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Singlet quenching of chlorophyll *a* by nitroaromatics. Effect of solvents on the electron transfer from excited singlet of chlorophyll *a* to nitroaromatic acceptors

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Key words: Singlet emission quenching; Chlorophyll *a*; Nitroaromatic; Charge transfer; Electron transfer; Solvent effect

Optical absorption and singlet emission properties of chlorophyll *a* in the presence of diverse nitroaromatic acceptors of varying ring size and electron affinity have been investigated. Chl *a* forms 1:1 intermolecular complexes with the acceptors and the nature of interaction between the donor Chl *a* and the acceptor has its origin in charge transfer. The K_{sv} values are found to vary linearly with the electrochemical reduction potentials of the acceptors. The oxidation potential of the excited singlet state of Chl *a*, Chl a^*/Chl a^+ is found to be 1.28 V vs. saturated calomel electrode. The rates of bimolecular quenching (k_q) increase linearly with the change in free-energy for the excited-state electron transfer reaction in the weakly exergonic region, indicating that the electron transfer reaction is predominantly controlled by the activation energy. In the moderate exergonic region, the k_q values increase exponentially, with ΔG_{23} values without the exhibition of inverted region even upto a ΔG_{23} value of -100 kJ·mol^{-1}. This has been ascribed to the presence of low-lying electronically excited state in the ion-pair. The ΔG_{23} values have been used to distinguish the various acceptors based on their ability to form ionic or covalent exciplexes with Chl *a*. Ten solvents of varying polarizability and viscosity have been employed to study the influence of the rates of excited state electron transfer in Chl *a*–tetranitrofluorenone system. The solvent reorganization energies are found to over-estimate the magnitudes of free-energy change for radical-pair formation, ΔG_{rip}. It is shown that specific solvent effects, viz. H-bonding and coordinating ability, contribute to the k_q values. It is found that solvents can interact specifically with Chl *a* exhibiting maximum efficiency of quenching of the singlet emission of Chl *a* by the acceptor.

Introduction

The most important reaction in photosynthesis is the light-induced electron transfer from the donor chlorophyll in its excited singlet state to an acceptor strategically positioned close to the donor. The rates of vectorial forward electron transfer depend on many factors, viz., distal separation between the donor and acceptor, the molecular geometry of donor-acceptor complex, the extent of orbital overlap between the donor and acceptor and the nature of the intervening medium. Much work has been concerned with the investigations of these parameters. The motivation of this study is to investigate the donor functions of the cell

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Abbreviations: Chl, chlorophyll; Pheo, pheophytin; SCE, saturated calomel electrode.

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free pigment, Chl *a*, in a systematic manner with a view to learning more about the properties of the ground- and excited-state complexes and their dependence on the rates of excited state electron transfer which may have relevance to the understanding of the natural photosynthetic process.

The photochemistry of Chl *a* has been the subject of many investigations. It is known that the singlet emission of Chl *a* is quenched by quinones and nitroaromatics wherein the donor function essentially rests on Chl *a*. These data have been interpreted as evidence for excited-state electron transfer, though no experimental evidence for radical-pair formation is available [1]. Our earlier studies [2,3] on the intermolecular complexes of plant pigments pointed out the charge transfer nature of the molecular interaction and the manner in which the presence of exciplexes in these systems modulates the rate of excited-state electron transfer reaction. It is of interest to investigate whether similar features prevail in the Chl *a*-acceptor systems and also to investigate the nature of the effects of the medium on the rates of excited-state electron transfer reactions.

It is known that solvents influence all the measurable properties of the electron donor-acceptor complexes in the ground and excited states. The photophysical properties and the lifetime of Chl *a* are shown to exhibit a remarkable solvent dependency [4]. The quenching of triplet state of Chl *a* by benzoquinone is affected by the viscosity and dielectric constant of the solvents [5]. However, studies on the singlet quenching of Chl *a* by 2,5-dimethyl-*p*-benzoquinone in different solvents [6] reveal that the polarity of solvents has very little influence in governing the magnitudes of quenching constants (K_{sv}). It is recognized that the solvent parameters bear a complex dependence on the emission of the fluorophore and quenching properties. The interaction of a solvent with a fluorophore can be described in terms of orientation polarizability which takes into account the high- and low-frequency polarizabilities. In order to study the effects of these parameters on the excited-state electron transfer from Chl *a* to nitroaromatics, we have investigated the singlet quenching reactions of Chl *a* with 2,4,5,7-tetranitrofluorenone in ten solvents.

Here, the electron donor-acceptor complexes of Chl *a* with several nitroaromatic acceptors have been investigated. Eight nitroaromatics of varying electron affinity are chosen for the study. The wide range of reduction potentials of the acceptors is helpful in delineating the electron transfer mechanisms. The purpose of this study is to reveal the importance of charge transfer complexation, both in the ground and excited states and the manner in which the charge transfer interaction influences the rates of excited state electron transfer reactions. The choice of solvents of varying viscosity and dielectric constants permit a detailed study of the factors that influence the rates of electron transfer reactions. This study will show that the free-energy change for electron transfer from the excited singlet state of Chl *a* is a function of the electron affinity of the acceptors. It is demonstrated that, in addition to the solvent parameters, viscosity and dielectric constant, the nature of solvent (ability to H-bond and/or coordinate) causes large changes in the bimolecular quenching rates.

