solvent effect on the quenching of the mono-L-aspartyl chlorin e_6 triplet state by metronidazole

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Received 31 July 1996; accepted 17 October 1996

Abstract

The quenching of the triplet state of mono-L-aspartyl chlorin e_6 (^{3}MACE*) by the metronidazole ground state (METRO) has been investigated in 14 different binary mixtures of water and solvent by 532 nm laser flash photolysis. This quenching is a diffusion-controlled process in water with a quenching constant of $k_Q^0 = 1.9 \times 10^9\ M^{-1}\ s^{-1}$. In neat solvent, the quenching of ^{3}MACE* by METRO is not observed. The quenching constant is reduced in binary mixtures. The dependence of k_Q^0 in the mixture on the solvent molar fraction may be expressed by

$$\ln k_Q = \ln k_Q^0 - \gamma_{solv} \times (M.F.)_{solv}$$

The activation barrier of quenching on going from water to water–solvent mixtures is unchanged and in the range 3–4 kcal mol^{-1}. The solvent effect is analysed in terms of specific solute–water–solvent interactions. A mechanism for the quenching of ^{3}MACE* by METRO is proposed.

Keywords: Electron transfer; Hydrogen bond; Metronidazole; Mono-L-aspartyl chlorin e_6; Solvent effect; Triplet state

1. Introduction

In the past decade, porphyrins (P) and their derivatives have been the subject of extensive study due to their use as photosensitizers in the so-called "photodynamic therapy" (PDT) of cancers [1,2]. The mechanism responsible for cellular destruction is believed to involve the intermediate formation of singlet molecular oxygen $^1O_2(^1\Delta_g)$ via the triplet excited state ($^3P^*$) of the photosensitizer (type II photo-oxidation) by energy transfer [3,4]

$$^3P^* + {}^3O_2 \rightarrow P + {}^1O_2 \qquad (1)$$

However, in some situations, other photochemical reactions of porphyrins can lead to the formation of very reactive species such as porphyrin radical cation ($P^{\cdot +}$). Thus we have reported previously that nitroimidazoles (RNO_2) can strongly enhance the photosensitizing capacity of porphyrins towards certain substrates, such as Trp, Tyr and Cys (type I photo-oxidation), by photoinduced electron transfer [4,5]

$$^3P^* + RNO_2 \rightarrow P^{\cdot +} + RNO_2^{\cdot -} \qquad (2)$$

$$P^{\cdot +} + \text{substrate} \rightarrow P + \text{product(s)} \qquad (3)$$

We have also shown that nitroimidazoles, such as metronidazole and misonidazole, react with triplet porphyrins in water (Eq. (2)) according to a diffusion-controlled reaction [4,5].

It is well known that the efficiency of porphyrin photosensitization (Eq. (1) and Eq. (2)) depends on a variety of factors, such as the tendency of many porphyrins to undergo aggregation in aqueous solution [6], the photobleaching of porphyrins [7] and the changes in energy of the first excited singlet state of porphyrins as a result of interaction with the medium [8]. Moreover, there is no doubt that the effectiveness of photoinduced electron transfer (Eq. (2)) between porphyrins and nitroimidazoles bearing a charge-localizing NO_2 group depends strongly on the microenvironment or chemical composition of the solution around the molecules at the collision complex state. However, few systematic studies of solvent effects on the efficiency of porphyrin photosensitization have appeared.

A major problem encountered with PDT is the photosensitizing light penetration into tissues. This penetration is at a

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Abbreviations: MACE, mono-L-aspartyl chlorin e_6; ^{3}MACE*, first excited triplet state of mono-L-aspartyl chlorin e_6; METRO, metronidazole.

