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THE EFFECT OF OXYGEN AND BENZOQUINONE CONCENTRATION ON THE REVERSIBLE SPECTROPHOTOMETRIC CHANGES ACCOMPANYING THE PHOTOOXIDATION OF PORPHYRINS AT LOW pH VALUES

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SUMMARY

Oxygen has little or no effect on the spectrophotometric changes accompanying the reversible photooxidation of porphyrins by benzoquinone at low pH values. High concentrations of benzoquinone inhibit these reversible changes. Spectral evidence is presented to indicate that the porphyrin may be undergoing different photooxidative processes at different pH values.

INTRODUCTION

I have recently reported that oxygen and the concentration of benzoquinone greatly affected the light-induced proton uptake accompanying the reaction of chlorophyll with benzoquinone in methanol[1]. This reaction was originally discovered by Linschitz and Rennert[2] and studied by Korn[3]. The presence of oxygen enhances proton uptake while high concentration of benzoquinone has an inhibitory effect. Proton uptake is the result of the association of protons at low pH with the semiquinone formed by the photooxidation of the chlorophyll. The source of the protons is assumed to be from the solvent. Evstigneev and Gavrilova have shown that the reversible photooxidations of chlorophyll[4] and magnesium phthalocyanine[5] at low pH values can be followed spectrophotometrically. The present study was undertaken to determine the effect of these two variables (O_2 and benzoquinone concentration) on the spectral changes accompanying the photooxidation of the two pigments by benzoquinone at low pH values. The results show that oxygen has little or no effect on the reversible photooxidations of chlorophyll while high concentrations of benzoquinone inhibit the reactions. Similar results were observed with the magnesium phthalocyanine–benzoquinone systems. The different light-induced spectral changes observed in the Soret band of chlorophyll at the different pH values suggest that chlorophyll *a* may be undergoing different photooxidative processes at the different pH values as was previously indicated[6].

EXPERIMENTAL SECTION

The preparation and purification of chlorophyll and *p*-benzoquinone have been described[6]. The magnesium phthalocyanine was the Eastman Kodak white

label. U.S.P. reagent quality ethanol (U.S. Industrial Chemical Co., New York, N.Y.) was distilled at atmospheric pressure while Baker's purified methanol was used directly. No precautions were taken to keep the alcohols dried.

The percentage of the pigment undergoing reversible photooxidation was determined by measuring the red band after irradiation. The time of measurement of the red band following cessation of irradiation was usually between 7 and 20 s later. Maximum recovery of the red band was usually attained within 20 min. The measurements were made with a Cary Model 14 recording spectrophotometer at room temperature at 666 and 669 nm for chlorophyll *a* and magnesium phthalocyanine, respectively. Air-free systems were obtained by flushing with nitrogen which was previously scrubbed with either alkaline pyrogallol or chromous chloride solutions. Methanol or distilled ethanol was used directly for systems studied in the presence of air. The pH values of the solutions were adjusted with 0.005–0.01 M HCl solutions in alcohol. The chlorophyll–benzoquinone systems were irradiated in 1-cm spectrophotometric cells for 30 s with the projection lamp setup described in ref. 7. A 500-W projection lamp was used in the present study. The magnesium phthalocyanine systems were irradiated for 1 min. The times of irradiation were less than those usually used in order to limit the irreversible photooxidations of the porphyrins in the aerated systems.

RESULTS AND DISCUSSION

When chlorophyll *a* is irradiated in the presence of benzoquinone in either aerated or deaerated methanol at low pH values, the absorption spectrum exhibits decreases in absorbance at the red and Soret bands and an increase in the 500-nm region. Typical absorption changes for the chlorophyll *a*–benzoquinone in aerated methanol at a pH of 3.0 are shown in Fig. 1. The absorption changes are attributed to a one electron oxidation of chlorophyll by benzoquinone with the formation of the benzosemiquinone. The regenerated spectrum(... in Fig. 1) displays an increase in absorbance at the Soret band indicating some pheophytinization at the lower pH values. These results are not unlike those observed by Evstigneev and Gavrilova[4] in deaerated ethanol.