Materials and Methods

The source of Chl *a* in the present study is *Amaranthus caudatus*. Extraction of pigments was carried out according to the method described by Svec [7], with a few modifications. Chlorophyll was precipitated with the addition of water, dioxan and methanol to the diethyl ether extract [8] to simplify the separation process. The precipitated chlorophyll was chromatographed according to the procedure of Petrovic and Kolarov [9]. The molar absorption coefficient of Chl *a* obtained in this manner was found to be 61 000 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$ at 662 nm in methylene chloride and the ratio of intensities of absorption bands in the blue and red regions was 1.31. These data agree well with those reported by Pennington et al. [10].

The nitroaromatic acceptors 1,4-dinitrobenzene, 1,3,5-trinitrobenzene, 2,4,6-trinitrotoluene, 2,4,7-trinitro-9-fluorenone, 2,4,5,7-tetranitro-9-fluorenone and 9-dicyanomethylene-2,4,7-trinitrofluorenone were procured from Aldrich Chemicals (U.S.A.) and recrystallized from appropriate solvents. 1,3-Dinitrobenzene was prepared in our laboratory according to the procedure of Vogel [11]. AR grade nitrobenzene was obtained from

BDH (India). Reagent-grade dichloromethane, obtained from BDH (India), was washed twice with 10% aqueous sodium carbonate followed by water. It was dried over anhydrous $CaCl_2$ and distilled. AR-grade chloroform, acetone, ethyl acetate, acetonitrile, benzene, diethyl ether, toluene, ethanol, methanol and 1,4-dioxan procured from BDH (India) were used as such. Cellulose for TLC was from Acme's Synthetic Chemicals (Bombay, India).

The spectrophotometers and the experimental techniques employed are the same as described in our earlier papers [2,3]. The intermolecular complexation was studied using absorption and emission spectral methods. The concentrations of the acceptors were 10 μM and 1 mM for the absorption and emission spectral studies, respectively.

Results

The complexation of Chl *a* with nitroaromatic acceptors in the ground state was followed by visible absorption spectroscopy by monitoring the red absorption maxima of Chl *a* on addition of increasing concentration of the acceptor. The absorption spectra of Chl *a* containing varying amounts of 9-dicyanomethylene 2,4,7-trinitrofluorenone are shown in Fig. 1. The spectra reveal a number of isosbestic points. The decrease in intensity of the red absorption band on addition of increasing amounts of the acceptors was analysed using the method developed by Nash [12]. The Nash plots (Fig. 2) are found to be linear, indicating that the stoichiometry of the molecular complexes formed in solution is predominantly 1 : 1. The binding constants, K, are evaluated from the Nash plots using linear least-square analysis. Attempts to recover unmodified Chl *a* quantitatively from the solutions containing Chl *a* and acceptors have not been successful. It has been possible to estimate the thermodynamic parameters of complexation, change in enthalpy (ΔH) and change in entropy (ΔS) from the K values measured at different temperatures (Table I).

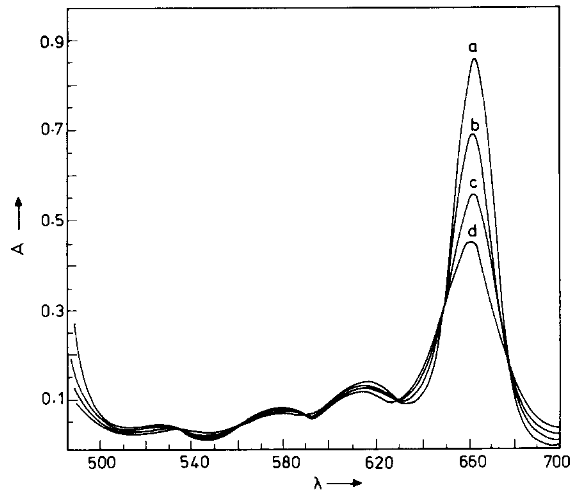

Fig. 1. Absorption spectrum of (a) Chl *a* (0.02 mM) and (b–d) Chl *a* containing varying concentrations (0.1 mM, 0.5 mM and 3.2 mM, respectively) of 9-dicyanomethylene-2,4,7-trinitrofluorenone in methylene chloride at 25 ° C.

Intensities of the emission bands of Chl *a* (669 nm and 716 nm (sh)) are quenched on addition of increasing concentrations of the acceptors. The quenching process follows the Stern-Volmer relation $I_0/I = 1 + K_{sv}[A]$, where I_0 and I represent the intensities of Chl *a* fluorescence in absence and in presence of a known concentration of the acceptor, respectively, and K_{sv} is the Stern-Volmer constant of bimolecular quenching. The K_{sv} values were obtained from the plots of I_0/I vs. [A]

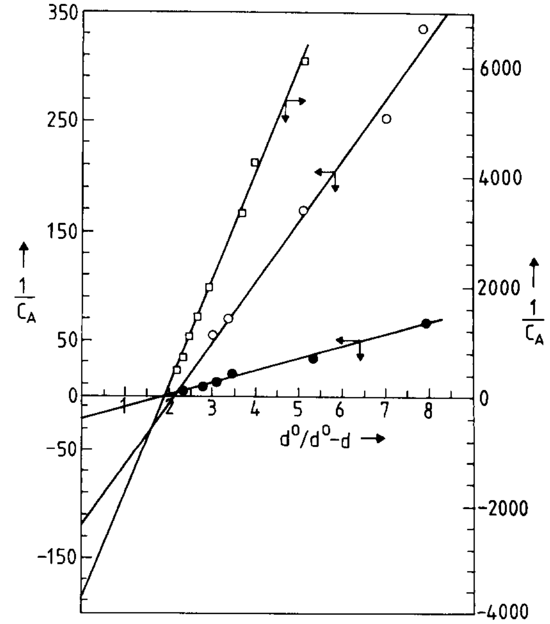

Fig. 2. Nash plots for the interaction of Chl *a* with 9-dicyanomethylene 2,4,7-trinitrofluorenone (□), 2,4,5,7-tetranitrofluorenone (○) and (●) *m*-1,3-dinitrobenzene in methylene chloride at 25 ° C.