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PII S1010-6030(96)04529-7

maximum in the 650–800 nm range. To replace porphyrins, whose absorption is too close to that of haemoglobin in tissues, so-called "second-generation photosensitizers" have been synthesized. Of these, chlorins are very promising new photosensitizers. Thus Spikes and Bommer [9] reported that, like porphyrins, mono-L-aspartyl chlorin e_6 (MACE) is a quite effective type II and, in some circumstances, type I (electron transfer to electrophilic substrates [4,5]) photodynamic photosensitizer. In cells, depending on their hydrophobic character, photosensitizers tend to localize in hydrophilic or lipophilic structures. At the site of localization, the solvation of the photosensitizers and substrates leads to the creation of a more or less polar environment. The latter may influence type I and type II processes.

The bimolecular reaction rate constants for the quenching of triplet states by oxygen ($^1O_2$ formation) are rather insensitive to the polar or non-polar nature of the solvent. The quenching is generally diffusion controlled. On the other hand, triplet quenching may also be due to electron transfer from a donor triplet state to an acceptor substrate or vice versa. In this case, large changes in the reaction rate constant as a function of the solvation of the reactants and products by more or less polar solvent molecules are not unexpected. Temperature studies of the solvent effects on the electron transfer may provide additional information about the steps involved in this process.

This paper reports a detailed laser flash photolysis study of the effects of solvent and temperature on the kinetic parameters of electron transfer between the triplet state of MACE (^{3}MACE*) and metronidazole (METRO) in various solvent–water mixtures.

2. Experimental details

2.1. Materials

MACE was a generous gift from Professor K.M. Smith (University of California at Davis). METRO was obtained from Janssen Chimica (Beerse, Belgium). Alcohols of the purest available grade were provided by Merck or by Fluka. *N*-Methylformamide, dimethylsulphoxide and 1,4-dioxan were supplied by Merck and acetonitrile by Janssen Chimica. These were used without further purification. Water was bidistilled in a quartz still from Quartex (Paris, France). Aqueous solutions were buffered with sodium phosphates (100 mM, pH 7.4). To avoid possible degradation or aggregation of MACE, solutions were prepared according to the procedure described by Spikes and Bommer [9].

2.2. Measurements

Absorption spectroscopy was performed with a UVIKON-941 spectrophotometer (Kontron Instruments, France). The laser flash photolysis apparatus, which utilizes a YAG laser YG44 provided by Quantel S.A. (Massy, France), has been described previously [10]. The optical cell, excitation path length and analysing light conditions (perpendicular to the excitation laser light path) were the same as those used by Reyftmann et al. [11]. The kinetics of the transients with $\tau > 50$ ns were digitized, averaged (at least ten pulses) and subsequently analysed using the Hewlett-Packard 98820 statistical library. The temperature of the cell holder was adjusted by a thermostatically controlled bath (Huber, Germany). Temperature measurements were carried out using a YSI-401 thermistor (Yellow Springs Instrument Co., USA) attached to the optical cell. The precision of such measurements was ±0.5 °C. All the experiments were carried out in argon-saturated solutions.

3. Results and discussion

3.1. Triplet state properties

The 532 nm laser flash photolysis was carried out in buffered water, neat alcohols, acetonitrile, dimethylsulphoxide, *N*-methylformamide, 1,4-dioxan and in binary solvent–water mixtures. Transient kinetic measurements were made at 440 nm and at 690 nm corresponding to the triplet–triplet absorption of MACE and its radical cation respectively [9,12]. In air-saturated water, ^{3}MACE* is quenched by ground state oxygen (Fig. 1(A); transient 1) with a bimolecular reaction rate constant of 1.3×10^9 M^{-1} s^{-1} [9]. Therefore, in order to avoid the quenching of ^{3}MACE* by O_2, studies were carried out with samples bubbled for 10 min with argon prior to laser flash photolysis. In argon-saturated water, ^{3}MACE* decays by first-order kinetics with a rate constant $k_T^0 \sim 10^4$ s^{-1} (Fig. 1(A); transient 2). On going from water to neat solvents, the changes in k_T^0 were within the accuracy of the kinetic analysis, i.e. k_T^0 was practically solvent independent.

