

## EFFECTS OF SOLVENT ON THE RATE OF BACTERIOCHLOROPHYLL a PHOTO-OXIDATION†

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### Summary

The photostability of bacteriochlorophyll a (BChl a) in organic media shows strong solvent effects on the rate of pigment photo-oxidation, but the lifetimes  $\tau_{\Delta}$  of singlet oxygen  $O_2(^1\Delta_g)$  do not account quantitatively for the observed effects on the photo-oxidation rate. The mechanistic aspects of BChl a photo-oxidation are complicated on the basis of the number (five or more) of isolable photoproducts. However, the spectrophotometric progress of the reaction in most solvents appears to be simple, exhibiting well-defined isosbestic points that are preserved essentially to reaction completion, which indicates that the stoichiometry, even though complex, remains constant.

We have carried out comparative studies of direct and rubrene-sensitized photo-oxidation of BChl a in nine solvents spanning a moderately wide range of dielectric constant, oxygen solubility,  $\tau_{\Delta}$  and solvent nucleophilicity. The last property affects the coordination state of the central magnesium ion of BChl a and the fluorescence properties of the pigment. The relative rates of pigment photodegradation show parallel trends in both the direct and the sensitized experiments. However, there is no correlation of initial photo-oxidation rates (in direct irradiations) or relative degradation rates (compared with rubrene disappearance in the sensitized experiments) either with solvent polarity or with  $\tau_{\Delta}$ . The only clear trend appears to be with the coordination number of BChl a magnesium. Thus, the rates are significantly slower when the central magnesium ion is hexacoordinated compared with pentacoordinated magnesium species. The sole exception to this trend is diethyl ether (pentacoordinated magnesium) in which BChl a is remarkably stable.

A possible explanation of our results is that the triplet energy  $E_T$  of BChl a lies close to the  $^1\Delta_g$  state of oxygen but varies with solvent. Thus, in hexacoordinating solvents,  $E_T$  may be less than  $7882 \text{ cm}^{-1}$ , but in penta-coordinating solvents (except diethyl ether)  $E_T \geq 7882 \text{ cm}^{-1}$ . This effect

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may be important in understanding the *in vivo* photostability conferred by proteins.

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## 1. Introduction

Early investigations of the photostability of bacteriochlorophyll a (BChl a) in organic solvents revealed significant solvent effects on the rate of pigment photo-oxidation. Thus, Smith and Calvin [1] reported an approximate fortyfold increase in the rate of BChl a photo-oxidation in acetone compared with the rate in diethyl ether. Recently, accurate values for the lifetimes  $\tau_{\Delta}$  of singlet oxygen  $O_2(^1\Delta_g)$  in various organic solvents have become available [2], but these fail to account quantitatively for the observed solvent effect on the photo-oxidation rates ( $\tau_{\Delta}(\text{Et}) = 30.4 \mu\text{s}$  (Et  $\equiv$  diethyl ether);  $\tau_{\Delta}(\text{Ac}) = 50.5 \mu\text{s}$  (Ac  $\equiv$  acetone)). Nevertheless, involvement of  $O_2(^1\Delta_g)$  in the photo-oxidation of BChl a is strongly implicated, on the basis of cumulative evidence concerning photo-oxidation of other synthetic and natural magnesium porphyrins [3 - 6]. Moreover, Krasnovsky [7] reported that BChl a is both a moderately effective sensitizer and a potent quencher of  $O_2(^1\Delta_g)$  luminescence. Sensitization of  $O_2(^1\Delta_g)$  formation by protoporphyrin [7], hematoporphyrin [8] and other tetrapyrrole macrocycles [9, 10] has also been established.

The mechanistic aspects of BChl a photo-oxidation appear to be complicated on the basis of the large number (five or more) of isolable photo-products [1, 11]. However, the spectrophotometric progress of the reaction in most solvents appears to be simple, exhibiting clear and well-defined isosbestic points that are preserved essentially to completion of the reaction; this indicates that the stoichiometry, even if complex, remains constant. Cox and Whitten [6] have demonstrated that protoporphyrin IX undergoes self-sensitized photo-oxidation via an attack by  $O_2(^1\Delta_g)$  on the vinyl side groups of the molecule. Also, Fuhrhop and Mauzerall [12] showed that photo-oxidation of magnesium octaethylporphyrin (MgOEP) leads to a single product (a magnesium formylbiliverdin) which results from addition of molecular oxygen at the methine bridge position, followed by O—O bond cleavage. The results of rate inhibition studies using  $\beta$ -carotene clearly suggest that the mechanism of MgOEP photo-oxidation involves  $O_2(^1\Delta_g)$  which is formed by energy transfer from the porphyrin triplet state. A similar mechanism involving  $O_2(^1\Delta_g)$  has been established in the self-sensitized photo-oxidation of magnesium tetraphenylporphyrin (MgTPP) [13].

