Quenching of Triplet Chlorophyll-*a* by Aromatic Nitrogen Compounds

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Flash excitation of chlorophyll-*a* in outgassed ethanol solution gives a high yield of the long-lived triplet state. Various types of aromatic nitrogen compound have been found to quench the triplet *via* an electron transfer reaction. Thus, nitrobenzenes and aromatic heterocyclic compounds act as electron acceptors with triplet chlorophyll-*a* and quenching leads to formation of chlorophyll-*a* radical cations and quencher radical anions. Decay of the transient absorption observed by flash spectroscopy is due to back electron transfer. On the other hand, aromatic amines react with triplet chlorophyll-*a* to yield the chlorophyll-*a* radical anion and the amine radical cation. Here, reduced chlorophyll-*a* decays *via* oxidation with an impurity within the system.

Chlorophyll plays a vital role in the natural photosynthetic process by virtue of its ability to bring about a charge separation. Little is known about how the chlorophyll mechanism works and many *in vitro* systems have been studied in order to gain further understanding. In polar solvents, chlorophyll-*a* exists as a monomer solvate for which the fluorescence quantum yield has been measured [1] at 0.32. In addition to fluorescence, efficient intersystem crossing to the triplet manifold occurs, a quantum yield for triplet formation of 0.64 has been determined by flash spectroscopy.[2] However, in dry, nonpolar solvents [3] low yields of both fluorescence and triplet formation are observed. Here, chlorophyll-*a* exists in a dimeric form and, upon excitation, the excited singlet state of the dimer undergoes dissociation into two ground state monomer species.[2] The energy of the lowest triplet state of chlorophyll-*a* was first estimated [4] to be about 120 kJ mol^{-1} by measuring the amount of delayed fluorescence at different temperatures. Recently, very weak phosphorescence was detected in ethanol at low temperature [5] which locates the triplet level at 125 kJ mol^{-1} in agreement with the earlier measurements.

In fluid solution, ion–pair formation occurs by triplet state reaction. Electron transfer reactions resulting in both photo-oxidation, with quinones [6] as electron acceptor, and photoreduction, with ascorbic acid [7] as donor, have been observed. Although many types of quencher react with both singlet and triplet excited states of chlorophyll-*a*, photoionisation from the excited singlet state has not been detected as yet. However, this is not the case with *in vivo* studies where it is the excited singlet state that is of prime importance. In this paper we have investigated the photochemical reaction between chlorophyll-*a* triplets and several aromatic nitrogen compounds in ethanol solution. The aim of the work has been to extend the available data regarding photoredox reactions of chlorophyll-*a* under *in vitro* conditions.

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EXPERIMENTAL

MATERIALS

Chlorophyll-*a* (Sigma Chemicals) and ethanol (Burrough A.R. grade) were used as received. *N*,*N*-Dimethylaniline, *N*-methylaniline, and aniline (Aldrich) were distilled from zinc dust under reduced pressure, the central fractions being collected. Nitrobenzene (B.D.H.) and pyridine (Aldrich) were distilled several times and found to be better than 99.5 % by g.l.c. analysis. Samples of 1,3- and 1,4-dinitrobenzene (Aldrich) were recrystallised from ethyl acetate and vacuum sublimed. Phenazine, 1,10-phenanthroline, and 2,2′-bipyridyl (Aldrich) were recrystallised from ethyl acetate and zone refined. Triphenylamine (B.D.H.) was recrystallised several times from petroleum spirits and stored in the dark under an atmosphere of nitrogen. *N*,*N*,*N*′,*N*′-Tetramethylphenylenediamine (TMPD) (Aldrich) was isolated from the hydrochloride salt and recrystallised from petroleum spirits. The purified material was stored in the dark under vacuum and a sample recrystallised immediately before use. All solutions were prepared and handled in a darkened room to prevent undue photodecomposition.

METHODS

All measurements were made with ethanolic solutions of chlorophyll-*a* (1×10^{-6} mol dm^{3}) after thorough outgassing by the freeze–thaw method. General methods were as reported previously,[8] and for all flash photolysis experiments, the photolysis lamps were filtered to remove light with $\lambda < 560$ nm.