TABLE I

MOLECULAR COMPLEXATION PARAMETERS OF CHLOROPHYLL *a* WITH NITROAROMATIC π-ACCEPTORS IN METHYLENE CHLORIDE

Abbreviations: NB, nibrobenzene; *m*DNB, 1,3-dinitrobenzene; *p*DNB, 1,4-dinitrobenzene; TNB, 1,3,5-trinitrobenzene; TNF, 2,4,7-trinitro-9-fluorenone; TENF, 2,4,5,7-tetranitro-9-fluorenone; CNTNF, 9-dicyanomethylene-2,4,7-trinitro-9-fluorenone; TNT, 1,3,5-trinitrotoluene.

Acceptor	EA [a]	Temp. (°C)	K ($\mathrm{dm^3 \cdot mol^{-1}}$)	$-\Delta G$ ($\mathrm{kJ \cdot mol^{-1}}$) at 25 °C	$-\Delta H$ ($\mathrm{kJ \cdot mol^{-1}}$) at 25 °C	$-\Delta S$ ($\mathrm{J \cdot mol^{-1} \cdot K^{-1}}$)
1 NB	1.30	22	0.01	–	–	–
2 *m*DNB	1.35	22	19 ± 1.2	7.2 ± 1.0	29.1 ± 3.0	7.4 ± 1.5
		10	30 ± 2.5			
		0	50 ± 4.8			
3 TNT	1.55	20	20 ± 3.0	7.3 ± 1.2	40.3 ± 3.8	11.3 ± 3.0
		10	35 ± 3.5			
		0	80 ± 4.0			
4 *p*DNB	1.75		– [b]	– [b]	– [b]	– [b]
5 TNB	1.75	20	55 ± 2.5	9.81 ± 1.1	27.2 ± 2.2	5.90 ± 1.2
		10	80 ± 3.5			
		0	125 ± 8.0			
6 TNF		24	90 ± 11	11.1 ± 2.1	81.3 ± 9.8	23.6 ± 5.0
		15	271 ± 22			
		10	400 ± 30			
7 TENF	2.20	22	116 ± 10	11.7 ± 1.8	37.4 ± 3.0	8.7 ± 1.2
		10	180 ± 20			
		0	274 ± 21			
8 CNTNF	2.45	22	3700 ± 200	20.2 ± 2.8	21.0 ± 1.8	0.3 ± 0.2
		14	4640 ± 200			
		7	6000 ± 310			
		0	7800 ± 350			

[a] The EA values are taken from Ref. 13.

[b] Values not determined.

(Fig. 3), using linear least-square analysis. The values of K_{sv} obtained in this fashion were found to be in agreement with those obtained when the excitation was carried out at an isosbestic point (630 nm in Fig. 1). The oxidation potential of the singlet excited Chl *a* was obtained by plotting the K_{sv} values against the reduction potentials (versus SCE) of the quenchers (Fig. 4). The intercept of the abscissa occurs at −1.28 V. This is in agreement with the value (−1.23 V) obtained using the expression [14].

$$E_{1/2}(\mathrm{Chl}\ a^{+}/\mathrm{Chl}\ a^{*}) = E_{1/2}(\mathrm{Chl}\ a^{+\cdot}/\mathrm{Chl}\ a) - E_{\infty} \qquad (1)$$

The first ring oxidation potential of Chl *a* in methylene chloride measured in our laboratory was found to be 0.62 V. The energy of emission band (E_{∞}) of Chl *a* in methylene chloride is 1.85 eV.

The lifetime of Chl *a* in methylene chloride is assumed to be 6 ns, since this value agrees well with the lifetime of Chl *a* in solvents of similar viscosity and dielectric constants [4]. The rate constants of bimolecular quenching (k_q), for the different acceptor-Chl *a* systems were obtained using this value. These k_q values are listed in Table II along with the first reduction potentials of the acceptors. The rate constant of the diffusion limited reaction (k_d) was estimated using the expression [17], $k_d = 8\ RTp/2000\ \eta$. The values of k_d were obtained using the viscosities of solvents at 25 °C [18] and assuming every collision to be

