SEP 11 1965

100-2088

FEB 8 1966

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LIGHT-INDUCED SINGLE ELECTRON TRANSFER REACTIONS BETWEEN CHLOROPHYLL *a* AND
QUINONES IN SOLUTION. III. ABSOLUTE QUANTUM YIELDS AS A FUNCTION OF
TEMPERATURE AND SOLVENT*

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Abstract

Absolute quantum yields for electron transfer have been determined as a function of temperature in chlorophyll *a*-*p*-benzoquinone solutions in alcoholic solvents. These range, at high quinone concentrations, from about 0.1 electron per quantum absorbed at -10°C , up to about one electron per quantum at -110°C , and then down to zero electrons per quantum at -196°C . The yield appears to be determined principally by solvent viscosity and dielectric constant. A mechanism is proposed to account for this and comparisons with photosynthetic electron transfer are made.

*This work was supported in part by a grant (AT(11-1)908) from the United States Atomic Energy Commission.

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FEB 11 1965

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Introduction

Previous work from this laboratory (1,2) has shown that illumination of deoxygenated chlorophyll (a and/or b) solutions in alcoholic solvents containing various oxidizing agents (for example, quinones, riboflavin, thiocetic acid) leads to the formation of free radical species, identified as the one-electron reduction product of the oxidizing agent used. In the quinone and thiocetic acid systems, these radicals are transient. They appear only when the light is on and disappear when the light is turned off. Optical studies on the quinone system reveal no net chemical changes as a result of illumination, except possibly due to side reactions, either in the chlorophyll or in the quinone. Thus, recombination processes leading to the starting materials must be the principal mode of radical decay. A mechanism of unpaired spin production has been proposed for these systems involving a reversible, temperature-independent, single electron transfer from an electronically-excited chlorophyll molecule to a molecule of oxidant. In view of the evidence which implicates similar processes in photosynthesis (3), we have carried out absolute quantum yield determinations for the chlorophyll a-p-benzoquinone system at various temperatures in the hope of obtaining further mechanistic insights into these reactions.

Materials and Methods

Electron paramagnetic resonance (EPR) spectra were obtained as described previously (1).

p-Benzoquinone (Eastman) was purified by sublimation. Chlorophyll a was obtained from spinach leaves by the method of Anderson and Calvin (4). The final eluate (approximately 0.045 g. of spectroscopically pure chlorophyll a in isooctane containing 0.25% *n*-propanol) was concentrated to a

volume of 10 ml. and stored as such in the freezing compartment of a refrigerator. Spectrophotometric observations, as well as the reproducibility of our quantum yield measurements, indicated no significant decomposition of the chlorophyll over a period of several months under these conditions. Aliquots of this solution, diluted 20-fold with alcohol, were used in the quantum yield determinations. In all experiments, the molar concentration of chlorophyll a was 2.5×10^{-4} and that of the p-benzoquinone was 3×10^{-2} .

The yield measurements involved two basic calibrations: the number of light quanta per unit time absorbed by the sample and the initial rate of free radical production under light-limited conditions. The light source was a 500 watt tungsten filament projection lamp focused into the cavity of the EPR spectrometer through a Bausch and Lomb 250 mm. grating monochromator, using a slit width of 5 mm. (bandwidth 33 m μ). This source was calibrated at 405 and 436 m μ with a potassium ferrioxalate actinometer (5). In order to achieve a calibration at 665 m μ , the wavelength used in the chlorophyll illumination experiments, the calibrated light source was used to determine the energy sensitivity of a Kipp thermopile at the two shorter wavelengths. A calculation was then made, assuming that the thermopile has a flat response in energy units over the visible spectral

range, of the energy sensitivity at 665 m μ . An essentially flat energy response was indicated by the fact that the calibrations at 405 and 436 m μ were within 2% of each other. This thermopile was then used to determine the number of light quanta per second emitted from the tungsten lamp at 665 m μ .

A geometry correction was made by illuminating, in the spectrometer cavity, an EPR sample tube, of the same kind used in the free radical measurements, containing actinometer solution with an optical density high

enough to absorb essentially all incident quanta. This permitted the calculation of the number of quanta per second at 665 m μ which reached the EPR tube (1.96×10^{15} quanta per second).