Radical anions of the three nitrobenzenes studied were prepared by addition of sodium to an outgassed solution of the compound in dimethoxyethane. This gave complete conversion to the radical anion, thus providing a direct measure of the extinction coefficients. The absorption spectra obtained by this method were in good agreement with those produced by one electron reduction using chemical reduction [9] and γ-ray irradiation [10] techniques. The radical cation of *N*,*N*-dimethylaniline was prepared by flash photolysis in polar solvent. In the presence of a thiazine dye, high yields of the amine radical cation can be generated[11, 12] by flash excitation and comparison with the extent of ground state bleaching of the dye allows calculation of the required extinction coefficients. Flash photolysis of phenazine in aqueous solution containing either potassium ferricyanide or ferrocyanide resulted in formation of the phenazine radical cation and radical anion respectively. The absorption spectra recorded by this technique showed excellent agreement with those produced by γ-irradiation in low temperature glasses.[13]

Phosphorescence spectra were recorded in ethanol glasses at 77 K using a Perkin–Elmer MPF 4 spectrofluorimeter. The solutions ($\sim 10^{-2}$ mol dm^{-3}) were purged with argon immediately before the experiment. Polarographic half-wave potentials ($E_{\frac{1}{2}}$) were obtained from literature sources.

RESULTS

In the absence of a quencher, flash photolysis of an ethanolic solution of chlorophyll-*a* (1×10^{-6} mol dm^{-3}) results in formation of the triplet state which decays by complex kinetics. The triplet [6] absorbs throughout the visible region but it is most conveniently monitored at 465 and 820 nm. Strong ground state bleaching is observed at 670 nm where the triplet state has negligible absorption relative to ground state chlorophyll-*a*. Addition of a quencher had no observable effect on the ground state absorption spectrum of chlorophyll-*a* but caused a decrease in the triplet state lifetime. The bimolecular quenching rate constants (k_T) were determined as reported previously [8] and are collected in table 1. At least six different concentrations of quencher were used for each system and the k_T values obtained have a reproducibility of ±5 %.

Once k_T is known, the concentration of quencher can be increased to a level at which the lifetime of triplet chlorophyll-*a* is comparable to the flash duration. Under these conditions, a long-lived transient absorption was observed which resembled that of the triplet state but showed several important differences depending upon which quencher was present. The spectra were obtained from five separate experiments carried out under identical reaction conditions. Reaction was reversible within detectable limitations and there was no consumption of chlorophyll-*a*.

TABLE 1.—DATA FOR QUENCHING TRIPLET CHLOROPHYLL-*a* IN ETHANOL SOLUTION

quencher	k_T/dm^3 mol^{-1} s^{-1}	E_T/kJ mol^{-1}	$-E_{\frac{1}{2}}$/V
nitrobenzene	2.6×10^6	250	1.15
1,3-dinitrobenzene	2.0×10^9	240	0.90
1,4-dinitrobenzene	3.6×10^9	245	0.69
aniline	2.0×10^3	285	−1.28
N-methylaniline	7.4×10^3	287	−1.03
N,*N*-dimethylaniline	3.0×10^4	290	−0.71
triphenylamine	8.5×10^5	290	−0.48
TMPD	2.7×10^6	215	0.10
pyridine	$<10^3$	340	1.82
2,2′-bipyridyl	4.5×10^5	278	1.69
1,10-phenanthroline	1.6×10^6	270	1.42
phenazine	1.1×10^7	190	1.17

Thus, flash excitation of chlorophyll-*a* (1×10^{-6} mol dm^{-3}) and 1,4-dinitrobenzene (5×10^{-4} mol dm^{-3}) gave the transient absorption spectrum shown in fig. 1. Both 1,3-dinitrobenzene and nitrobenzene gave identical effects. Transient decay followed second order kinetics at all wavelengths, the decay rate constants were 2.5×10^9, 5.4×10^9, and 3.2×10^9 dm^3 mol^{-1} s^{-1} for 1,4-dinitrobenzene, 1,3-dinitrobenzene, and nitrobenzene respectively. Except with TMPD, quenching by aromatic amines is inefficient. However, care must be taken when using TMPD to prevent the facile ionisation to Wurtzer's blue which occurs in polar solvents. Therefore, *N*,*N*-dimethylaniline (2.0 mol dm^{-3}) was chosen for the spectrographic studies and the transient absorption spectrum obtained is given in fig. 2. Decay of this transient