Fig. 2 shows the results of a series of experiments carried out to study the effect of oxygen on the reversible photooxidation of chlorophyll *a* by benzoquinone. In the determination of the percent of reversible photooxidation, the assumption was made that the photochemical transient does not absorb appreciably the red light used to monitor the system. The percent of reversible photooxidation is shown to be approximately the same in either aerated or deaerated systems. The plotted data do show some inhibition by oxygen in the pH range of 3.0–3.8. Oxygen is assumed to be without an effect since the standard of error of the data is greater than indicated by their spread. Values of the percent of reversible photooxidation can vary as much as 5 % at the same pH value. This variation is probably related to the decrease of reproducibility of the glass electrode in organic solvents. These results are in contrast to those observed by Evstigneev and Gavrilova[4] where oxygen had an inhibitory effect at low pH values and low temperatures. Complete reversibility was not always attained with the deaerated systems and this may be attributed to a "two electron" photooxidation of chlorophyll by residual oxygen. The percent of this irreversible

photooxidation is relatively constant over the pH range studied in either the deaerated or aerated system. The pH values of the systems shown in Fig. 2 were measured before the run. Studies where the measurements of pH followed the run gave maximum values of 30 and 34 % for the reversible photooxidation for the aerated and deaerated systems, respectively. The lower values observed in Fig. 2 are the result of partial pheophytinization.

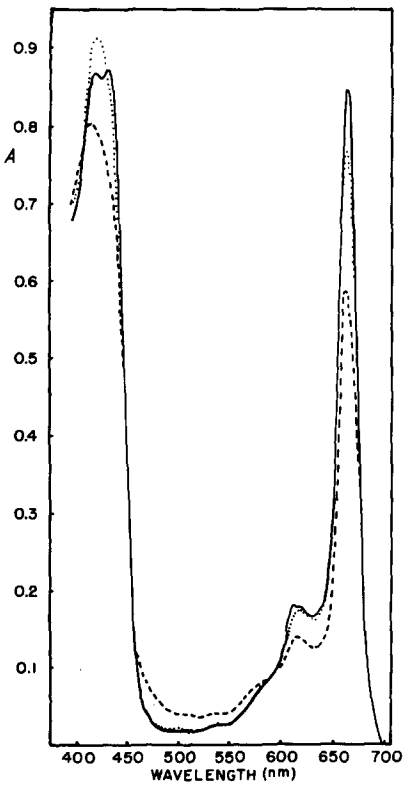

Fig. 1. Spectral changes accompanying the reversible photooxidation of chlorophyll *a* by benzoquinone in aerated methanol at room temperature. ———, original spectrum; – – –, light-induced; · · · · · ·, regenerated. Chlorophyll *a* = $1.3 \cdot 10^{-5}$ M, benzoquinone = $2.6 \cdot 10^{-3}$ M, pH 3.0.

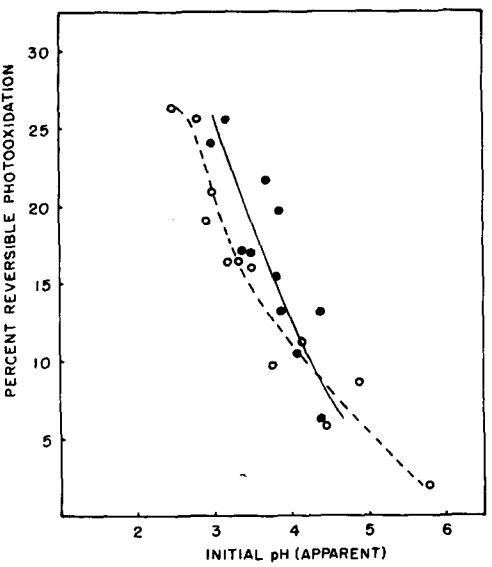

Fig. 2. The effect of oxygen on the percent of chlorophyll *a* undergoing reversible photooxidation at various pH values. Abscissa represents initial solution pH. ●—●, deaerated methanol; ○- - -○, aerated methanol. Chlorophyll *a* = $1.4 \cdot 10^{-5}$ M, benzoquinone = $2.6 \cdot 10^{-3}$ M. Solutions irradiated for 30 s at room temperature.

These results indicate that oxygen does not interfere with the removal of an electron from chlorophyll in the presence of benzoquinone at lower pH values. Oxygen must therefore interact in some way with the light-induced chlorophyll–benzoquinone system to effect the enhancement of proton uptake. ESR measurements of the aerated chlorophyll–benzoquinone system at low pH showed only a small light-induced broad signal similar to the one observed at neutral pH values. At the present time, one can only conclude that a species is formed in these light

sensitive systems at low pH which is quite basic and does not give rise to an ESR signal.