3.2. Quenching of MACE triplet state by METRO in water

In the presence of METRO, the initial, fast, triplet decay is followed by a slow decay (Fig. 1(B); transient 1). The decay of the short-lived triplet species follows the expected concentration dependence (Fig. 2)

$$k_T = k_T^0 + k_Q[\text{METRO}] \tag{4}$$

where k_T and $k_T^0 = 1/\tau_T$ are the rate constants of the transient decays measured in the presence and absence of METRO respectively and k_Q is the quenching constant. In water, the quenching of ^{3}MACE* by METRO occurs with $k_Q^0 = (1.9 \pm 0.2) \times 10^9$ M^{-1} s^{-1} at 22 °C. This value was found to be in good agreement with that reported previously [4,9]. We have attributed this quenching to electron transfer from ^{3}MACE* to METRO. Indeed, the appearance of a long-lived transient species at 690 nm (Fig. 1(C); transient 1) reflects the formation of the chlorin ring radical cation ($Chl^{\cdot +}$) whose transient absorption extends to the red and infrared [12].

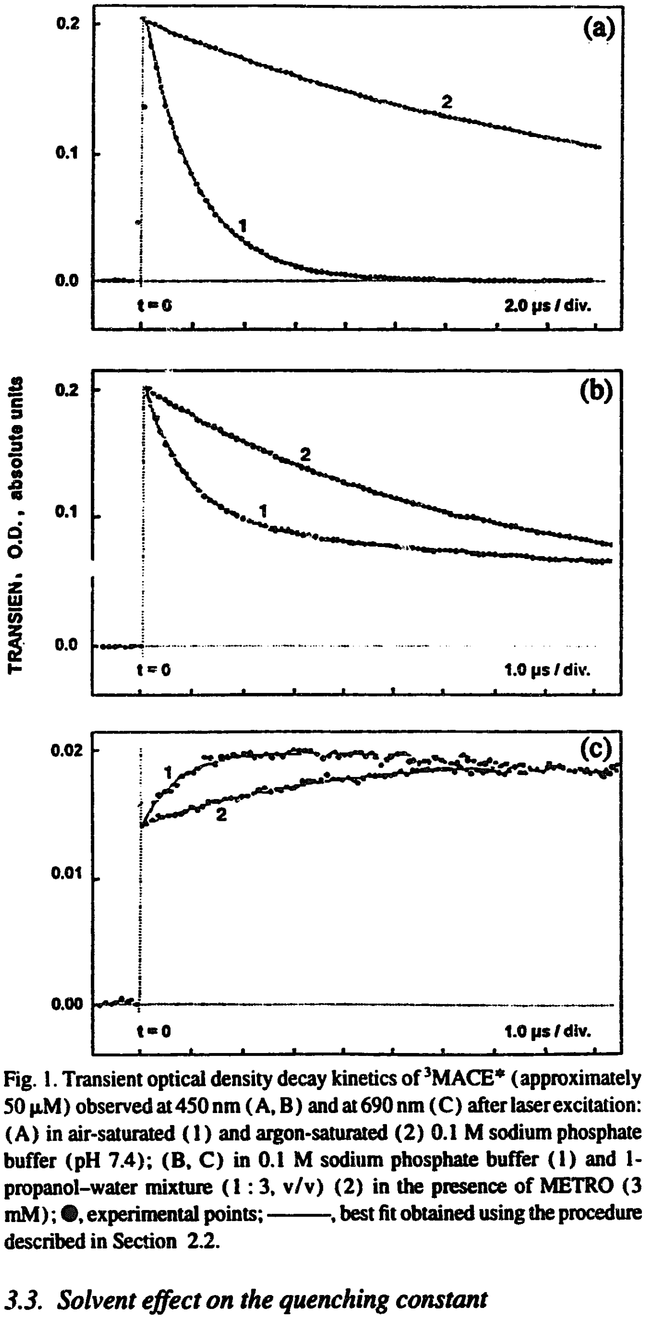

Fig. 1. Transient optical density decay kinetics of ^{3}MACE* (approximately 50 µM) observed at 450 nm (A, B) and at 690 nm (C) after laser excitation: (A) in air-saturated (1) and argon-saturated (2) 0.1 M sodium phosphate buffer (pH 7.4); (B, C) in 0.1 M sodium phosphate buffer (1) and 1-propanol–water mixture (1 : 3, v/v) (2) in the presence of METRO (3 mM); ●, experimental points; ———, best fit obtained using the procedure described in Section 2.2.

3.3. Solvent effect on the quenching constant