The chlorophyll a concentration used in the unpaired spin determinations gave an optical density of approximately three at 665 m μ in the EPR sample tube. Thus, it was assumed that all of the incident light was absorbed in the sample.

Diphenylpicrylhydrazyl (DPPH; Aldrich) was used as an unpaired spin standard. This was recrystallized from carbon disulfide and the purity was determined spectrophotometrically (6). A solution of this material in the appropriate alcoholic solvent was then used to calibrate the EPR spectrometer.* Double integrations were performed to obtain a value of unpaired spins per unit area of chart paper on the recorder.

Temperature corrections of spin concentration were made by running DPPH solutions in the various solvents as a function of temperature to obtain a calibration of the spectrometer sensitivity and by applying the standard reciprocal temperature dependence of EPR signal height.

To determine the initial rates of radical production, chlorophyll a-p-benzoquinone solutions in an alcoholic solvent were illuminated in the EPR spectrometer at various temperatures with monochromatic light (665 m μ) of known intensity. A double integration of the steady-state EPR signal thus obtained permitted a calculation of the steady-state unpaired electron concentration. Rise and decay curves were recorded using a Sanborn model 150 recorder. An overall instrument response time of approximately 0.3 second was used. This was just fast enough to allow reasonably accurate

* Microwave saturation curves were run on these solutions and on the chlorophyll-quinone solutions and a value for the microwave attenuation was chosen so that no power saturation occurred.

measurements of initial rates to be made. The actinic light was turned on and off by actuating a solenoid-controlled camera shutter. Neutral density filters were used to ascertain that the radical production rates were linearly proportional to light intensity. The initial rate of formation of radicals was obtained graphically from these rise curves.

The quantum yield was calculated from the ratio of the spins formed per second to the number of light quanta absorbed per second. It is estimated that the overall accuracy of these measurements is approximately 25%. The yields have not been corrected for the occurrence of radical decay during the initial rise period (see below).

Fluorescence intensity measurements on chlorophyll solutions were carried out using a previously-described fluorimeter (7). Excitation was at 3650 Å. At this wavelength and at the concentrations used, less than 10% of the exciting light is absorbed by quinone.

Results and Discussion

Some typical curves for radical formation and decay are shown in Figure 1. The determination of initial rates from these curves was probably the limiting factor in the accuracy of the quantum yield measurements.

In Figure 2 is plotted the radical concentration one second after the light was turned on (which is a satisfactory approximation to the initial rate) as a function of light intensity. The linear relationship confirms the validity of our approach and is also consistent with a mechanism, such as the one proposed earlier (1), in which radical production proceeds from a singly-excited state of chlorophyll.

The values of the absolute quantum yields and the steady-state radical concentrations obtained at several temperatures and in several solvents are shown in Table 1. In Figure 3, the data obtained in ethanol are plotted.

Absolute Quantum Yields of Electron Transfer in Chlorophyll-Benzoquinone

Solutions in Alcoholic Solvents at Various Temperatures

Table 1

<u>Solvent</u>	<u>T, °C</u>	<u>Viscosity, poise*</u>	<u>Quantum Yield**</u>	<u>Steady-state radical concentration (unpaired spins)</u>
ethanol	-10	2.1	0.10	1.4×10^{14}
ethanol	-50	6.3	0.052	0.8×10^{14}
ethanol	-90	28	0.33	4.2×10^{14}
ethanol	-110	75	0.70	9.0×10^{14}
ethanol	-150	----	0.13	1.9×10^{15}
ethanol	-196	----	0.0	0.0
methanol	-90	8.5	0.13	1.3×10^{14}
n-butanol	-10	7.6	0.05	0.8×10^{14}
n-butanol	-50	36	0.08	3.8×10^{14}

*These values were obtained by plotting data obtained from the "Handbook of Chemistry and Physics", 44th edition, Chemical Rubber Co., 1962 and interpolating to the proper temperature.

**These figures represent averages of from two to four determinations.

In order to obtain reasonably good measurements, it was necessary to use large molar excesses of quinone. However, under these conditions and at low temperatures, quite high yields were obtained, approaching unity at about -100°C in ethanol.