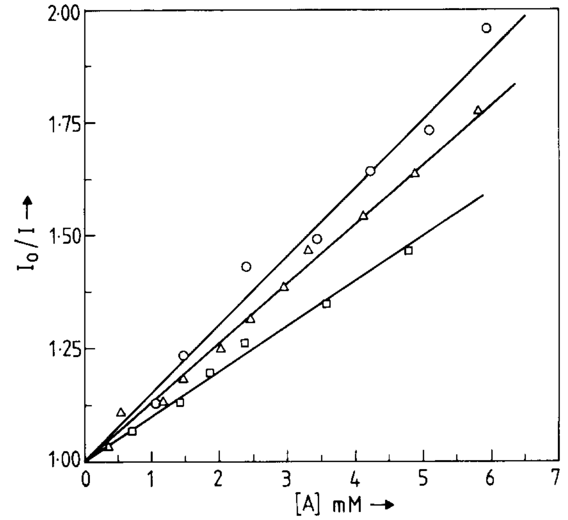

Fig. 3. Stern-Volmer plots of Chl *a*-nitrobenzene (△), Chl *a*-1,3,5-trinitrobenzene (○) and Chl *a*-9-dicyanomethylene-2,4,7-trinitrofluorenone (□). The concentrations of the acceptors on the *x*-axis are different. The values shown have to be multiplied by 10 for nitrobenzene, while the factors of multiplication are 1 and 0.1 for 1,3,5-trinitrobenzene and 9-dicyanomethylene-2,4,7-trinitrofluorenone, respectively.

effective ($\rho = 1$). The diffusion controlled rate for methylene chloride turns out to be $2.38 \cdot 10^{10}$ $\mathrm{M}^{-1} \cdot \mathrm{s}^{-1}$. The rates of bimolecular quenching of Chl *a* by different acceptors are close to being diffusion controlled within the limits of experimental error (±15%). No attempts have been made to partition the static and dynamic parts of quenching process. The k_q value for CNTNF is greater by an order of magnitude than the value of k_d. This is possibly due to the effective contribution from the static quenching (K) to k_q value which competes with diffusional rate owing to the high oxidizing ability of CNTNF. The log k_q values are plotted against the electron affinity values of the acceptors in Fig. 5. It is seen that the k_q values increase with increasing electron affinity values.

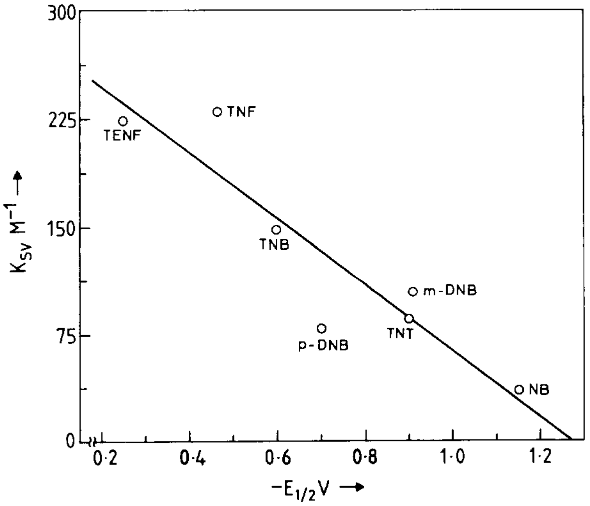

Fig. 4. Plot of the K_{sv} values of singlet quenching of Chl *a* versus reduction potentials of the acceptors.

TABLE II

EXCITED STATE COMPLEXATION PROPERTIES OF CHLOROPHYLL *a* WITH NITROAROMATIC π-ACCEPTORS IN METHYLENE CHLORIDE AT 25°C

Abbreviations: see Table I.

Acceptor	$-E_{1/2}$ [a] (V)	K_{sv} (M^{-1})	$k_q \times 10^9$ ($\mathrm{M}^{-1} \cdot \mathrm{s}^{-1}$)	$-\Delta G$ ($\mathrm{kJ \cdot mol}^{-1}$)
1 NB	1.15	36.9	6.15	−8.68
2 *m*DNB	0.91	105	17.5	15.4
3 TNT	0.90	85.3	14.2	16.4
4 DNB	0.70	78.5	13.1	35.7
5 TNB	0.60	148	24.7	45.4
6 TNF	0.46	258	34.8	59.8
7 TENF	0.25	224	37.3	80.1
8 CNTNF	0.10	989	165	95.5

[a] The polarographic reduction potentials of all the acceptors are in acetonitrile with respect to SCE [15,16]. The reduction potential for TNT is measured in methylene chloride using Pt button as working electrode against SCE.

The possible kinetic scheme for the quenching mechanism can be written as:

$$\begin{array}{ccccc} \mathrm{Chl}\,a^* + \mathrm{A} & \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} & \mathrm{Chl}\,a^* \ldots \mathrm{A} & \underset{k_{32}}{\overset{k_{23}}{\rightleftharpoons}} & \mathrm{Chl}\,a^+ \ldots \mathrm{A}^- \\ \downarrow 1/\tau_F^0 & & & & \downarrow k_{30} \end{array}$$

Scheme I

where $\mathrm{Chl}^*a \ldots \mathrm{A}$ and $\mathrm{Chl}^+a \ldots \mathrm{A}^-$ represent the accounter complex and the geminate ion-pair, respectively. It can be shown that k_q is related to the other rates as:

$$k_q = \frac{k_{12}}{1 + \frac{k_{21}}{k_{30}}\left[\exp\left(\frac{\Delta G_{23}}{RT}\right)\exp\left(\frac{\Delta G_{23}^0}{RT}\right)\right]} \qquad (2)$$

where ΔG_{23} and ΔG_{23}^0 are the activation free energy and the standard free energy difference, re-

