Chemiluminescence Arising from the Action of $^1\Delta_g$-Molecular Oxygen on Chlorophyll-a

HARTMUT FUHR* and JOACHIM STAUFF

Institut für Physikalische Biochemie der Universität Frankfurt/Main

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Dedicated to THEODOR WIELAND *on the occasion of his 60th birthday*

Chlorophyll-a, singlet oxygen ($^1\Delta_g$) chemiluminescence, kinetics

Singlet oxygen ($^1\Delta_g$) was generated by a microwave discharge and bubbled through a solution of chlorophyll-a in dibutylphtalate at approximately 10-20 torr. It not only excited the dye to its first singlet state but also produced oxidized species which generated a very long lasting weak chemiluminescence. From quenching experiments and reaction kinetic data a possible mechanism for the generation of the excited species could be derived and supported by a hybrid-analog computer simulation.

Introduction

Since the early work of KAUTSKY[1] energy-transfer from electronically excited chlorophyll a to ground state oxygen generating singlet oxygen has been demonstrated. KRASNOVSKII and LITVIN[2] showed that solutions of chlorophyll-a in different solvents produced a long lasting afterglow when irradiated with light of the appropriate wavelength — but only when oxygen was present. They explained this observation with the presence of intermediates of peroxides and/or peroxy-radicals, formed in the reaction between the electronically excited photosensitizer and oxygen. However, this effect can possibly be attributed to the generation of singlet oxygen via energy-transfer from the excited chlorophyll-a molecule and its subsequent attack of the sensitizer itself. Further speculation suggests that this type of reaction might possibly be connected with the known afterglow of plant cells like algae[3], from leaves or in chloroplasts. Furthermore, it might be a possible mechanism in the damage of plants by air pollutants, *i. e.*, peroxyacetyl nitrate, which is a powerful phytotoxicant[4], has been proven to generate singlet oxygen when hydrolysed [5].

We now are able to present results which show that the interaction of chlorophyll-a with externally generated singlet oxygen does lead to a long lasting afterglow process which is entirely different from the simultaneous observation of physical energy-transfer from $^1\Delta_g$ $^1\Delta_g$-oxygen pairs to ground-state chlorophyll-a[6]. An attempt to describe the complex afterglow mechanism in a kinetic scheme is made by comparing experimentally accesible data with a mathematical optimization method utilizing a hybrid-analog computer (EAI 680).

Requests for reprints should be sent to Herrn Prof. Dr. J. STAUFF, Institut für Physikalische Biochemie der Universität, *D-6000 Frankfurt a. M.-Niederrad 1,* Sandhofstr.

* Present adress: Department of Chemistry, University of California, Riverside, California.

Experimental

Singlet oxygen was generated by passing ultra high purity grade (> 99.99 %) oxygen through a microwave discharge at pressures between 4 and 20 torr[7]. A film of mercuric oxide was coated just behind the discharge zone to destroy atomic oxygen. The gas stream was bubbled through the solutions under investigation which were placed in a silver plated, light shielded chemiluminescence cuvette. The light-detector used was a red sensitive photomultiplier (RCA 7265) which was cooled by evaporating liquid nitrogen in a specially designed housing. A shutter mechanism which allowed optical filters to be interchanged was fitted between the brass cuvette housing and the photomultiplier housing. All the spectra were taken with sharp cut-off filters (Schott & Gen., Mainz). The low intensities of the generated chemiluminescence excluded the use of a monochromator.

Chlorophyll-a was prepared following the method of STRAIN *et al.* from fresh spinach leaves[8]. Phaeophytin-a was derived from chlorophyll-a by the method of WILSON and NUTTING[9] and chlorophyllin-a was purchased from Mann Res. Lab. Inc., New York. The solvent used in most cases, because of its low volatility under reactions, was dibutylphtalate

(DBPh). Dimethylacetamide (DMA) and diethylacetamide (DEA) also were used.