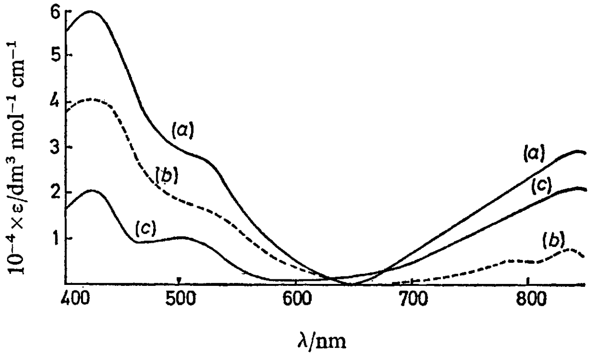

FIG. 1.—(*a*) Derived transient absorption spectrum 200 μs after flash excitation of chlorophyll-*a* (1×10^{-6} mol dm^{-3}) in ethanol containing 1,4-dinitrobenzene (5×10^{-4} mol dm^{-3}); (*b*) chlorophyll-*a* radical cation in ethanol; and (*c*) 1,4-dinitrobenzene radical anion in dimethoxyethane.

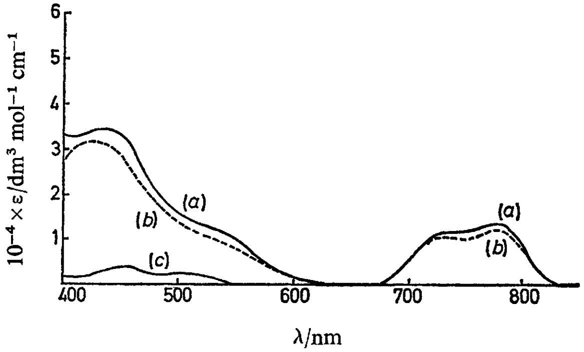

FIG. 2.—(*a*) Derived transient absorption spectrum 100 μs after flash excitation of chlorophyll-*a* (1×10^{-6} mol dm^{-3}) in ethanol containing *N*,*N*-dimethylaniline (2 mol dm^{-3}); (*b*) chlorophyll-*a* radical anion in ethanol; and (*c*) *N*,*N*-dimethylaniline radical cation in ethanol.

was monitored at 800 nm and was found to follow first order kinetics with a unimolecular rate constant of 1.1×10^4 s^{-1}. Similar first order rate constants were found with aniline, *N*-methylaniline, and TMPD. The transient absorption spectrum obtained by flash excitation in the presence of phenazine (1×10^{-2} mol dm^{-3}) is shown in fig. 3. Decay of the transient absorption follows second order kinetics at all wavelengths. 1,10-Phenanthroline and 2,2′-bipyridyl gave identical results, the observed decay rate constants were found to be 6.0×10^8, 8.0×10^8, and 8.5×10^8 dm^3 mol^{-1} s^{-1} for phenazine, 1,10-phenanthroline, and 2,2′-bipyridyl respectively.

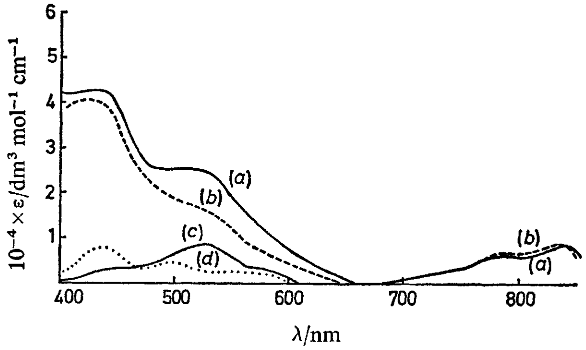

FIG. 3.—(*a*) Derived transient absorption spectrum 250 μs after flash excitation of chlorophyll-*a* (1×10^{-6} mol dm^{-3}) in ethanol containing phenazine (1×10^{-2} mol dm^{-3}); (*b*) chlorophyll-*a* radical cation; (*c*) phenazine radical anion in water; and (*d*) phenazine radical cation in water.

Phosphorescence was used to estimate the energy of the lowest triplet state of each quencher (table 1). In most cases, the 0, 0 band of the triplet emission could be located easily but the nitrobenzenes gave very little phosphorescence. For these compounds, the reported triplet energy is a rough approximation only.

DISCUSSION

Although there are several important processes whereby a quencher can deactivate a triplet excited state in fluid solution, we need only consider quenching mechanisms based on energy transfer, electron transfer, and photochemical product formation.