A decrease in the quenching constant k_Q is observed on addition of any of the investigated solvents to an aqueous solution containing MACE and METRO (Fig. 1(B); transients 1 and 2). This decrease parallels a reduction in the rate constant of $Chl^{\cdot +}$ formation (Fig. 1(C); transients 1 and 2). It should be noted that, in the presence of METRO in neat solvents and in water in the absence of METRO, the values of the rate constant of ^{3}MACE* deactivation are similar. This observation strongly suggests that the quenching of ^{3}MACE* by METRO is not observed in neat solvents other than water.

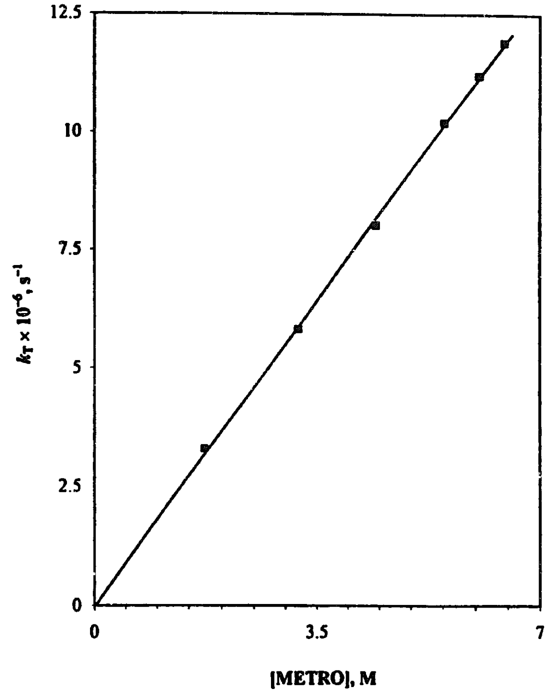

Fig. 2. Stern–Volmer plot for the quenching of ^{3}MACE* by METRO in 0.1 M sodium phosphate buffer (pH 7.4); [MACE] = 50 µM; temperature, 22 °C.

In all the solvent–water mixtures investigated, the rate constant for the quenching of ^{3}MACE* by METRO is at a maximum in water and exponentially decreases as a function of the solvent molar fraction (Fig. 3). The decrease in the quenching constant (k_Q) can be represented by the following equation

$$\ln k_Q = \ln k_Q^0 - \gamma_{solv} \times (M.F.)_{solv} \qquad (5)$$

where γ_{solv} is a proportionality coefficient depending on the kind of solvent and $(M.F.)_{solv}$ is the molar fraction of solvent in the aqueous mixture. The γ_{solv} parameters obtained for the different solvent–water mixtures investigated are collected in Table 1.

It should be noted that Eq. (5) does not hold when the quantity of solvent in water is less than approximately 5 vol.%. It seems likely that significant changes in the water structure occur on addition of a few per cent of solvent. In other words, with low amounts of solvent in water, microheterogeneous structures are formed in solvent–water mixtures, whereas increasing the amount of solvent leads to more homogeneous structures.