In this study, we measured the rate constants of BChl a reaction with  $O_2(^1\Delta_g)$  by observing the competition for  $O_2(^1\Delta_g)$  by BChl a and another acceptor molecule of known reactivity. We have used the well-characterized sensitizer-acceptor rubrene (tetraphenylanthracene) to determine relative reaction rates in several organic solvents. Rubrene is ideally suited for this purpose, as its rate constant for reaction with  $O_2(^1\Delta_g)$  has been determined in many solvents. Stevens and Perez [14] have shown that the rate varies by

less than a factor of 3 in eight organic solvents of varying viscosity and polarity. Also, the visible absorption spectrum of rubrene does not overlap that of BChl a in the 450 - 800 nm range; thus, either pigment can be excited selectively in the presence of the other. Even though the triplet energy of BChl a [15 - 17] is probably below that of rubrene [18], energy transfer to BChl a has been minimized in our work by the use of low BChl a concentrations (10  $\mu$ M or less) and oxygen-saturated solutions ( $[O_2] \geq 5$  mM).

Another objective of this work was to determine the importance of electron transfer in BChl a photo-oxidation. Connolly *et al.* [15], on the basis of their very low estimate of the triplet energy of BChl a, suggested that the lowest triplet state of the pigment ( $^3$ BChl a) may be quenched by oxygen to give the BChl a radical cation and superoxide anion. This would provide an additional oxidative pathway when BChl a is excited directly, provided that the ions undergo subsequent net reaction. Recent electron paramagnetic resonance experiments using spin traps for superoxide have shown that measurable amounts of  $O_2^-$  are generated when many synthetic metalloporphyrins are irradiated in organic solvents [19]. Electron transfer to oxygen apparently can compete effectively with energy transfer when the triplet states of magnesium, zinc and copper hematoporphyrins are quenched by oxygen in aqueous solution [8]. Therefore, it might be expected that the rates of BChl a photo-oxidation will be enhanced when the pigment is excited directly compared with rubrene-sensitized experiments.

## 2. Experimental methods

Rubrene (Aldrich) and BChl a (Sigma, from *Rhodospseudomonas sphaeroides* or Chromatium D) were used as supplied. The solvents were obtained from Burdick and Jackson Laboratories except for *p*-dioxane and acetone which were from Baker Chemical Company (Analyzed Reagent grade). The solutions were prepared by adding solid BChl a to solutions containing rubrene to achieve maximum absorbances of about 0.5 both in the  $Q_y$  band of the chlorophyll (about 770 nm) and in the visible bands of rubrene.

Solutions were oxygen saturated by bubbling through a hypodermic syringe needle inserted through a vented rubber septum cap in long-stemmed optical cuvettes of 1.00 cm path length. Sensitized reactions were carried out in the sample holder of a Hewlett-Packard model 8450 UV-visible spectrophotometer using the 488 nm line of an argon ion laser (Spectra Physics model 162A). The laser light was passed through a diverging lens (focal length, -2.5 cm) placed 25 cm from the cell so that an aperture 1.0 cm in diameter drilled in the side of the cell holder was filled. The light source for BChl-a-sensitized reactions was a 450 W xenon arc lamp passed through a monochromator (Spex Minimate) with slits set at 12 nm bandwidth. A 550 nm cut-off filter was used to eliminate higher order wavelengths.

Except for solutions containing diethyl ether, constant oxygen concentrations were maintained by bubbling during the irradiation periods; this technique also served to stir the solutions. Diethyl ether solutions were bubbled before illumination and were magnetically stirred during illumination to minimize evaporation losses. Experiments carried out in diethyl ether-acetonitrile mixtures were not oxygen saturated, but were irradiated under air-saturated conditions with magnetic stirring.

The rates of pigment disappearance were calculated directly from the absorbance changes at the long wavelength absorption maxima for rubrene and BChl a in each of the solvents studied. Since both BChl a and its photo-products are nearly transparent in the region of rubrene absorption, spectral interference is negligible during the initial stages of the reaction. We estimate from the BChl-a-sensitized photo-oxidation experiments in the absence of rubrene that the contribution to the absorbance changes between 450 and 550 nm due to the BChl a photoproducts is less than 3% for an approximate 30% change in absorbance at about 770 nm.

Relative rate constants for the reaction of BChl a and  $O_2(^1\Delta_g)$  were calculated from the relationship [20]

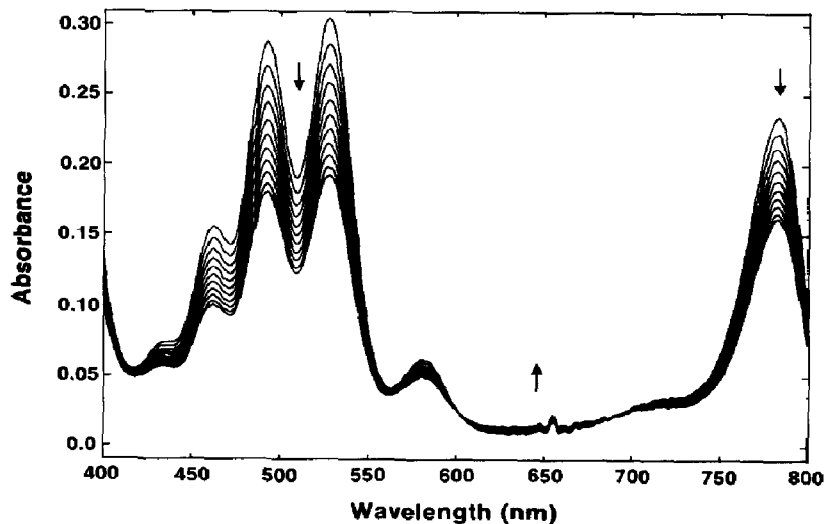
$$\frac{\ln(A_0^B/A^B)}{\ln(A_0^R/A^R)} = \frac{\ln([B]_0/[B])}{\ln([R]_0/[R])} = \frac{k_x^B}{k_x^R} \quad (1)$$