Results and Discussion

A. General

When an oxygen stream containing singlet oxygen was bubbled through solutions of chlorophyll-a in DBPh or DMA, the $^1\Delta_g$ $^1\Delta_g$-dimol emission band at 634 nm was considerably quenched compared to the control run of singlet oxygen through the pure solvent. However, new emission bands appeared in the region of 665-715 nm. The spectra have been published recently[6] and it was pointed out that besides the emission band in the chlorophyll-a fluorescence region (685 nm) a band of even higher intensity was observed at 700-715 nm. This band can be explained by emission from an electronically excited chlorophyll-a oxidation product. This conclusion was also reached by comparing the absorption spectra of the chlorophyll-a solutions before and after the treatment with singlet oxygen (Fig. 1). A new absorption

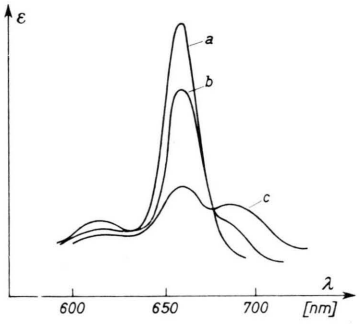


Fig. 1. Absorption spectrum of chlorophyll-a in DBP, a: before treatment, b: after immediately 20' treatment with $^1\Delta_g$–0_2, c: experiment b after standing for 4hours at room temperature.

band appeared at 697 nm which increased with the duration of singlet oxygen treatment. After the microwave discharge was stopped the absorption bands of chlorophyll-a continued to disappear (shown for the red part of the absorption spectrum) and the new band increased in the afterglow-phase. This showed undoubtedly that a primary oxidation product must have been generated during the discharge period which later slowly transformed chlorophyll-a into a new compound absorbing at 697 nm. Taking into account the small spectral shift between the red absorption shoulder of the chlorophylls and their fluorescence maxima it seems likely that the emission to the red of 700 nm was caused by the substance absorbing at 697 nm. Attempts to analyze the reaction products were only partially successful. Chromatography on cellulose coated glass plates (Merck, DC) with petroleum ether-propanole did not give a total separation of chlorophyll-a from the reaction product absorbing at 697 nm. However, the spot showed with KI/acetic acid/starch solution a bright color thus indicating the presence of peroxides or hydroperoxides being present. The peroxide reaction with *p*-aminodimethylaniline was positive as well but attempts to prove acids or aldehydes failed.

The first assumed generation of chlorophyll-d (oxidation of the ethylene group at ring I of the chlorophyll-a molecule) which absorbs in the region of our reaction product could not be confirmed. We generated chlorophyll-d by oxidation of chlorophyll-a with $KMnO_4$ following the method of HOLT and MORLEY[10] and were able to isolate it from chlorophyll-a by the same chromatographic method described above for the chlorophyll-a/singlet oxygen reaction. Comparison of spectra showed no accordance of spectral bands. Another attempt to produce the oxidized species of chlorophyll-a was made in a reaction where singlet oxygen was generated in a heterogeneous system by the $NaOCl/H_2O_2$ method (aqueous solution + chlorophyll-a in DBPh). The reaction showed a negative result. This can be explained by the strong inhibitory effect of water on the reaction (see Table I) which was found earlier.

Table I. Influence of the addition of effectors on the luminescence of the chlorophyll-a/singlet oxygen reaction.

Substance	Effect	Added before treatment with singlet O_2	Added after treatment
tetramethyl-ethylene	R. with $^1\Delta_g$-O_2	inhibition of luminescence	no effect
β-carotene	R. with $^1\Delta_g$-O_2	inhibition of luminescence	no effect
tri-tert-butyl-phenole	radical scavenger	inhibition of luminescence	inhibits immediately irrevers.
hydroquinone	scavenger, reductant and inhibitor for peroxide dec.	inhibition of luminescence	inhibits immediately irrevers.
dimethylaniline	initiator for peroxide dec.	inhibition of luminescence	inhibits slowly, irrev.
H_2O	?	inhibits partially	inhibits slowly, irrev.
chorophyll-a	—	no effect	inhibits * slowly, irrev.
$^1\Delta_g$-O_2	—	no effect	inhibits immediately, reversibly
benzoquinone	strong oxidant	enhancement of luminescence	enhancement

* Only at conc. of $> 10^{-4}$ M.