Since the lifetime of ^{3}MACE* does not vary on going from water to neat solvents, we exclude the possibility of an interaction between ^{3}MACE* and the water or solvent molecules that might lead to deactivation of ^{3}MACE* [13–15]. Furthermore, in neat solvents in the presence of METRO, the formation of a collision complex between ^{3}MACE* and METRO probably does not result in electron transfer since, as noted above, the rate constant of ^{3}MACE* deactivation is about the same as the rate constant of ^{3}MACE* deactivation in water or in neat solvents in the absence of METRO. The modulation of the electron transfer rate between ^{3}MACE* and METRO by water molecules may thus be the determining factor governing the quenching constant (Eq. (5)).

Table 1
Solvent effect on the γ_{solv} parameter obtained from Eq. (5), molar volumes $(MV)_{solv}$ of solvents used in the preparation of various binary solvent–water mixtures and energies of activation (E_a) for some solvent–water mixtures

Number	Solvent	$(MV)_{solv} \times 10^3$ (M^{-1})	γ_{solv} (Eq. (5))	E_a (kcal mol^{-1})
1	Water	18.0	0	3.9±0.5
2	Methanol	40.6	2.1±0.2	3.2±0.5
3	Acetonitrile	52.2	3.6±0.5	
4	1,2-Ethanediol	55.8	6.4±0.5	
5	2-Fluoroethanol	57.8	5.5±0.2	
6	*N*-Methylformamide	59.1	6.5±0.5	3.6±0.25
7	2-Chloroethanol	67.0	9.6±1.0	
8	Dimethylsulphoxide	71.0	10.3±0.5	
9	2,2,2-Fluoroethanol	72.0	9.7±1.0	
10	1,3-Propanediol	72.3	10.5±0.5	
11	1-Propanol	75.1	15.0±2.0	3.2±0.5
12	2-Propanol	77.1	12.4±1.5	
13	1,4-Dioxan	85.5	12.9±1.5	3.2±0.5
14	1-Butanol	91.5	22.3±4.0	3.0±0.5
15	2-Butanol	91.8	20.1±4.0	

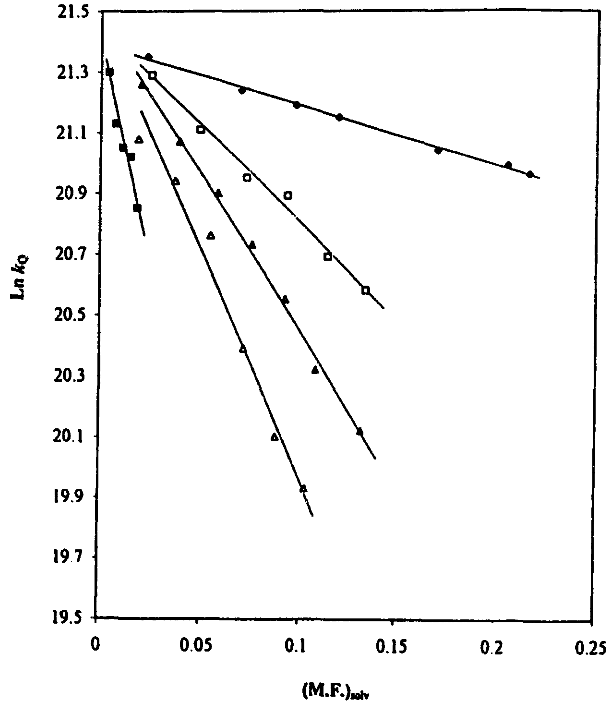

Fig. 3. Variation of the quenching constant k_Q of the MACE triplet state by METRO as a function of the solvent concentration in water: ◆, methanol; □, 1,2-ethanediol; ▲, dimethylsulphoxide; △, 1-propanol; ■, 1-butanol. Temperature, 22 °C.

3.4. Temperature dependence of the quenching

The Arrhenius plots obtained for the different binary solvent–water mixtures are shown in Fig. 4. The values of the activation energy (E_a) can be readily obtained from the Arrhenius plots using the relationship

$$k_Q = A\exp(-E_a/RT) \qquad (6)$$

where A is a pre-exponential (frequency) factor. The values of E_a are given in Table 1.