Triplet energy transfer becomes important when the energy of the donor exceeds that of the acceptor by at least 12 kJ mol^{-1}. However, the phosphorescence measurements show that this situation does not arise here since chlorophyll-*a* triplet, which would act as donor, is at much lower energy than the triplet level of the quenchers. Similarly, all the photolysis experiments are reversible and there is no permanent product formation. Thus, we might expect quenching to occur by electron transfer: either full electron transfer leading to ion-pair formation or partial electron transfer leading to enhanced non-radiative decay of the excited state.

Both 1,3- and 1,4-dinitrobenzene quench triplet chlorophyll-*a* at a rate close to the diffusion controlled limit but nitrobenzene is a much less efficient quencher. The observed k_T values correlate with the polarographic half-wave potential (table 1) which is a measure of the ease of reduction of the quencher. This correlation is consistent with a charge transfer mechanism in which the nitroaromatic compound acts as an electron acceptor[14]

$$\mathrm{Chl_T} + \mathrm{Q} \underset{k_b}{\overset{k_f}{\rightleftharpoons}} (\mathrm{Chl} \ldots \mathrm{Q}) \xrightarrow{k_q} \text{quenching}. \tag{1}$$

For such a process, the observed triplet quenching rate constant is given by:

$$k_T = \frac{k_f k_q}{k_b + k_q}. \tag{2}$$

The rate constant for formation of a collision complex (k_f) should be close to diffusion controlled so that k_T is probably determined by the magnitude of k_q relative to k_b. Where $k_q \gg k_b$, the expression is simplified:

$$k_T \sim k_f \tag{3}$$

and this is the case found with 1,3- and 1,4-dinitrobenzenes. For situations where $k_q < k_b$, the expression becomes:

$$k_T = K k_q \tag{4}$$

so that k_T is affected by factors which influence both k_q and the equilibrium constant K ($K = k_f/k_b$). The standard free energy for complex formation (ΔG) is related to K:

$$\Delta G = -RT \ln K \tag{5}$$

and, for quenching a given excited state by a series of similar quenchers, ΔG is also related to the half-wave potential, or more correctly the electron affinity, of the quencher. Thus, the various relationships indicate that, where quenching is not diffusion-limited, a plot of $\ln k_T$ against $E_{\frac{1}{2}}$ should be linear with a slope of $-1/RT$. This requirement is fulfilled only by nitrobenzene, its lower k_T value indicates that $k_b > k_q$ and hence K is much smaller than found with dinitrobenzene quenchers.

Flash spectroscopy (fig. 1) shows a long-lived absorption which is present after the triplet has decayed. This long-lived transient shows an excellent match to a compilation of the chlorophyll-*a* radical cation and the nitro compound radical anion. Therefore, we can attribute the quenching reaction, at least in part, to full electron transfer

$$\mathrm{Chl_T} + \mathrm{NB} \rightarrow \mathrm{Chl}^{\dot{+}} + \mathrm{NB}^{\dot{-}}. \tag{6}$$

At all wavelengths, the transient absorption decays *via* second order kinetics and leads to regeneration of starting materials. This decay process can be expressed in terms of simple back electron transfer and, as is often the case with photoredox reactions, this process is at least as efficient as the forward reaction

$$\mathrm{Chl}^{\dot{+}} + \mathrm{NB}^{\dot{-}} \rightarrow \mathrm{Chl} + \mathrm{NB}. \tag{7}$$

Aromatic amines are often used as electron donors in photoredox reactions. They are inefficient quenchers of triplet chlorophyll-*a* (table 1) but the observed k_T values do correlate with the half-wave potential of the amine. Flash spectroscopy shows that photolysis leads to full electron transfer whereby chlorophyll-*a* radical anions and amine radical cations are formed. The absorption spectra of chlorophyll-*a* radical anions and cations are very similar but the region around 700-850 nm is sufficiently different to allow assignment of the observed spectrum. With TMPD as quencher, flash excitation of chlorophyll-*a* leads to unambiguous formation of $\text{TMPD}^{+\cdot}$ which has a characteristic absorption spectrum around 600 nm.[15] The extinction coefficient of this species is higher than that of reduced chlorophyll-*a* in this region of the spectrum. However, it should be noted that TMPD undergoes facile ionisation in polar solvents so that analysis based on this compound is not entirely satisfactory. Using *N*,*N*-dimethylaniline as quencher, we again observe formation of the chlorophyll-*a* radical anion (fig. 2). The amine radical cation shows little absorption relative to reduced chlorophyll-*a* and is not well resolved. However, the experimental results indicate quite clearly that full electron transfer occurs and it seems very unlikely that an amine radical anion could be formed. Thus, we express the quenching reaction:

$$\text{Chl}_T + \text{Am} \rightarrow \text{Chl}^{-\cdot} + \text{Am}^{+\cdot}. \tag{8}$$

