Environmental Effects On Electron Transfer From Chlorophyll

Triplet to Quinone: Role of Dielectric Constant, Viscosity

And Quinone Structure In Cellulose Acetate Films*

MASTER

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*This work was supported in part by a U.S. Department of Energy Research Contract.

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ABSTRACT

The effects of environmental parameters on chlorophyll triplet quenching and electron transfer to quinones have been investigated in a system consisting of donor and acceptor incorporated into a cellulose acetate film which was subsequently exposed to solvent. Triplet quenching by a diffusional mechanism was found to occur in the dry film, with steric effects being a major determinant of quencher effectiveness. No formation of separated radicals was found under these conditions, probably because the high viscosity prevented separation of the initially formed radical-ion pair. When the film was subsequently exposed to water, triplet quenching became more effective and separated radical production occurred. This is attributed to effects of decreased microviscosity and increased dielectric constant. Both steric effects and quinone redox potential were found to influence radical yields. Rate constants for reverse electron transfer were independent of quinone redox potential. When solvents other than pure water were used, radical yields were observed to increase with the dielectric constant. This is ascribed to an increase in the ease of separation of the radical-ion pair.

INTRODUCTION

Previous studies (1) of electron transfer from the triplet state of chlorophyll to quinones in fluid, homogeneous solutions have established the following mechanism for the major reaction pathway:

In order to more fully understand the factors which control these processes, as well as to investigate means of increasing the efficiency of light energy conversion by chlorophyll, we have examined electron transfer under a wide variety of experimental conditions, including various solvent environments (2), and incorporation of reactants into heterogeneous systems such as lipid bilayers (3) and cellulose acetate films (4). Such studies have shown that it is possible to influence triplet quenching (process [1]) by solvent viscosity and quinone redox potential, to enhance the ratio of process [2] to process [3] (radical pair separation vs. geminate recombination) by increasing the solvent dielectric constant, and to reduce the rate of process [4] (radical recombination) by increasing solvent viscosity and by allowing the reaction to occur in a heterogeneous medium.

In the present work we have examined the effects of viscosity, dielectric constant and quinone structure on the photophysical and photochemical processes occurring in a heterogeneous system consisting of

chlorophyll and quinone dissolved together in a cellulose acetate film matrix and placed in contact with either pure water or a water-organic solvent mixture. This has allowed us to obtain further insights into the role of environmental parameters in energy conversion by chlorophyll, especially as related to the influence of a high viscosity polymeric molecular environment on excited state quenching and photoinduced electron transfer. Such insights may have significance regarding our understanding of certain aspects of photosynthesis, as well as the potential use of chlorophyll as a solar energy transducer in vitro.

MATERIALS AND METHODS

The chlorophyll a used in these experiments was extracted from fresh spinach leaves, using the method of Strain and Svec (5), and its purity checked by visible absorbance spectroscopy. Benzoquinone, tetrachloro-p-benzoquinone, 2,5-dichloro-p-benzoquinone and methyl-p-benzoquinone were purified by vacuum sublimation; 2,6,-di-t-butyl-p-benzoquinone and duroquinone were used without further purification. AR grade acetone was further purified by vacuum distillation, and was stored over 3Å molecular sieves to reduce water content. Cellulose acetate (acetyl content 39.8%) was used without further purification.

Sample preparation, the laser photolysis apparatus and experimental techniques were as described previously (2, 4). The sample excitation wavelength was 660nm and the laser pulse width was approximately 10ns. Triplet quenching was measured by analysis of decay kinetics using a strip of cellulose acetate film approximately 3cm x 1cm x 0.005cm, containing both chlorophyll and the desired concentration of quinone, which was placed in an evacuated cuvette. Concentrations of quinones within the film were determined by measuring chlorophyll absorbances at 665nm in the

solutions from which the films were made and in the films themselves, and applying the correction factor thereby determined to the known starting concentration of quinone in solution. Upon completion of the triplet quenching measurements, the film was immersed in 5ml of degassed water or other solvent for a variable period of time after which further measurements of triplet and radical concentrations and decay kinetics were made.