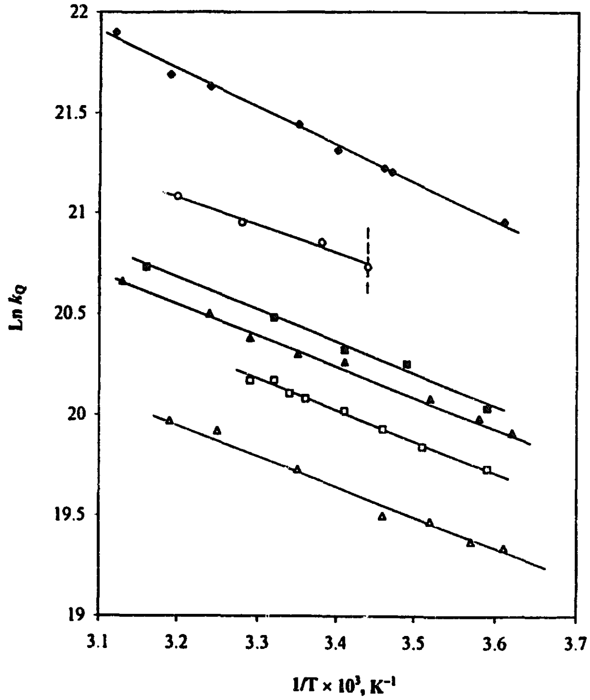

Fig. 4. Arrhenius plots for the quenching constant of ^{3}MACE* by METRO in 100 mM sodium phosphate buffer (◆), methanol (70% v/v)–buffer mixture (■), 1-propanol (40% v/v)–buffer mixture (△), 1,4-dioxan (29% v/v)–buffer mixture (▲), *N*-methylformamide (32% v/v)–buffer mixture (□) and 1-butanol (8.8% v/v)–buffer mixture (○). In the last case, at a temperature below 18 °C (vertical line), the separation of an aqueous solution of 1-butanol into two liquid phases occurs.

The temperature dependence of the quenching constant (Fig. 4) reveals that the activation barrier in water is approximately 4 kcal mol^{-1}. This barrier is the same as that expected for the diffusion of water molecules (approximately 5 kcal mol^{-1}) as estimated from Murov et al. [16]. Similar E_a values are obtained in solvent–water mixtures (Table 1). In view of these data, we suggest that the decrease in the quenching constant in water–solvent mixtures is related to the effective collision frequency between the ^{3}MACE*–METRO complex and the water molecules.

3.5. Some comments on the quenching mechanism

The present experimental data demonstrate the following:

1. the absence of interaction of ^{3}MACE* with water or solvent molecules that might lead directly to deactivation of ^{3}MACE*, whereas the quenching constant of ^{3}MACE* by METRO is at a maximum in water;
2. the absence of quenching of ^{3}MACE* by METRO in neat solvents;
3. the energy of activation of quenching is practically unchanged on going from water to neat solvents and is close to the energy of activation expected for the diffusion of water molecules.

Now a question arises: how can the mixing of water with solvent so strongly perturb the photophysics of the quenching process?

Under conditions in which microhomogeneous binary solvent–water mixtures are obtained, the relative change in the H_2O concentration ($\delta_{[\mathrm{water}]}$) can be represented by $\delta_{[\mathrm{water}]} = ([\mathrm{water}]^0 - [\mathrm{water}])/[\mathrm{water}]^0$, where $[\mathrm{water}]^0$ and $[\mathrm{water}]$ are the concentrations of water molecules in aqueous solution (55 M) and in the solvent–water mixture respectively. On the other hand, $\delta_{[\mathrm{water}]}$ is given by

$$\delta_{[\mathrm{water}]} = (\mathrm{MV})_{\mathrm{solv}} \times [\mathrm{solvent}] \qquad (7)$$

where $(\mathrm{MV})_{\mathrm{solv}}$ is the molar volume of the added solvent to water. From Eq. (5) and Eq. (7), it follows that

$$\ln k_Q = \ln k_Q^0 - (\gamma_{\mathrm{solv}}/(\mathrm{MV})_{\mathrm{solv}})\,\delta_{[\mathrm{water}]} \qquad (8)$$