where  $A_0$  and  $A$  represent the absorptions measured before irradiation and at some time after irradiation respectively. Here, R stands for rubrene and B for BChl a, the quantities in brackets are molar concentrations and  $k_x^M$  is the bimolecular rate constant for reaction of molecule M with  $O_2(^1\Delta_g)$ . This treatment assumes that reaction with  $O_2(^1\Delta_g)$  is solely responsible for the disappearance of both pigments and that the absorbance changes at each observation wavelength accurately reflect the changes in the respective pigment concentrations. Good linear plots were obtained in nearly all cases with least-squares slopes that are independent of the specific wavelengths used in the analysis. Non-linear behavior was consistently observed for oxygen-saturated acetonitrile and methylene chloride solutions, for which we report initial rates only.

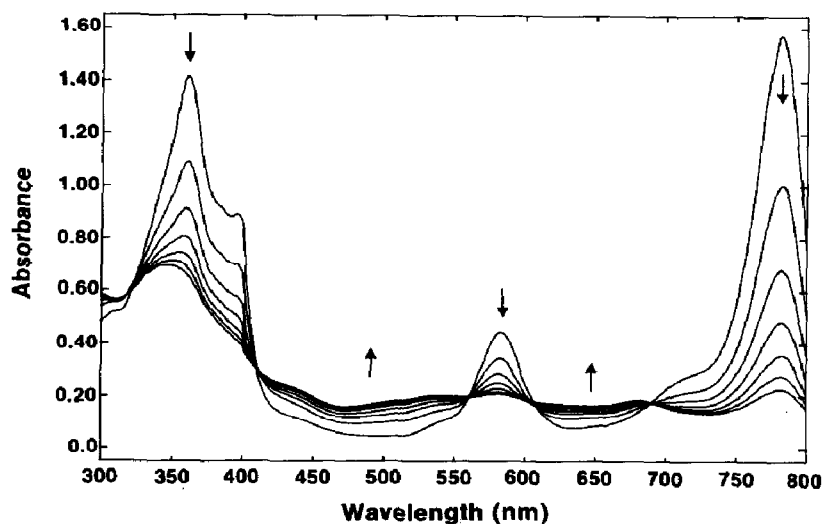
A potential complication is that the fluorescence spectrum of rubrene overlaps the  $Q_x$  absorption band of BChl a ( $\lambda_{max} \approx 570 - 610$  nm). Therefore, a small steady state concentration of  $^3BChl$  a could be generated by reabsorption of rubrene emission, even though this emission is partially quenched by oxygen. This would present a difficulty in interpreting the results of the rubrene-sensitized reactions in terms of  $O_2(^1\Delta_g)$  reaction rates if  $^3BChl$  a leads to measurable pigment degradation. The intensity of BChl a fluorescence in a typical solution containing rubrene in argon-purged tetrahydrofuran (THF) shows an approximate 20% contribution due to absorption by rubrene.

### 3. Results

The spectrophotometric progress of the rubrene-sensitized photo-oxidation of BChl a is similar to the self-sensitized reaction (Fig. 1), and the isosbestic points (in benzene) at 332, 407, 565, 602 and 694 nm are preserved. Data from each run were analyzed according to eqn. (1); typical results are plotted in Fig. 2. The slopes were obtained by a linear least-squares method with the intercept constrained to the origin and are listed



(a)



(b)

Fig. 1. Spectrophotometric progress of photo-oxidation of BChl a in oxygen-saturated benzene: (a) rubrene sensitized (30 s irradiations; 488 nm; argon ion laser excitation); (b) BChl a sensitized (10 min irradiations; broad band excitation ( $\lambda_{\text{ex}} \geq 550$  nm)).