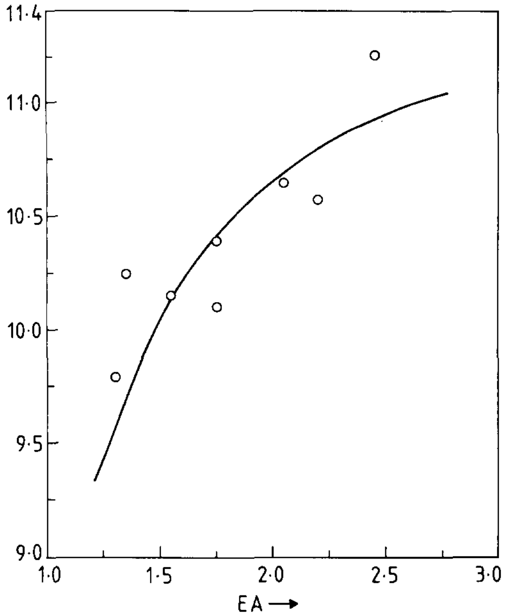

Fig. 5. Plot of log k_q versus electron affinity values of the acceptors (refer to Table I).

spectively, for the electron-transfer step. The ΔG_{23} is related to ΔG^0_{23} according to Rehm and Weller [19] as follows:

$$\Delta G_{23} = \left\{ \left[\frac{\Delta G^0_{23}}{2} \right]^2 + [\Delta G_{23}(0)]^2 \right\}^{1/2} + \frac{\Delta G^0_{23}}{2} \tag{3}$$

where $\Delta G_{23}(0)$ is the activation free energy when $\Delta G^0_{23} = 0$. The ΔG^0_{23} can be expressed in terms of half wave potentials as follows:

$$\Delta G^0_{23}(\mathrm{kJ \cdot mol^{-1}}) = -96.48[E_{1/2}(\mathrm{Chl}^+a/\mathrm{Chl}^*a) + E_{1/2}(\mathrm{A/A^-})] + \frac{e^2}{\varepsilon r_{\mathrm{DA}}} \tag{4}$$

where $E_{1/2}(\mathrm{Chl}^+a/\mathrm{Chl}^*a)$ and $E_{1/2}(\mathrm{A/A^-})$ are the reduction potentials (in eV) for the excited singlet state of Chl *a* and the quencher, respectively. The last term $e^2/(\varepsilon r_{\mathrm{DA}})$ account for the coulombic potential between the reactants upon formation of the ion-pair. The contribution from this term is evaluated by assuming the donor and acceptor molecules to be spherical. The radii of acceptor molecules were obtained from their crystal structures [20–23]. Radius of Chl *a* was obtained from the crystal structure of ethyl chlorophyllide *a* [24]. The term r_{DA} in Eqn. 4 denotes the radius of the ion pair which is taken as the sum of the radii of Chl *a* and the acceptor and is the dielectric constant of the solvent ($\varepsilon = 9.08$ for methylene chloride). The ΔG^0_{23} values calculated using Eqn. 4 in the present study are associated with an error of $\pm 10\%$. The dependence of the bimolecular quenching rates on the ΔG^0_{23} values is shown in Fig. 6.

Before we consider the effect of solvents on the singlet quenching features of Chl *a* by nitroaromatic compounds, it is worthwhile to study the optical spectral features of Chl *a* in different solvents. An earlier study concerning this aspect was reported by Seely and Jensen [25]. The absorption and emission spectra of Chl *a* are recorded in ten solvents of varying viscosity and dielectric constant in the present study. The Stokes shift in different solvents is compared with the polarizability of the solvents (Table III). We chose to study the quenching process of Chl *a* by tetranitrofluorenone, in view of tetranitrofluorenone's being a strong π-acceptor and its favourable solubility in these solvents. The Stern-Volmer plots are linear and the K_{sv} values are calculated using the least square method of analysis. The k_q values were estimated from the known lifetimes of Chl *a* in different solvents [4]. The product of the rate of diffusion controlled process and the lifetime of Chl *a* in different solvents is presented in Table IV. This constant, K^*_{sv}, represents the quenching

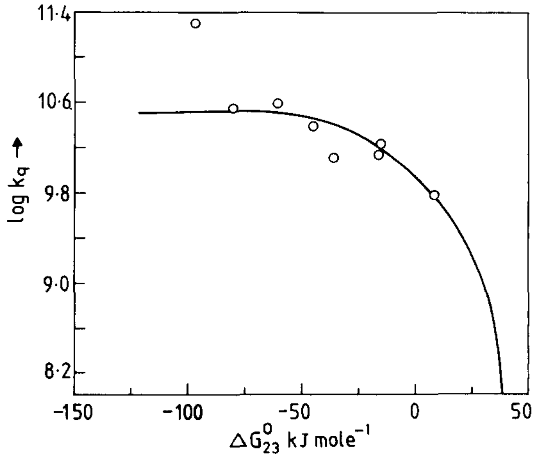

Fig. 6. Plot of log k_q versus ΔG^0_{23} for the singlet emission quenching of Chl *a* in methylene chloride at 25 ° C.