Furthermore, some experiments were made with chlorophyllin-a and phaeophytin-a in order to study the reactivity of singlet oxygen towards these chlorophyll-a analog substances. An afterglow with the same characteristic decay curves and similar spectral distribution was recorded in both cases. The phytol-group as well as the central Mg-ion can therefore not be involved in the light producing reaction.

B. Afterglow reaction

When the microwave power was turned off (oxygen flow remained unaltered) the intensity of the luminescence decreased instantly to less than 1 % of its stationary value during the discharge period. However, a further monotonic decay of the chemiluminescence occured for low oxygen pressures or for short treatment times with singlet oxygen only. Longer discharge periods or higher oxygen pressures led to a minimum/maximum course of the afterglow as indicated in Fig. 2. The spectral distribution of the afterglow emission was published earlier[6] and has emission bands between 665 and 715 nm.

C. Kinetics

To elucidate the reaction mechanism leading to the generation of excited molecules the kinetics of the

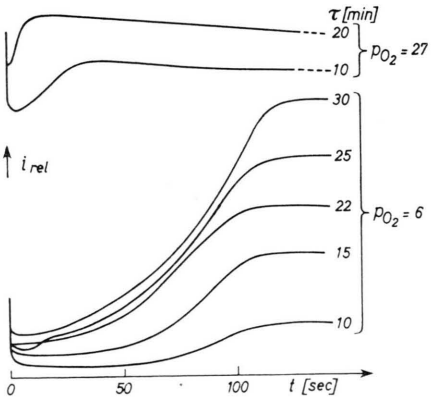


Fig. 2. Examples of the time course of the relative afterglow intensity (i_{rel}) of chlorophyll-a, treated with $^1\Delta_g$–O_2. The numbers indicate the duration of treatment τ. The lower set of curves are recorded at $p_{O_2} = 6$ mm, the upper set at $p_{O_2} = 27$ mm (see text).

afterglow curves were studied extensively. For this purpose the concentration of chlorophyll-a, the oxygen pressure and the duration of treatment ($= \tau$) were varied. For low chlorophyll-a concentrations no rise of the luminescence intensity was found in the afterglow phase, but concentrations of 10^{-5} to 10^{-4} mole/l showed a minimum-maximum course of the intensity-time curves. The maximum intensity reached clearly depended upon O_2-pressure (p_{O_2}) and τ as demonstrated in Fig. 2. With increasing oxygen pressure but constant values of τ the maxima were reached faster. As indicated in Fig. 2 the curves then started out at a much higher level compared to the case at lower p_{O_2}. The decay of the luminescence intensity followed a pseudo first order kinetics with the decay constant depending upon τ and p_{O_2}. Its value ranged from 10^{-4} to 10^{-3} sec^{-1} and after several hours chemiluminescence could still be detected.

The maximum afterglow intensity, i_{max}, indicates a stationary state and characterizes a distinct ratio of the reacting species. Therefore, the dependence of i_{max} upon the variables determining the time course should deliver some information of importance. In Fig. 3 i_{max} is plotted as a function of p_{O_2} with τ as a constant. The plot obtained by this procedure is somewhat intriguing in that only the curve at $\tau = 5$ min shows a third power dependence on p_{O_2}. (Dotted line indicates the dependence of the cubic root of i_{max} on p_{O_2}.) With increasing τ a negative square term of p_{O_2} shows increasing influence on i_{max}. (For analysis of the curves see below.)