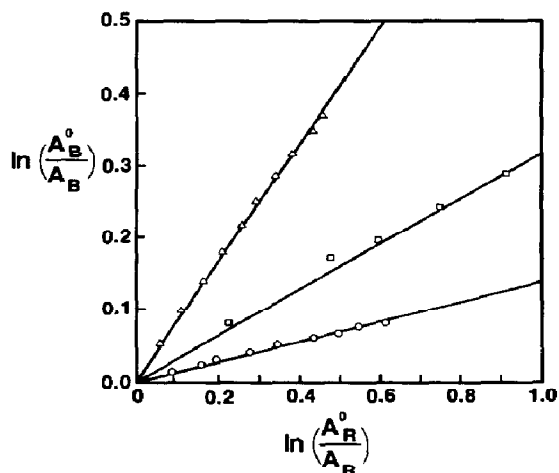


Fig. 2. Rates of rubrene-sensitized photo-oxidation of BChl a according to eqn. (1) in benzene ( $\Delta$ ), acetone ( $\square$ ) and pyridine ( $\circ$ ).

TABLE 1

Relative rate of rubrene-sensitized photo-oxidation of bacteriochlorophyll a in oxygen-saturated organic solvents

Solvent	Mg coordination number <sup>a</sup>	$\epsilon^b$	$\tau_{\Delta}^c$ ( $\mu\text{s}$ )	$k_T^B/k_T^R$
Methylene chloride	5	(9.1)	82.9	1.12 <sup>d</sup>
Acetonitrile	5	$\approx 37$	58.3	$1.03 \pm 0.02^e$
Benzene	5 <sup>f</sup>	2.3	31.2	$0.82 \pm 0.02$
Acetone	5	20.7	50.5	0.41 <sup>d</sup>
Pyridine	6	12.3	(16) <sup>g</sup>	$0.138 \pm 0.03$
Dioxane	6	2.2	26.7	$0.044 \pm 0.001$
Diethyl ether	5	4.3	30.4	$0.035 \pm 0.005$
THF	6	7.6	20 <sup>h</sup>	$0.015 \pm 0.002$

<sup>a</sup>See ref. 21.

<sup>b</sup>Dielectric constant, see ref. 22.

<sup>c</sup>See ref. 2.

<sup>d</sup>Initial rates.

<sup>e</sup>Air-saturated solutions.

<sup>f</sup>Axial ligand is probably H<sub>2</sub>O [21].

<sup>g</sup>See ref. 23.

<sup>h</sup>See ref. 9.

in Table 1. It can be seen that the relative rates in the fastest (methylene chloride) and slowest (THF) solvents differ by a factor of at least 50. Also, in solvents in which the magnesium coordination number is five (based on the position of the Q<sub>x</sub> absorption maximum) [21, 24, 25], the rates are roughly comparable and are consistently higher than in the hexacoordinating solvents, diethyl ether being the sole exception.

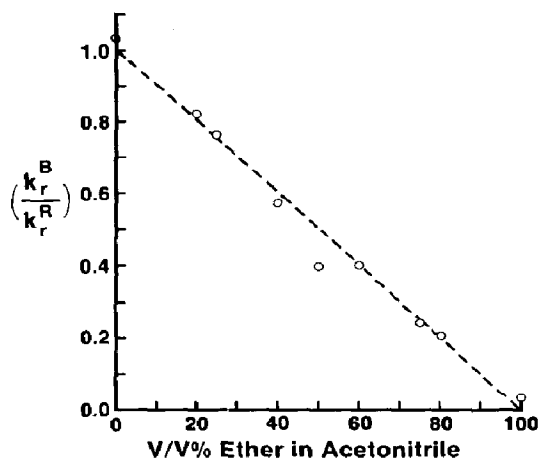


Fig. 3. Effect of added diethyl ether in acetonitrile on the rates of rubrene-sensitized photo-oxidation of BChl a: ---, theoretical curve for  $K_{eq} = 2.0$  for eqn. (2) and normalized relative rates.

Since diethyl ether solutions showed rates more consistent with those obtained in the hexacoordinating solvents, we investigated a possible equilibrium [24] between diethyl-ether- and acetonitrile-ligated BChl a in mixed solvent systems. The effect of adding diethyl ether to acetonitrile solutions of rubrene and BChl a is shown in Fig. 3, where relative reaction rates have been plotted against the concentration of diethyl ether in acetonitrile. The observed trend in the data can be accommodated in terms of an equilibrium between two pentacoordinated magnesium species:



TABLE 2

Relative rates of self-sensitized and rubrene-sensitized photo-oxidation of bacteriochlorophyll a

Solvent	Mg coordination number	$k_r^B/k_r^R$ <sup>a</sup>	$k_r^B[S \equiv B]/k_r^B[S \equiv R]$ <sup>b</sup>
Methylene chloride	5	1.47 <sup>c</sup>	1.31
Acetonitrile	5	$0.88 \pm 0.29$	$0.85 \pm 0.27$
Benzene	5	$1.68 \pm 0.14$	$2.04 \pm 0.13$
Acetone	5	$0.43 \pm 0.01$	1.06
Pyridine	6	$0.66 \pm 0.05$	$4.78 \pm 0.29$
Dioxane	6	0.09 <sup>c</sup>	2.04
Diethyl ether	5	0.05 <sup>c</sup>	1.40
THF	6	$0.11 \pm 0.07$	$7.3 \pm 3.8$

<sup>a</sup>Self-sensitized experiments.