TABLE III

STOKES SHIFT OBSERVED FOR CHLOROPHYLL *a* IN SOLVENTS OF VARYING POLARIZABILITY

Solvent	$\nu_a - \nu_f$ (cm^{-1})	Δf [a]
1 Diethyl ether	181.8	16.8
2 Acetonitrile	203.9	30.6
3 Methylene chloride	170.0	25.6
4 Acetone	215.0	28.4
5 Benzene	145.5	0.3
6 Toluene	112.2	1.8
7 Ethyl acetate	191.2	20.0
8 Chloroform	223.5	15.0
9 Ethanol	224.1	28.9
10 Methanol	234.8	30.8

[a] $\Delta f = \frac{\varepsilon - 1}{2\varepsilon + 1} - \frac{n^2 - 1}{2n^2 + 1}$.

constant when the process is controlled only by diffusion.

Discussion

The binding constants of 1 : 1 molecular complexes of Chl *a* with different acceptors are in the range of 20–3700 l · mol^{-1}. It is found that the magnitude of the binding constants bear more or less linear dependency with the electron affinity values of the acceptors. The decrease in enthalpy of complexation varies from -10 to -80 kJ · mol^{-1}. This range of values of ΔH suggests that the complexes formed can be described as weak charge transfer complexes [26]. In addition, the ΔG and ΔS values observed for the complexation process lie in the range of -7.0 to -20 kJ · mol^{-1} and -0.3 to -23.6 J · mol^{-1} · K^{-1} respectively, as expected for the charge transfer complexes. These observations indicate that there is considerable charge transfer in the ground-state complexes of Chl *a* with nitroaromatic acceptors.

The acceptors employed in the present study are found to quench efficiently the singlet emission of Chl *a*. The acceptors which have a greater tendency to form ground state complexes (larger K values) exhibit larger K_{sv} values. This suggests that exciplex formation is one of the mechanisms of the fluorescence quenching in these systems [27]. The x-intercept in Fig. 4 indicates the value of the redox potential, Chl $a^{+\cdot}$/Chl a^*, to be -1.28 V, in agreement with the value obtained using Eqn. 1. The rates of bimolecular quenching increase with increasing electron affinity values of the acceptors (Fig. 5), suggesting the possible charge transfer in the excited state. The rates of bimolecular quenching can be related to the change in free energy for the excited state electron transfer reaction. Assuming $\Delta G_{23} \approx \Delta G^0_{23}$ in Eqn. 2, it can be shown that:

$$k_q \approx \frac{k_{12} k_{30}}{2 k_{21}} \exp\left(-\frac{\Delta G^0_{23}}{RT}\right) \qquad (5)$$

TABLE IV

EXCITED-STATE COMPLEXATION PROPERTIES OF CHLOROPHYLL *a* WITH 2,4,5,7-TETRANITRO-9-FLUORENONE IN SOLVENTS OF VARYING POLARITY AT 25 ° C

Solvent	K_{sv} (M^{-1})	$k_q \times 10^9$ (M^{-1}s^{-1})	$k_d \times 10^9$ (M^{-1}s^{-1})	K^* [a] (M^{-1})	$-\Delta G_{r8p}$ [b] (eV)
1 Diethyl ether	490	83.5	44.6	262	1.22
2 Acetonitrile	594	80.8	28.7	172	–
3 Methylene chloride	224	37.3	23.8	143	1.07
4 Acetone	223	37.1	30.9	186	−1.01
5 Benzene	33.3	5.6	15.2	91	1.49
6 Toluene	137	22.6	16.8	102	1.46
7 Ethyl acetate	100	16.7	22.2	136	1.14
8 Chloroform	593	98.8	16.9	101	1.19
9 Ethanol	121	20.2	8.3	43	−1.01
10 Methanol	378	69.5	16.7	91	−1.03

[a] $K^*_{sv} = K_{sv} \cdot \tau^0_F$.

[b] ΔG_{rip} values are determined with respect to the most polar solvent, acetonitrile.

This shows that the k_q values should vary exponentially with ΔG^0_{23} values. Fig. 6 depicts the dependence of k_q values on ΔG^0_{23}. It can be recognized that the initial rise in k_q values in the weakly exergonic region (under -40 kJ · mol^{-1}) is somewhat linear implying that the process is controlled by activation energy. At the moderate exergonic region, the k_q values rise rapidly, reaching a limiting value. The expected dependence of the rate is to decrease with increasing exothermicity for highly exothermic electron transfer reactions [28]. The plot (Fig. 6) does not show any inversion up to a ΔG^0_{23} value of -100 kJ · mol^{-1}, while the anticipated decrease should occur around -80 kJ · mol^{-1}. It is not possible to carry out the reaction at exothermic regions greater than -100 kJ · mol^{-1}, since Chl *a* undergoes oxidation on interaction with quenchers bearing high reduction potentials. We are unable to find a definite answer for the absence of Marcus inverted region for the following reasons. (i) Reactions with large exergonoicities have not been possible in the present systems. The nonlinear relationship between the rate and free energy change of the reaction is valid over a range where $[G_{23}] \gg G_{23}(0)$ [29]. (ii) The true relationship of the rate to energy is generally marked by the diffusion control limit and complexation between the colliding reactants. Contributions from the solvent reorganization and the distance parameters to the energy of the reaction should be considered [30]. (iii) There is a possibility of the ion pair having low-lying electronically excited states [6]. Initially, the electron will be transferred to this state rather than to the ground state directly.