RESULTS AND DISCUSSION

1. Triplet quenching in dry and wet films

When quinones are incorporated into cellulose acetate films containing chlorophyll, in the absence of solvent one observes triplet state quenching but no detectable production of radical species (4). We attribute the lack of radical formation to the high viscosities within the film matrix which act to prevent radical pair separation (4). We have now looked at this process more closely. In the dry films, triplet quenching is manifested by a decrease in the triplet decay time. Some typical results obtained with 2,5-dimethyl benzoquinone at various concentrations are shown in Fig. 1. The fact that triplet lifetime is dependent upon the quinone concentration indicates that diffusional interactions are occurring, as opposed to a static quenching process involving nearest-neighbor effects. Decay kinetics, however, are not exponential in the presence of quinone, even at quite high concentrations, showing that the kinetic mechanism of the quenching process is complex. This is probably a reflection of heterogeneity in the microenvironments of the quinone and chlorophyll species within the film. If one uses the triplet decay half-time as a measure of the effectiveness of quenching, it is possible to obtain a linear Stern-Volmer plot over

a reasonably wide range of quinone concentrations. This is shown in Fig. 2. For benzoquinone, a quenching constant of $2.9 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$ can be calculated from these data. This can be considered to represent an average value for k_q among the heterogeneous microenvironments.

When the quinone-containing films are exposed to water, triplet lifetimes quickly become appreciably shorter than in the dry film (within the time required for mixing and measurement, which is 1-2 min.) Thereafter, lifetimes decrease more slowly, reaching an equilibrium state in approximately 25 min. If one uses data measured as soon after mixing as possible, one obtains a Stern-Volmer quenching constant for benzoquinone of $2 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$ (Fig. 2) and, upon equilibration, a constant of $3.7 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$ (4). These values can be compared to a quenching constant of $3.4 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$ determined for benzoquinone in ethanol solution at room temperature (1). Thus, diffusion of quinone in the dry film is approximately 10^4 times slower than in fluid solution, and approximately 10^2 times slower than in a water-equilibrated film. We interpret these results as follows: In the dry film, viscosities are high, not high enough to completely eliminate the diffusion of quinone, but high enough to suppress the production of separated radical species (chlorophyll diffusion apparently does not occur within these films (4), presumably because of the larger size and the long hydrocarbon tail of the chlorophyll molecule). Water penetrates the film quickly, reducing the microviscosity and increasing the dielectric constant of the chlorophyll-quinone environments (see below for further discussion of this effect). Upon longer exposure to water the film swells (this can be observed visually), and microviscosities are reduced still further.

These viscosity effects are manifested in increased Stern-Volmer quenching constants. It is significant in this regard that radical formation can be observed immediately upon addition of water (this is probably a mixed viscosity and dielectric effect) and that radical yield increases and reaches a maximum during the same time period that triplet quenching becomes more rapid (see below for further details). This latter effect is presumably a consequence of the increasing ease of separation of the radical-ion pair as microviscosity decreases.

We have examined several different quinones in order to determine the effect of redox potential and the nature of ring substitution on triplet quenching. The results are shown in Table I. In both the dry and wet films (the latter referring to results obtained prior to equilibration), triplet quenching seems to be determined mainly by structural effects rather than by quinone potential. Thus, duroquinone and tetrachloroquinone are approximately of equal effectiveness as quenchers, whereas benzoquinone is considerably better. This is most likely a result of frictional and steric effects occurring within the highly viscous polymer-solvent matrix.

2. Radical formation in solvated films

As we have shown previously (4), C^+ formation and second order decay due to recombination with Q^- are readily observable under conditions in which a quinone solution in water is placed in contact with a cellulose acetate film containing chlorophyll. A similar situation exists under the present conditions: both quinone and chlorophyll initially present within the film and subsequent addition of solvent.

The determination of triplet to radical conversion efficiencies is somewhat complicated, however, by the extraction of quinone into the solvent. We have found that, over the time course of our experiments (approximately 25 minutes), the amount of quinone extracted never exceeds 25% and, for quinones such as the tetrachloro derivative, is less than 10%. Radical yields as reported here have been corrected for this effect.

In order to determine whether radical formation in a water-equilibrated film is simply related to the extent of chlorophyll triplet quenching, we have compared these two quantities for the benzoquinone system. In Fig. 3 is shown a plot of the fraction of triplet quenched vs. radical concentration, measured at a variety of benzoquinone concentrations in equilibrated samples. Although considerable experimental scattering was observed (probably due to film inhomogeneity), a linear relationship is apparent. This indicates that there is a single mechanism for radical production which is reflected in the decrease of the triplet lifetime by the addition of quinone and, therefore, that no complications are occurring due to vastly different chlorophyll microenvironments.

In Table I is also shown the results of determinations of triplet to radical conversion efficiencies for several quinones. It is apparent that both redox potential and structural parameters influence this quantity. Thus, tetrachloroquinone is more effective than benzoquinone, which in turn is more effective than dimethylquinone. This ordering reflects redox potentials. However, di-t-butyl quinone yields no measurable radical even though it is capable of quenching the chlorophyll triplet. Similarly, duroquinone is not able to generate

radical species upon triplet quenching. These results must be due to steric restrictions upon radical pair separation within the polymer-solvent matrix, inasmuch as the effect of redox potential is relatively small. We have also determined that the rate constants for radical recombination are essentially invariant with quinone potential, as was found for fluid solvents (1).