<sup>b</sup>[S  $\equiv$  B], sensitized by BChl a (broad band monochromatic light; 770 - 782 nm); [S  $\equiv$  R], sensitized by rubrene (argon ion laser; 488 nm).

<sup>c</sup>Initial rates.

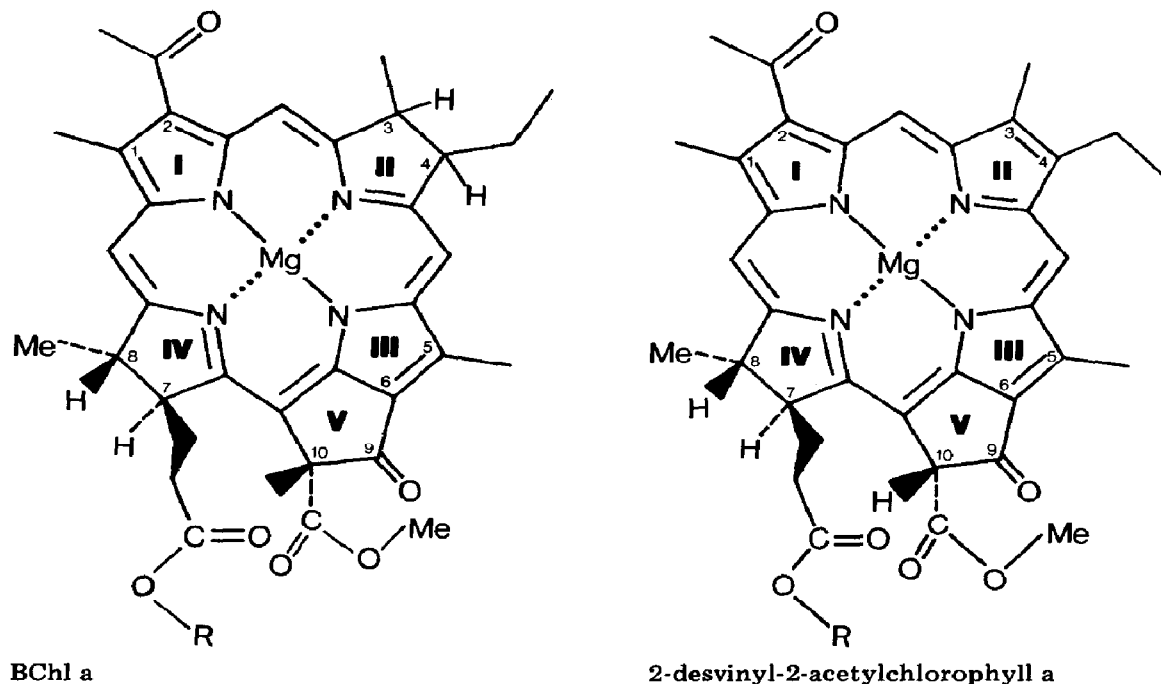
where An denotes acetonitrile. For eqn. (2) as written,  $K_{eq} \approx 2.0$ , as determined from the data in Fig. 3.

The relative rates obtained for self-sensitized photo-oxidations are always faster than the rubrene-sensitized rates. This is shown in Table 2, where the ratios of the two rates are given. While this ratio is generally larger for the hexacoordinating solvents, no definite trend can be seen with solvent polarity as might be expected if electron transfer processes were involved.

## 4. Discussion

### 4.1. Mechanism of reaction

Earlier attempts to isolate and identify the products of BChl a photo-oxidation showed that one of the products is the chlorin, 2-desvinyl-2-acetylchlorophyll a, formed by dehydrogenation of ring II:



It is not clear that  $O_2(^1\Delta_g)$  is involved in this mode of reaction, however. Smith and Calvin [1] have shown that the chlorin, which exhibits absorption maxima at 390, 437 and 678 nm, can be obtained in 90% yield on equimolar addition (in acetone) of 2,3-dichloro-5,6-dicyanoquinone, although photo-oxidation of BChl a never gave yields of the chlorin higher than about 20%. Kim [11] isolated several products of BChl a photo-oxidation by thin layer chromatography; four of these had closely similar absorption spectra with maxima at 385, 435 and 675 nm in methanol. Another of



the major products showed only broad featureless absorption in the visible region. The low yields obtained suggest that chlorin formation is a minor reaction pathway and that a competing process is involved, perhaps electrophilic attack by  $O_2(^1\Delta_g)$  at the methine bridge positions. This has been shown to be the exclusive mode of reaction with  $O_2(^1\Delta_g)$  for MgOEP [12] and MgTPP [13] to give metal-substituted bilitriene photoproducts. Brown *et al.* [26] have shown that BChl c methyl pheophorbides undergo the same reaction; presumably, the methyl substituent at the  $\delta$  methine carbon in BChl c activates the adjacent double bond toward electrophilic attack. The same researchers have also proved using labeled  $^{18}O_2$  that both oxygen atoms inserted in the photoproduct derive from a single oxygen molecule, consistent with an  $O_2(^1\Delta_g)$  mechanism. The spectrophotometric progress of both rubrene- and self-sensitized reactions is consistent with formation of one or more products in which the conjugation of the porphyrin ring system has been interrupted.