If the decrease in the quenching constant on going from water to solvent–water mixtures is only related to the decrease in water concentration, it follows from Eq. (8) that

$$\gamma_{\mathrm{solv}} = \mathrm{constant} \times (\mathrm{MV})_{\mathrm{solv}} \qquad (9)$$

Fig. 5 shows that the γ_{solv} parameter increases with increasing $(\mathrm{MV})_{\mathrm{solv}}$, but more steeply than predicted by Eq. (9). In fact, Eq. (9) only takes into account the effect of $(\mathrm{MV})_{\mathrm{solv}}$ of the added solvents on the water concentration. In our opinion, the observed curvature should be related to properties of the solvent other than the molar volume, e.g. to the "polarity" in the more general definition. Thus the solvent polarity may be described in terms of the dielectric constant (Kirkwood function), refractive index and/or empirical parameters of polarity/polarizability and specific water–solvent–solute interactions. In water–solvent mixtures, a good correlation between γ_{solv} or $\ln k_Q$ and any one of the above solvent parameters is not observed.

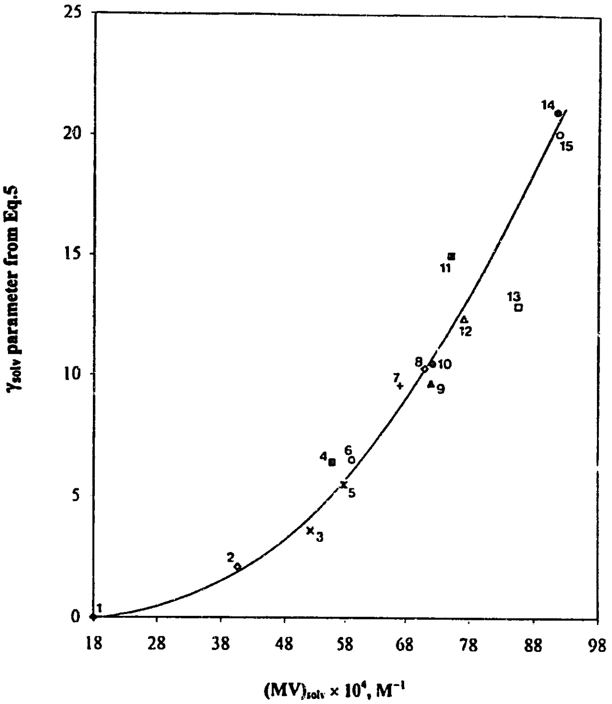

Fig. 5. Correlations between γ_{solv} values in binary solvent–water mixtures and $(\mathrm{MV})_{\mathrm{solv}}$ values of solvents used in the mixtures. Numbers correspond to the solvents shown in Table 1.

Thus, considering the Kirkwood function, the dielectric continuum model used is not valid when solvent–solvent specific interactions, such as hydrogen bonding, occur [17]. On the empirical scale of polarity (such as Dimroth–Reichardt's $E_T(30)$ or Kamlet–Taft's solvatochromic parameters) [17], the paucity of data on water–solvent mixtures precludes any statistical use and the calculations with weighted parameters related to pure solvents often give poor results. However, it seems that, at least qualitatively, at a given water concentration, the lower the solvent polarity, the higher its hydrophobicity and the inhibition of electron transfer. Thus the action of solvents on water may not only result from a change in concentration, but also from a change in the activity of water.