It is to be noted that the yield in ethanol drops slightly between about -10°C and -50°C and that, as the temperature is lowered still further, it rises rather sharply to a maximum at about -100°C and falls to zero at -140°C . The fact that the reaction can still occur at these low temperatures indicates that the process of electron transfer from excited chlorophyll to quinone probably has little or no activation energy.

p-Benzoquinone is known to be a very effective quencher of chlorophyll fluorescence at room temperature (8). Bimolecular quenching constants are of the order of 10^{10} liter mole $^{-1}$ second $^{-1}$ and seem to be viscosity-controlled. We have carried out experiments in which the fluorescence intensity of chlorophyll a solutions in ethanol was measured at various temperatures with and without added p-benzoquinone. The concentration levels were the same as were used in the EPR experiments. At -80°C , the quinone quenched approximately 35% of the chlorophyll fluorescence; at -130°C , approximately 45% of the emission was quenched. Thus, the two types of measurements, fluorescence emission and free radical formation yields, are consistent with each other. This would suggest that the principal quenching mechanism involves an electron transfer from chlorophyll to quinone.

The following considerations give some insight into the factors which are affecting the radical formation yields. The unpaired spin decay curves (after the light is turned off) obey bimolecular kinetics over a fairly wide range of light intensities. Some typical data are shown in Figure 4. This suggests that radical recombination is rate-limiting. In

Figure 5 we have plotted the bimolecular rate constants obtained from such data as a function of temperature over the range -50°C to -150°C . In the same Figure is a similar plot of the viscosity of ethanol.^{*} We consider the agreement between the temperature coefficients of these two quantities to be satisfactory, which suggests that the radical recombination process $(\text{Chl}^{\dagger} + \text{Q}^{\cdot-}?)$ is viscosity-controlled.^{**}

It is convenient to consider the remainder of the data in terms of the following mechanism:

$(\text{R}_1 + \text{R}_2)^{\dagger}$ is assumed to be a radical-ion pair which has some excess

* Another solvent parameter which is temperature-dependent is the dielectric constant. However, the temperature coefficient of this quantity is much smaller than that of the viscosity. For example, in ethanol, over the temperature range -10°C to -110°C , the dielectric constant changes by a factor of approximately 1.3 whereas the viscosity changes by a factor of approximately 37. See below for a further discussion of the possible effects of solvent dielectric.

** It is interesting to note that the magnitude of the bimolecular decay constants is considerably less than one would expect for a diffusion-controlled process. For example, k_p ($\frac{8RT}{30\eta}$) at -10°C in ethanol should be approximately 10^9 liter mole $^{-1}$ second $^{-1}$, whereas the experimental value is approximately 10^7 liter mole $^{-1}$ second $^{-1}$. This suggests that orientation factors (perhaps involving electron orbital overlap) are limiting the recombination rates.

kinetic energy and which is in a solvent cage. This was suggested by our earlier work (1) and can be rationalized in terms of a situation in which the light quantum has more than enough energy to achieve the actual transfer of the electron and therefore the radical-ions produced are in an electronically-excited state. Internal conversion processes would then cause a localized heating of the products and the surrounding solvent.

$(R_1 + R_2)$ represents the thermally-equilibrated radical pair still in the solvent cage while $R_1 + R_2$ are the separated radical-ions.

In the temperature range -50°C to -110°C , the steady-state radical concentration is approximately proportional to the square root of the solvent viscosity. This is shown in Figure 6. This would indicate that, at least in this temperature range, the solvent viscosity has very little affect on either the rate at which the reaction products separate from one another (i.e., reaction 5, escape from the solvent cage), or the rate of encounter (reaction 4) of an excited chlorophyll molecule with a quinone molecule (perhaps because of loose complex formation) or the recombination within the solvent cage (reaction 6). The result of this would be a situation in which the viscosity-dependence of the steady-state radical concentration is determined mainly by the recombination rate. A more rigorous derivation of this conclusion can be obtained if one solves the above mechanism for the steady-state radical concentration. This gives the following expression:

$$(R)^2 = 1/k_7 (k_5 - k_6) k_4 (\text{Chl}^*) (Q)$$

Thus, if k_4 , k_5 and k_6 are roughly viscosity-independent, one would expect that the steady-state radical concentration would increase as the square root of the viscosity (since reactions 1, 2 and 3 are all unimolecular, one would guess that the excited-state chlorophyll concentration would also be viscosity-independent). As we shall see below, the effect of solvent viscosity is different at temperatures below -110°C .