#### 4.2. Effect of magnesium coordination number

The results of both rubrene-sensitized and self-sensitized photo-oxidations indicate that, in general, BChl a with hexacoordinated magnesium is relatively stable toward reaction with  $O_2(^1\Delta_g)$ . We have confirmed the qualitative observations of Smith and Calvin [1], although we find that the ratio of the rate constant in acetone to that in diethyl ether is about 10 compared with about 40 reported by Smith and Calvin for the overall rates. The exceptional stability of BChl a in diethyl ether (thought to be a pentacoordinating solvent [21]) remains for now an anomaly. We do not think it likely that knowledge of the absolute rate constants for rubrene photo-oxidation in each solvent would substantially alter the order of reactivities given in Table 1. This conclusion is based on the results of Stevens and Perez [14], cited above, and also on the report of Wilkinson and Brummer [27], who determined that the "best value" for the rate constant of rubrene photo-oxidation by  $O_2(^1\Delta_g)$  is  $k_r^R = 4.16 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$  (within a factor of 1.27 at the 95% confidence limit). This value, which was obtained by a statistical treatment of 34 measurements reported by different laboratories for 11 solvents, can be used to set limits on BChl a photo-oxidation rates as  $6.24 \times 10^5 \text{ M}^{-1} \text{ s}^{-1} \leq k_r^B \leq 4.66 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$  in the solvents listed in Table 1.

A possible explanation for the apparent protective effect of hexacoordinating solvents (and possibly diethyl ether) is that physical quenching of  $O_2(^1\Delta_g)$  by ground state BChl a is promoted at the expense of chemical quenching in these solvents due to a lowering of the energy level  $E_T$  of  $^3\text{BChl a}$ . The mechanism of physical quenching of  $O_2(^1\Delta_g)$  by porphyrinic molecules is not well understood. Cox and Whitten [6] have estimated that 90% of quenching encounters between  $O_2(^1\Delta_g)$  and protoporphyrin IX result in physical rather than chemical quenching. Also, Krasnovsky [7] has shown that porphyrins with relatively high triplet energies physically quench  $O_2(^1\Delta_g)$  luminescence at rates approaching the diffusion-controlled limit.

Thus, reverse energy transfer



is not required for efficient physical quenching, although the energy gap between  ${}^3M$  and  $O_2(^1\Delta_g)$  appears to affect the magnitude of the rate constant  $k_q$ . Thus, chlorophyll a (Chl a), which has a triplet energy of about  $10\,400\text{ cm}^{-1}$  [28], quenches  $O_2(^1\Delta_g)$  with  $k_q \approx 7 \times 10^9\text{ M}^{-1}\text{ s}^{-1}$ , both in  $\text{CCl}_4$  [7] and in benzene [29], while BChl a, which has a lower  $E_T$  (see below), is a somewhat less efficient quencher ( $k_q \approx 10^9\text{ M}^{-1}\text{ s}^{-1}$  [7]). In addition, Krasnovsky [7] showed that BChl a is about threefold less efficient than Chl a in sensitizing formation of  $O_2(^1\Delta_g)$ . These data suggest that the triplet state of BChl a lies close to, and in some solvents possibly lower than, the  ${}^1\Delta_g$  level of oxygen.

Our photo-oxidation data can be interpreted, at least in part, in terms of a relatively strong perturbation of  $E_T$  of BChl a which accompanies a change in the coordination number of the central chlorophyll magnesium and, perhaps, the nature of the ligand(s) as well. Solov'ev *et al.* [28] have shown that the phosphorescence spectra of both Chl a and Chl b are red shifted by about  $400\text{ cm}^{-1}$  on additional magnesium ligation. Similar spectral shifts have been observed for magnesium and zinc etioporphyrins [30]. Thus, process (3) should become faster as  $E_T$  of  ${}^3\text{BChl a}$  is lowered in hexacoordinating solvents. This is depicted in Fig. 4, where the lower limit of  $E_T$  (level a) was inferred from the kinetics of energy transfer and electron transfer quenching of  ${}^3\text{BChl a}$  [15], while the upper limit (level b) was estimated from the temperature dependence of E-type delayed fluorescence [16]. *Ab initio* calculations for ethyl bacteriochlorophyllide a [17] place  $E_T$  of the pigment isoenergetic with  $O_2(^1\Delta_g)$  within  $\pm 1200\text{ cm}^{-1}$ . The broken lines in Fig. 4 represent the upper and lower limits which we estimate would be consistent with a solvent effect on  $E_T$ . Thus, in pentacoordinating solvents,  $E_T$  may lie near the upper limit (about  $8300\text{ cm}^{-1}$ ), while in hexacoordinating solvents (and perhaps in diethyl ether, as well), the suggested lower