The amines exhibit low k_T values, which suggests that $k_q < k_b$. Now, for formation of an ion–pair, k_q is related to the half-wave potential of the quencher. This arises because the charge transfer mechanism involves indirect mixing of the initial and final forms of the complex *via* a charge transfer state ($\text{Chl}^-\ \text{Am}^+$) and the extent of mixing depends upon the energy difference between the charge transfer state (E_{CT}) and the initially excited chlorophyll-*a* triplet state.[16] The energy of the charge transfer state is given by:[17]

$$E_{CT} = IP_{Am} - EA_{Chl} - C \tag{9}$$

where IP_{Am} is the ionisation potential of the amine, EA_{Chl} is the electron affinity of chlorophyll-*a* triplets, and C includes a term for the coulombic stabilisation energy of the complex. For a given excited state and solvent, the change in energy of the charge transfer state should be determined mostly by IP_{Am} for which the half-wave potential provides a quantitative measure. Thus, both K and k_q depend upon $E_{\frac{1}{2}}$ but our data do not allow determination of the individual rate constants.

Decay of reduced chlorophyll-*a* was monitored at 800 nm since the amine radical cation does not absorb in this region. For all amines studied, decay followed first order kinetics and resulted in regeneration of ground state chlorophyll-*a*. This probably reflects oxidation by some impurity in the system. Attempts to further purify the solvent and reactants had no effect upon the rate of decay which remained at an average $(1.4 \pm 0.4) \times 10^4\ \text{s}^{-1}$.

Aromatic heterocyclic compounds can act as electron donors or acceptors depending upon the reaction conditions. Radical cations and anions of phenazine are readily generated by flash photolysis in the presence of potassium ferricyanide and ferrocyanide respectively. Both 1,10-phenanthroline and 2,2′-bipyridyl behave identically and we were interested to see whether these compounds would react with triplet chlorophyll-*a* as electron donors or acceptors.

The data in table 1 show that aromatic heterocyclic compounds are poor quenchers of triplet chlorophyll-*a*, in fact no quenching was observed with pyridine. The observed k_T values are low but do follow the trend in half-wave potential of the quencher. Thus, for these compounds $k_q < k_b$, as found with aromatic amine quenchers. However, the data are restricted to three quenchers so that the validity of a ln k_T against $E_{\frac{1}{2}}$ plot is considered to be questionable. Flash spectroscopy with phenazine shows a long-lived transient absorption present after decay of the triplet. In the 700-850 nm region, the observed transient absorption spectrum matches quite well with that of the chlorophyll-*a* radical cation. At shorter wavelengths, the transient spectrum shows a better fit to a compilation of chlorophyll-*a* radical cations and phenazine radical anions than to a mixture of reduced chlorophyll-*a* and phenazine radical cations. A similar result was found with 1,10-phenanthroline and 2,2′-bipyridyl so that it appears that heterocyclic compounds prefer to act as electron acceptors with triplet chlorophyll-*a*

$$\mathrm{Chl_T + ArN \rightarrow Chl^{\overset{+}{\cdot}} + ArN^{\overset{-}{\cdot}}}. \qquad (10)$$

At all wavelengths, decay of the transient followed second order kinetics due to back electron transfer.

Thus, triplet chlorophyll-*a* participates in photoredox reactions with a variety of different aromatic nitrogen compounds. Reaction leads to full electron transfer and, by careful choice of quencher, both oxidised and reduced chlorophyll-*a* can be generated. When the product of reaction is the chlorophyll-*a* radical cation then decay of the ion–pair proceeds rapidly by simple back electron transfer. However, the chlorophyll-*a* radical anion is rapidly oxidised by some impurity within the system. This oxidation reaction leads to reformation of ground state chlorophyll-*a* without loss and may reflect an intrinsic instability of reduced chlorophyll-*a*.

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