It is also possible to account for the observed increase in the quantum yield in ethanol over the temperature range -10°C to -110°C in terms of this picture. Thus, inasmuch as the observed rate of radical production and the rate of radical disappearance due to recombination appear to have comparable magnitudes (see Figure 1), the initial rate that we measure will undoubtedly be lowered by the occurrence of the back reaction during the initial portion of the recording of the rise curve. Therefore, as one reduces the rate of radical recombination by increasing the viscosity, one should observe a parallel increase in the observed initial rate of spin production and hence an increase in the observed quantum yield.

The larger value for the yield at -10°C in ethanol as compared to -50°C would suggest that other factors, as yet unknown, may come into play at higher temperatures.

It remains now to account for the drop-off in yield at the very lowest temperatures used here. In this range, the solvent viscosity is very high. At these viscosities, it might be expected that some of the product molecules will not have sufficient energy to escape from the solvent cage. These will recombine rapidly causing an apparent decrease in the observed initial rate (and thus a decrease in the observed quantum yield). If one carried this analysis still further, one would expect that those product molecules which are at the higher end of the kinetic energy distribution would take longer to recombine or, possibly, would find themselves so far apart after the reaction that they would be unable to diffuse sufficiently far to recombine at all. This concept of the opposing effects of very high solvent viscosities fits very nicely with the following two observations. First, it explains the fact that the steady-state radical

concentration maximizes at a lower temperature than does the quantum yield (see Figure 3). Second, it accounts for the occurrence, below -110°C but above -155°C in ethanol, of a substantial radical signal, of the same shape as the light-induced signal, which remains in the dark after an illumination period and which disappears upon melting the solvent and recooling in the dark ("frozen-in radicals").

At the very lowest temperatures (-195°C), presumably none of the products can escape from the solvent cage (or perhaps the excited chlorophyll molecules either cannot diffuse to the quinone or cannot orient themselves properly with respect to the quinone) and so both the quantum yield and the steady-state radical concentration drop to zero.

The above concepts of the effects of solvent viscosity are in agreement with our earlier work (1) and with the results of other investigators (9, 10, 11).

In order to obtain some insight into the role of other solvent properties in determining quantum yields, some runs were made in methanol and in n-butanol. These data are also given in Table 1. The fact that the yield in methanol at -90°C is roughly the same as the ethanol yields at -10°C and -50°C is probably a reflection of the similarity in viscosities of the two media at these temperatures. However, the somewhat higher methanol value may be due to other factors, for example, dielectric constant. This is further supported by the generally lower yields obtained in n-butanol. Inasmuch as the products of electron transfer in these systems should be radical-ions, it is not unexpected that a lower dielectric constant medium would result in a lower quantum yield by permitting more efficient radical recombination to occur.

Conclusion

It is apparent from the above discussion that there are two principal factors which influence the quantum yield in the present system. The first involves the necessity of getting the reactants (excited chlorophyll and quinone) together so that an electron transfer process can occur. The second factor has to do with preventing recombination events (these are of importance because there must be a considerable amount of light energy stored in the free radical products). In the present system, we have achieved these goals by, first, using a large molar excess of quinone, and, second, by increasing the solvent viscosity and dielectric constant. It is tempting to speculate that the photosynthetic system accomplishes these ends somewhat differently. The highly-organized structure of the chloroplast lamellae may have reaction sites composed of built-in donor-acceptor pairs which are in resonance contact with the large bulk of the chlorophyll which serves as a light-collecting device. The lamellar structure may also provide a mechanism for moving the transferred electron away from the reaction site by having a solid-state conducting medium surrounding the site and/or by having electron transport chains (cytochromes, etc.) connected to the site. All of these structurally-determined devices would serve to increase the efficiency of photosynthetic electron transfer.

Acknowledgements

We are indebted to Drs. A. K. Banerjee and D. C. Bhatnagar for assistance in carrying out the fluorescence measurements and to Professor L. S. Forster for many helpful discussions.

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FIG. 1

FIG. 2

QUANTUM YIELD (UNPAIRED SPINS PER QUANTUM)

FIG. 3

FIG. 4

FIG. 5

FIG. 6