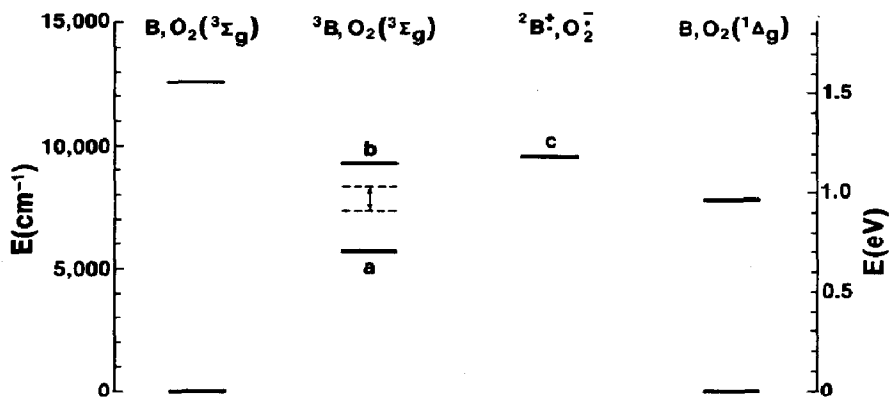


Fig. 4. Energy level diagram of the BChl a-oxygen system: level a, see ref. 15; level b, see ref. 16; level c, see refs. 31 - 35.

limit (about  $7300\text{ cm}^{-1}$ ) would permit favorable energy transfer from  $\text{O}_2(^1\Delta_g)$ . Connolly *et al.* [21] found that the fluorescence lifetimes of BChl a are longer in hexacoordinating solvents than in pentacoordinating solvents and suggested that intersystem crossing is somewhat (about 10%) less efficient in the former case, assuming internal conversion to be invariant; this is consistent with a larger excited singlet-triplet energy gap. Tait and Holten [36] measured the unimolecular component of the decay of  $^3\text{BChl a}$  and found it to be faster in solvents in which the central magnesium is hexacoordinated; they attributed this effect, in part, to a lowered  $E_T$ . In the absence of phosphorescence data, careful studies of the solvent dependence of the rates of  $\text{O}_2(^1\Delta_g)$  quenching by ground state BChl a and quenching of  $^3\text{BChl a}$  by oxygen (and by other energy acceptors) will be required to determine the importance of solvent effects on  $E_T$  of BChl a.

## 5. Conclusions

Our results demonstrate that BChl a reacts with  $\text{O}_2(^1\Delta_g)$  to give several products, only one of which has been identified [1, 11]. Furthermore, selective irradiation of BChl a sensitizes formation of  $\text{O}_2(^1\Delta_g)$  in all solvents examined, as demonstrated by the BChl-a-sensitized photo-oxidation of rubrene. This indicates that the triplet energy of BChl a is near that of  $\text{O}_2(^1\Delta_g)$  (about  $7880\text{ cm}^{-1}$ ), as suggested by Krasnovsky [7]. However, the rate constant for chemical quenching of  $\text{O}_2(^1\Delta_g)$  by ground state BChl a appears to depend on the ligating properties of the solvent. Thus, when the chlorophyll central magnesium ion is hexacoordinated (*e.g.* in pyridine, THF, dioxane) the rate of pigment oxidation is slower than for pentacoordinated magnesium species. We tentatively ascribe this effect to enhanced physical quenching via more favorable energy transfer from  $\text{O}_2(^1\Delta_g)$  to BChl a (process (3)). The sole exception to this trend is diethyl ether. All available evidence [21, 24, 25] points to the ability to distinguish between pentacoordinated and hexacoordinated BChl a magnesium by the wavelength maximum of the  $Q_x$  transition, which coincides with trends in the fluorescence lifetimes. Thus, the remarkable photostability of BChl a in diethyl ether remains to be explained.

The enhancement of BChl a photo-oxidation rates in the self-sensitized experiments probably is not due to electron transfer. First, there is no clear trend in any of the data with solvent polarity. Second, the electrochemical potentials available in the literature for BChl a [31 - 34] and oxygen [35] indicate that, in all solvents in which comparisons can be made, the ground state reaction



should be endergonic by about 1.2 eV (see Fig. 4, level c). Since  $\text{O}_2(^1\Delta_g)$  and probably  $^3\text{BChl a}$  [15, 17] lie at least 0.2 eV lower, electron transfer oxidation of BChl a should be endergonic except for reaction between excited

chlorophyll species and  $O_2(^1\Delta_g)$ , which can be ruled out in our BChl-a-sensitized experiments on kinetic grounds.

We can also exclude excited singlet state reactions of BChl a, either with the solvent or with dissolved oxygen, since the trends we observe in the enhanced photo-oxidation rates (Table 2) do not coincide with the solvent dependence of the fluorescence lifetimes [21]. Furthermore, the pigment is extremely stable in rigorously degassed pyridine and THF (the solvents with the most markedly enhanced photo-oxidation rates) even under intense pulsed laser excitation; thus, reaction of excited state BChl a (either singlet or triplet) with the solvent itself is of negligible importance in these experiments. We conclude that, in addition to oxidation of BChl a by  $O_2(^1\Delta_g)$ , there is at least one additional oxidative pathway that remains to be identified.