It is possible to distinguish the several acceptors employed in this study based on the relative magnitude of ΔG^0_{23} values. The weakly oxidizing acceptors, nitrobenzene, 1,4-dinitrobenzene, 1,3-dinitrobenzene, 1,3,5-trinitrotoluene and 1,3,5-trinitrobenzene exhibit a lower value of ΔG^0_{23}, whereas the strongly oxidising acceptors, 2,4,7-trinitrofluorenone, 2,4,5,7-tetranitrofluorenone and 9-dicyanomethylene tetranitrofluorenone, exhibit a higher value of ΔG^0_{23}. These could be rationalised in terms of the relative proximity of the ion-pair energy surface (Chl $a^+ \ldots A^-$) to the minimum of (Chl*, A) energy surface. We observed a featureless EPR signal corresponding to Chl$^+$ in a methylene chloride solution containing 1 : 1 mole proportion of Chl *a* and 9-dicyanomethylene trinitrofluorenone. Such a signal is not observed in the solutions containing Chl *a* and weakly oxidizing acceptors. It seems to suggest that the charge-transfer complexes formed by the strongly oxidizing acceptors with Chl *a* are ionic in nature, thereby providing a favourable pathway for the excited state electron transfer process. The large formation constants (K and ΔG) as well as dissociation rates (larger k_q and ΔG^0_{23}) lend support to the above reasoning.

The charge transfer complexes of Chl *a* formed with the weakly oxidising acceptors have a transient existence, owing to their partial covalent character. The enhancement of charge transfer complexation can be seen from the broadening and shifting of absorption bands with decreasing temperature (Fig. 7). The complexes are more stable towards dissociation as manifested by the lower ΔG and ΔG^0_{23} values. These observations suggest that the formation and dissociation of such complexes both in the ground and excited states are slow, in comparison with the complexes of Chl *a* with strongly oxidizing acceptors.

Many solvent parameters, static polarizability,

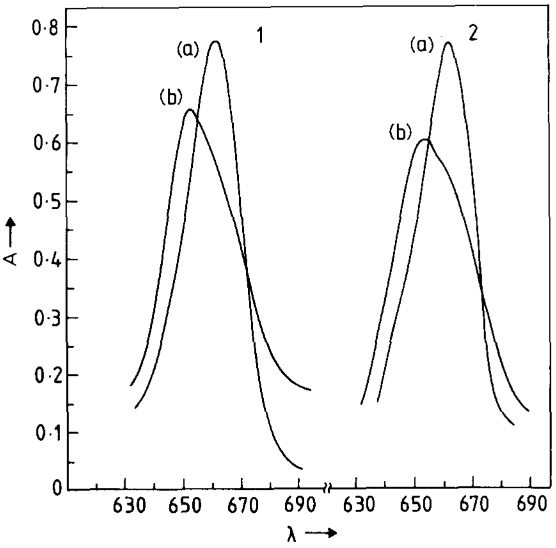

Fig. 7. (a) The $Q_{y(\infty)}$ absorption band of Chl *a* (0.2 mM) and (b) on addition of 60 mM trinitrofluorenone in methylene chloride at 25 °C (1). Absorption spectra of (1) at 10 °C is shown in (2).

viscosity, reorientation of solvent molecules around the excited-state dipoles, vibrational trapping and specific interaction of solvent molecules, are known to influence the rates of excited-state electron transfers and the free energy changes accompanying these reactions. The difference in energies of the absorbed and emitted light ($\nu_a - \nu_f$), i.e., the Stokes shift, of Chl *a* in different solvents can be related to the orientation polarizability of the solvents [31] as

$$\nu_a - \nu_f = \frac{e^2}{hc}\left(\frac{\varepsilon - 1}{2\varepsilon + 1} - \frac{h^2 - 1}{2n^2 + 1}\right) + \left(\frac{\mu^* - \mu}{a^3}\right)^2 + \text{constant} \qquad (6)$$

where *a* is the radius of the cavity in which the fluorophore resides, μ^* and μ are its dipole moments in the excited and ground states, respectively. It is found that the Stokes shift ($\nu_a - \nu_f$) observed for Chl *a* in different solvents increases with the static polarizability of the solvents (Table III). Interestingly, the k_q values appear to bear a similar dependence on the static polarizability of the solvents as that found for ($\nu_a - \nu_f$) values. This shows that factors that contribute to the Stokes shift also influence the rates of bimolecular quenching. The major contribution of the solvent properties of the Stokes shift in addition to the solvent polarizability are the dissipation of vibrational energy and specific solvent effects. It is found that an increase in the static component of the polarizability of solvents cause relatively a larger increase in the Stokes shifts than in the k_q values. We tend to believe that the vibrational relaxation of the excited state and coordinating and/or H-bonding tendencies of the solvents towards Chl *a* are responsible for the solvent polarizability dependence of k_q values.

We shall consider the effect of viscosity of the solvents on the k_q values. It is seen (Table IV) that the k_q values observed in different solvents are of the same order of magnitude as those obtained for the diffusion controlled process (k_d). It is found that the k_q values bear a linear dependence on the reciprocal viscosities of the solvents (Fig. 8). However, the rate constants in the solvents, ethanol, methanol, chloroform and acetonitrile, are found to be much larger than those observed in other solvents. The anomalous behaviour in the alcohol solvents has been attributed to the influence of high-frequency dielectric relaxation of the solvent dipoles [32]. It may be noted that these solvents are known to exhibit a coordinating and/or H bonding tendency with Chl *a*. We believe that such specific effects of these solvents can also lead to the deviation in the linearity observed in the plot (Fig. 8).