In fluid solutions, we have previously shown (2) that radical yield is determined largely by solvent dielectric constant. In order to determine whether a similar relationship exists in the heterogeneous film systems, we have measured radical yields in films containing both chlorophyll and benzoquinone exposed to a variety of solvents. Because of problems associated with film dissolution and/or extraction of chlorophyll, we used only mixed solvent systems containing water plus an organic solvent. Dielectric constants were assumed, as previously (2), to be a weighted average of the values for the pure solvents. The results of these experiments are shown in Fig. 4. It is apparent that, as was the case in homogeneous solution, there is a linear relationship between radical yield and solvent dielectric and that the line extrapolates to a limiting value for the dielectric constant in the region of 10. We again interpret this as reflecting the effect of dielectric constant on the efficiency of separation of the initially-formed radical-ion pair.

CONCLUSIONS

We conclude from the above results that cellulose acetate as a matrix for chlorophyll photochemical reactions behaves in a relatively simple and readily interpretable fashion, and in fact is remarkably

analogous to fluid, homogenous solutions. Its properties predominantly reflect its high internal viscosity and complex microenvironments which act to slow down and introduce heterogeneity into triplet quenching, to restrict radical pair separation, and to reduce the rate of radical recombination (4). We have shown that it is possible to obtain rather high triplet to radical conversion efficiencies in the presence of high dielectric constant solvents (approximately 40% for water-formamide), while maintaining relatively slow recombination rates (rate constants on the order of $10^6 \text{ M}^{-1} \text{ s}^{-1}$, about 10^3 times less than in fluid solutions). Because of these properties, polymer films would seem to offer a useful and relatively easily manipulated system for designing energy conversion devices based on the types of reactions known to occur in green plant photosynthesis. Furthermore, since the nature of the environmental effects we have observed on the mechanistic steps involved in chlorophyll excited state electron transfer appear to be very similar in systems as diverse as fluid solutions and polymer films, it may well be that analogous phenomena play a role in the even more complex environment of the chloroplast.

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Table 1

TRIPLET*QUENCHING AND RADICAL YIELDS WITH VARIOUS QUINONES IN CELLULOSE ACETATE FILMS.

Quinone	$-E_{1/2}(v)$	$k_q (\times 10^5 \text{ M}^{-1} \text{ s}^{-1})$ (dry)	$k_q (\times 10^6 \text{ M}^{-1} \text{ s}^{-1})$ (wet)	Maximum* Percent Conversion of C_T to C_T^+ (wet, equil)
tetra-Cl-BQ	-0.01	0.6	0.26	40
2,5-diCl-BQ	+0.18	0.97	0.5	-
BQ	+0.51	2.9	2.0	30
CH ₃ -BQ	+0.58	0.97	0.9	-
2,5-diCH ₃ -BQ	+0.67	0.7	0.67	21
2,6-di-t-butyl-BQ	+0.73	0.5	0.12	0
Duroquinone	+0.84	0.5	0.16	0

(Chl) = $1 \times 10^{-3} \text{ M}$; Quinone half-wave potentials for one electron reduction are those reported by Peover (6) for acetonitrile solutions.

* These values were determined from the dependence of C_T^+ yield on quinone concentration at constant chlorophyll concentration and with water as solvent.

LEGENDS FOR FIGURES

Fig.1: Semi-log plots of chlorophyll triplet decay curves under anaerobic conditions in the absence of solvent at various concentrations of 2,5-dimethyl benzoquinone within film.

- no quinone present
- X $2 \times 10^{-2}M$
- o $1 \times 10^{-1}M$
- 0.16M

Fig. 2: Stern-Volmer plots for benzoquinone quenching of chlorophyll triplet in dry and wet cellulose acetate films.

τ_0 for dry film is 1.4ms; τ_0 for wet film is 1.04ms.

Fig. 3: Plot of chlorophyll cation radical concentration as a function of fraction of triplet quenched at various benzoquinone concentrations.

Fig. 4: Plot of fraction of triplet converted to radical by benzoquinone as a function of solvent dielectric constant.

- X water-ethanol mixtures
- o pure water
- water-formamide mixture (50% v/v)

A least squares fit of these data yields an intercept on the x-axis of 7.1, with a correlation coefficient of 0.995.

FRACTION OF TRIPLET CONVERTED TO RADICAL