Further experiments are planned to answer some of the questions we have raised and also to attempt to understand the effects of molecular environment on the stability of the photosynthetic pigments *in vivo* as well as *in vitro*.

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## References

- 1 J. R. Lindsay Smith and M. Calvin, *J. Am. Chem. Soc.*, **88** (1966) 4500.
- 2 M. A. J. Rodgers, *J. Am. Chem. Soc.*, **105** (1983) 6201.
- 3 J. Barrett, *Nature (London)*, **215** (1967) 733.
- 4 J.-H. Fuhrhop, P. K. W. Wasser, J. Subramanian and U. Schrader, *Justus Liebigs Ann. Chem.*, (1974) 1450.
- 5 K. M. Smith, S. B. Brown, R. F. Troxler and J.-J. Lai, *Photochem. Photobiol.*, **36** (1982) 147.
- 6 G. S. Cox and D. G. Whitten, *J. Am. Chem. Soc.*, **104** (1982) 516.
- 7 A. A. Krasnovsky, Jr., *Photochem. Photobiol.*, **29** (1979) 29.
- 8 S. Cannistraro, G. Jori and A. Van de Vorst, *Photobiochem. Photobiophys.*, **3** (1982) 353.
- 9 J. R. Hurst, J. D. McDonald and G. B. Schuster, *J. Am. Chem. Soc.*, **104** (1982) 2065.
- 10 A. A. Krasnovsky, Jr., *Chem. Phys. Lett.*, **81** (1981) 443.
- 11 W. S. Kim, *Biochem. Biophys. Acta*, **112** (1966) 393.
- 12 J.-H. Fuhrhop and D. Mauzerall, *Photochem. Photobiol.*, **13** (1971) 453.
- 13 T. Matsuura, K. Inoue, A. C. Ranade and I. Saito, *Photochem. Photobiol.*, **31** (1979) 23.
- 14 B. Stevens and S. R. Perez, *Mol. Photochem.*, **6** (1974) 1.
- 15 J. S. Connolly, D. S. Gorman and G. R. Seely, *Ann. N.Y. Acad. Sci.*, **206** (1973) 649.
- 16 V. A. Shuvalov and W. W. Parson, *Biochim. Biophys. Acta*, **638** (1981) 50.
- 17 J. D. Petke, G. M. Maggiora, R. E. Christofferson and L. L. Shipman, *Photochem. Photobiol.*, **32** (1980) 399.
- 18 W. G. Herkstroeter and P. B. Merkel, *J. Photochem.*, **16** (1981) 331.

- 19 G. S. Cox, D. G. Whitten and C. Gianotti, *Chem. Phys. Lett.*, 67 (1979) 511.
- 20 T. Wilson, *J. Am. Chem. Soc.*, 88 (1966) 2898.
- 21 J. S. Connolly, E. B. Samuel and A. F. Janzen, *Photochem. Photobiol.*, 36 (1982) 565.
- 22 A. J. Gordon and R. A. Ford (eds.), *The Chemist's Companion*, Wiley, New York, 1972, pp. 4 - 13.
- 23 K. I. Salokhiddinov, I. M. Buteva and B. M. Dzhagarov, *Opt. Spectrosc.*, 47 (1979) 487.
- 24 T. A. Evans and J. J. Katz, *Biochim. Biophys. Acta*, 396 (1975) 414.
- 25 T. M. Cotton and R. P. Van Duyne, *J. Am. Chem. Soc.*, 103 (1981) 6020.
- 26 S. B. Brown, K. M. Smith, G. M. F. Bisset and R. F. Troxler, *J. Biol. Chem.*, 255 (1980) 8063.
- 27 F. Wilkinson and J. G. Brummer, *J. Phys. Chem. Ref. Data*, 10 (1981) 808.
- 28 K. N. Solov'ev, S. S. Dvornikov, V. N. Knyukshuto and A. E. Turkova, *Zh. Prikl. Spektrosk.*, 38 (1983) 87.
- 29 C. Tanielian and C. Wolff, *J. Chim. Phys.*, 78 (1981) 855.
- 30 D. G. Whitten, I. G. Lopp and P. D. Wildes, *J. Am. Chem. Soc.*, 90 (1968) 7196.
- 31 J. C. Goedheer, G. H. Horreus deHaas and P. Schuller, *Biochim. Biophys. Acta*, 28 (1958) 278.
- 32 J.-H. Fuhrhop and D. Mauzerall, *J. Am. Chem. Soc.*, 91 (1969) 4174.
- 33 P. A. Loach, R. A. Bambara and F. J. Ryan, *Photochem. Photobiol.*, 13 (1971) 247.
- 34 T. M. Cotton and R. P. Van Duyne, *J. Am. Chem. Soc.*, 101 (1979) 7605.
- 35 C. K. Mann and K. K. Barnes, *Electrochemical Reactions in Non-aqueous Systems*, Dekker, New York, 1970, p. 505.
- 36 C. D. Tait and D. Holten, *Photobiochem. Photobiophys.*, 6 (1983) 201.