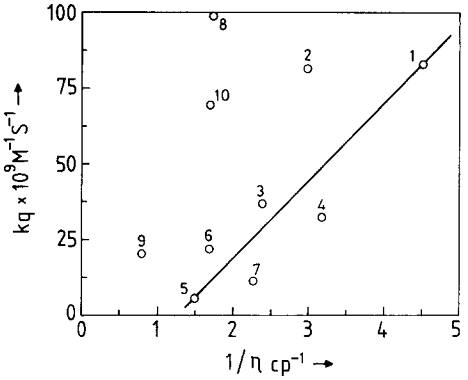

Fig. 8. Plot of the bimolecular quenching rates of singlet emission of Chl *a* by 2,4,5,7-tetranitrofluorenone versus reciprocal viscosity values of different solvent.

An attempt has been made here to investigate the role of solvent reorganisation energies in governing the magnitudes of the free energy change during the excited state electron transfer process. There exists a close correlation between the solvent polarisation and the solvent reorganisation energies [33]. The vibrational trapping energy (ψ_0) could be related to solvent reorganization energy. ψ_0 can be calculated from the radii of the donor and acceptor, the donor acceptor distance and, the static and optical dielectric constant of the solvents. The free energy of formation of the radical pair (ΔG_{rip}) is dependent on the redox potential of the donor and acceptor and the solvent reorganization energy [33].

$$-\Delta G_{\text{rip}} = E_s - E^{\text{ox}} + E^{\text{red}} + \Delta G^{\text{sol}} \qquad (7)$$

$$\Delta G^{\text{sol}} = \frac{e^2}{\varepsilon_1}\left(\frac{1}{r} - \frac{1}{a}\right) - \frac{e^2}{\varepsilon_0 r} \qquad (8)$$

where *a* and *r* are the ionic distance and the ionic radius, respectively. ε_0 is the dielectric constant of the solvent in which the redox studies have been carried out, viz., acetonitrile. ε_1 is the dielectric constant of the solvent for which ΔG_{reorg} has been evaluated. In order to arrive at the ΔG_{rip} values in different solvents, it is suggested [34] that a most polar solvent could be taken as the reference and the ΔG_{rip} values in different solvents could be evaluated relative to this polar solvent. We chose acetonitrile as the reference solvent and computed the values of ΔG_{rip} in different solvents (Table IV). The cyclic voltammogrammes of Chl *a* in different solvents reveal that the first ring oxidation potential occurs at 0.62 V in acetone and methylene chloride, 0.60 V and 0.52 V (with reference to saturated calomel electrode) in acetonitrile and methanol respectively. The estimated $-\Delta G_{25}^{0}$ values in different solvents -0.92, -0.64 and -1.04 eV, respectively, in acetone, methylene chloride and methanol. These values do not agree with the calculated ΔG_{rip} values (Eqns. 7 and 8) in these solvents, implying the possible over-estimation of the latter. We believe that specific solvent effects may be one of the contributing factors to these anomalies.

It has been possible to arrive at the influence of solvents on the efficiency of quenching. The K_{sv}^{*} values (Table IV) obtained as the product of k_{q} and τ_{F}^{0} can be compared with the experimentally evaluated K_{sv} values. The ratio of these two values is a measure of the efficiency of the quenching process. It is found that most of the solvents have a marked effect on the quenching efficiency (100%). Benzene is the only solvent which exhibits lower efficiency of quenching. Interestingly, the solvents that can induce specific interaction with Chl *a* have the efficiency of quenching higher than 100%. We attribute this to the abilities of these solvents to coordinate and/or H-bond with the Chl *a*.

Conclusions

The present study demonstrates the mechanism of fluorescence quenching of Chl *a* by various nitroaromatic compounds of moderate oxidizing abilities. A comparison of the data obtained in the present study with our earlier work suggests that Chl *a* is a stronger oxidizing agent than Pheo *a*. Studies on the fluorescence quenching of ZnPheo *a* by different nitroaromatic acceptors (unpublished data) suggest the importance of Mg in the redox reactions. This study suggests that the major contribution to the bimolecular quenching of Chl *a* is the electron transfer from the excited singlet state of Chl *a* to the acceptors. The rates of bimolecular quenching do not show any inversion in the highly exothermic region as predicted by Marcus. The ΔG_{23}^{0} values observed for quenching of the singlet emission of Chl *a* by several acceptors are useful in providing the possible pathway for the quenching process. The weakly oxidizing acceptors from covalent exciplexes with Chl *a* while the exciplexes formed by strongly oxidizing acceptors have considerable ionic character. Solvents of differing static polarizability and viscosity influence the magnitudes of the rates of bimolecular quenching and the ΔG_{rip} values. Increasing static polarizability of the solvent molecules leads to an increase in the k_{q} values and the spectral shifts (Stokes shifts) of Chl *a*. In addition to the solvent reorganization energy, specific solvent properties, viz., H-bonding and co-ordinating ability, contribute significantly to the k_{q} values and to the efficiency of the singlet emission quenching of Chl *a* by the acceptors. These studies suggest that the dielectric profile of the membrane and its constituents could alter the fluorescence behaviour of the membrane-bound Chl *a* and thus its excited-state properties. The relevance of this lies in the photosynthetic electron transport reactions.

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