With the above assumption linking the decrease in the quenching constant to a decrease in the water concentration and activity, these changes would modify the collision frequency between water molecules and the ^{3}MACE*–METRO complex, but not the activation barrier of quenching [18,19].

We suggest that, during the lifetime of the ^{3}MACE*–METRO collision complex, the formation of a hydrogen bond probably takes place between METRO and water molecules, inducing electron transfer from ^{3}MACE* to METRO, and the addition of solvent to water modifies the frequency of formation of the hydrogen bond.

The effectiveness of METRO as an electron acceptor is determined by the presence of the charge-localizing nitro group in this molecule. Indeed, the quenching of the triplet states of porphyrins and their derivatives by nitroimidazoles [5] and 1,4-benzoquinone [9], but not by histidine [5] or imidazole [20], has been observed. It is well known that if a solvent, through hydrogen bonding, promotes the electron density shift required in the reaction, the rate of reaction will be increased by this solvent [18]. For neat alcohols, the hydrogen bonding ability of the OH group increases in the following sequence [21,22]; long chain alcohols <

$CH_3OH < H_2O$. Thus it may be expected that the electron density shift and, consequently, the solvent effect will be at a maximum in water. This hypothesis is strengthened by the fact that the nitro substitution, required for electron transfer, tends to increase the hydrophobic character of nitroaromatic compounds [23]. In pure alcohols, the hydrophobic character may be increased as a result of the interaction between the aliphatic chain of the alcohol and the imidazole ring of METRO. Thus the hydrogen bonding between the H_2O molecules and the nitro group of METRO may withdraw electron density from the imidazole ring of METRO to the hydrogen bond [21,24]. This will result in an increasing electron affinity of METRO for $^3MACE^*$. In pure solvents, the shift in electron density to the hydrogen bonds between the solvent molecules and METRO is probably not sufficient. In this case, the reorganization of the collision complex to an optimal conformation is induced by the replacement of solvent molecules by water in the formation of a hydrogen bond with the NO_2 group of METRO.

Hence the following reaction steps, leading to the optimal conformation of the collision complex in water, may be suggested

$$^3\mathrm{MACE}^* + \mathrm{METRO} \rightarrow [^3\mathrm{MACE}^* \cdots \mathrm{RNO_2}] \qquad (10)$$

$$[^3\mathrm{MACE}^* \cdots \mathrm{RNO_2}] + \mathrm{H_2O} \rightarrow [^3\mathrm{MACE}^* \cdots {}^{\delta+}\mathrm{RNO_2^{\delta-}} \cdots {}^{\delta+}\mathrm{HO}^{\delta-}\mathrm{H}]_{opt} \qquad (11)$$

$$[^3\mathrm{MACE}^* \cdots {}^{\delta+}\mathrm{RNO_2^{\delta-}} \cdots {}^{\delta+}\mathrm{HO}^{\delta-}\mathrm{H}]_{opt} \rightarrow \mathrm{MACE}^{\cdot +} + \mathrm{RNO_2^{\cdot -}} + \mathrm{H_2O} \qquad (12)$$

Thus hydrogen bond formation between H_2O and the NO_2 group of METRO (Eq. (11)) shifts some electron density to the hydrogen bond. This shift strengthens the electron acceptor property of METRO, allowing the organization of the collision complex into the optimal conformation (Eq. (11)) from which electron transfer probably occurs (Eq. (12)). In pure alcohols and *N*-methylformamide, which have weaker hydrogen bonding ability than water, and in aprotic solvents, such as acetonitrile, dimethylsulphoxide and 1,4-dioxan, the reaction described by Eq. (11) is not effective or is not possible.

Acknowledgements

We are pleased to thank Dr. Larry K. Paterson (Radiation Laboratory, Notre Dame University, IN, USA) for helpful discussions. One of us (S.V.K.) was supported by a Postdoctoral Position from the Ministère de l'Enseignement Supérieur et de la Recherche (France).

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