A series of experiments was performed at high benzoquinone concentrations ($2.9 \cdot 10^{-1}$ M) in order to observe the effect of benzoquinone concentration on the spectral changes accompanying the reversible photooxidation of chlorophyll *a* in aerated and dearated methanol. The high concentrations of benzoquinone completely inhibited the reversible spectral changes. These results show that the concentrations have the same quenching effect for the reversible photooxidation as they do for the reversible proton uptake.

TABLE I

PERCENT REVERSIBLE PHOTOOXIDATION OF MAGNESIUM PHTHALOCYANINE BY DIFFERENT CONCENTRATIONS OF BENZOQUINONE IN AERATED AND DEAERATED ETHANOL

pH values of maxima in parentheses. Magnesium phthalocyanine = $4.4 \cdot 10^{-6}$ M.

Benzoquinone concn (*M*)	*Percent reversible photooxidation*	
	Aerated	*Deaerated*
$2.0 \cdot 10^{-5}$	18 (3.3)	17 (3.7)
$3.7 \cdot 10^{-4}$	28 (3.4)	25 (3.2)
$2.6 \cdot 10^{-3}$	24 (3.2)	25 (3.2)
$2.9 \cdot 10^{-1}$	0	0

The effect of oxygen and benzoquinone concentration on the reversible spectral changes displayed by the magnesium phthalocyanine–benzoquinone system are reported in Table I. The data show that the two variables exhibit the same effect as found with the chlorophyll system. An optimum concentration of benzoquinone for the reversible spectral changes exists in the vicinity of $3.7 \cdot 10^{-4}$ M. These results are reminiscent of those observed for the proton uptake of the chlorophyll system[6]. The maximum values of the percent of the reversible photooxidation of magnesium phthalocyanine occurred within the pH range of 3.0 to 4.0. The profiles of the curves are similar to those observed by Evstigneev and Gavrilova[5] where a decrease in photooxidation occurs at the lower pH values. This decrease is probably the result of the increase of association between magnesium phthalocyanine molecules at low pH.

Previous work[6] has indicated that chlorophyll may undergo different photooxidative processes at different pH values. Fig. 3 shows the Soret bands of the irradiated solutions of the chlorophyll–benzoquinone systems at pH values of 5.7, 4.7 and 3.7. At pH 5.7 (Fig. 3A), the light-induced Soret band exhibits a greater absorbance at 428 nm than at 415 nm while the reversed is observed at pH 4.7 (Fig. 3B). This decrease absorbance at 428 nm is further intensified at the lower pH of 3.7 (Fig. 3C) where partial pheophytinization is observed in the regenerated spectrum. Similar spectra are also observed in the aerated systems.

These differences in the Soret bands show that different forms of the oxidized intermediates of chlorophyll may possibly be present in these systems. It is assumed that the different pH values are not influencing the spectrum of only one intermediate but that different intermediates are formed at the different pH values.

This assumption appears be to reasonable since the parent chlorophyll spectra are not greatly affected by pH and the cation radicals of chlorophyll formed at the lower pH values would probably not be affected by increases in acidity. Different photo-

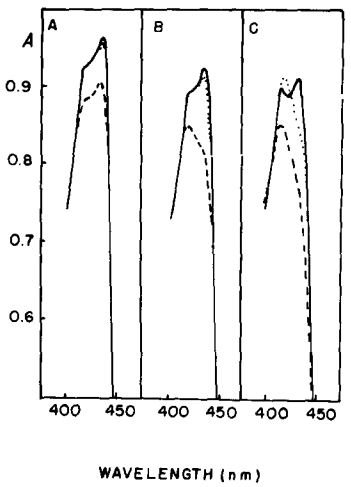

Fig. 3. The effect of pH on the light-induced Soret band accompanying the reversible photooxidation of chlorophyll *a* by benzoquinone in deaerated methanol. A. pH 5.7. B. pH 4.7. C. pH 3.7. ———, original spectrum; – – – –, light-induced; · · · · · ·, regenerated. Chlorophyll *a* = $1.4 \cdot 10^{-5}$ M, benzoquinone = $2.6 \cdot 10^{-3}$ M.

oxidative processes are also evident by the observation by Ke *et al.*[8] where the light-induced interaction between chlorophyll and ubiquinone was quenched by oxygen while the present paper shows that oxygen does not influence the spectral changes accompanying the reversible photooxidation of chlorophyll at these low pH values. These different processes may also be responsible for the different decays of the chlorophyll radical observed by Kelly and Porter[9] in the acid, neutral and alkaline systems of chlorophyll and duroquinone. These studies show the importance of pH in the study of these light-induced interactions.

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