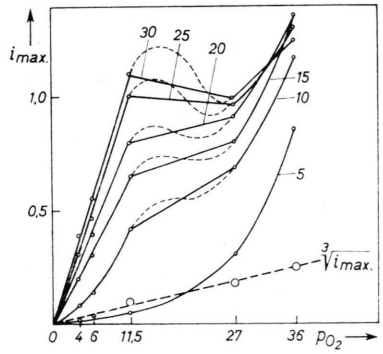


Fig. 3. Relative maximal intensity i_{max} of the afterglow in dependence of p_{O_2}. Parameter: duration of treatment τ.

In order to obtain more information about the intermediate steps of oxidation reactions, it was useful to study the influence of characteristical substances like radical scavengers or peroxide decomposition enhancers/inhibitors on one measurable parameter of the reaction - in this case, the luminescence. Table I shows the results obtained from some experiments with different compounds which were added to the chlorophyll solution either before or after the treatment with singlet oxygen.

The addition before the treatment lead in every case with one exception — benzoquinone to a suppression of the afterglow. Benzoquinone increased the velocity of the light producing process and the maximum intensity but did not change the spectral distribution of the emitted light. When the same substances were added during the afterglow phase, they showed remarkable differences in their effectivity. Tetramethylethylene and β-carotene are substances which react fast with singlet oxygen. During the discharge phase they trap or quench $^1O_2$ and therefore prevent chlorophyll from being oxidized. Their inefficiency during the afterglow phase proves that singlet oxygen is not involved in this period of the reaction. Benzohydroquinone and tri-tert-butylphenol are known radical scavengers. Benzohydroquinone is commonly used as inhibitor for the decomposition of peroxides[11]. Inhibitors which are diminishing the luminescence at a lower rate were dimethylaniline, H_2O and surprisingly chlorophyll-a itself. Moreover, $^1\Delta_g$-O_2 inhibits the luminescence if bubbled through the solution in the afterglow phase for a short time; after stopping the gas stream (or turning off the microwave discharge) the luminescence intensity drops to the same minimum value as in the beginnung of the course and then rises to the same value as before. This behaviour is demonstrated in Fig. 4a. The most puzzling behaviour is observed when the chemiluminescence was quenched by addition of fresh chlorophyll-a. A short treatment with singlet oxygen again lead to the development of luminescence (see Fig. 4b). The only enhancing substance was benzoquinone; it was able to activate the luminescence in every case even if hydroquinone had been added before and the luminescence was totally quenched.

A solution of chlorophyll-a in DBPh which had been treated three months before with singlet oxygen still contained peroxides, easily detectable by chromatographic analysis. The solution generated luminescence when only 10^{-5} M benzoquinone were added. This behaviour leads to the conclusion that at least two kinds of peroxides are produced. One being mainly responsible for the generation of immediate

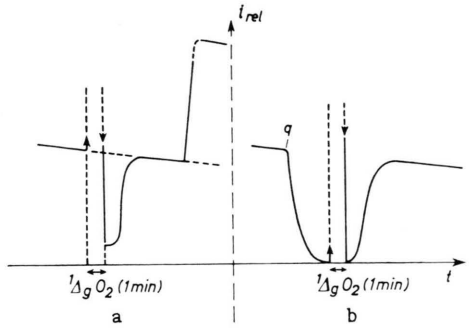


Fig. 4. a. Influence of $^1\Delta_g$-0_2 on the chemiluminescence-intensity of oxidized chlorophyll-a. The arrow indicates the period of $^1\Delta_g$-O_2 treatment.
b. Influence of $^1\Delta_g$-O_2 on the chemiluminescence intensity of oxidized chlorophyll-a quenched beforehand by chlorophyll-a at q.

afterglow, the other being more stable and not participating in the afterglow reactions to a greater extent. However, it can be activated by a strong oxidant like benzoquinone and will not be used up during a long storage period.

KRASNOVSKII *et al.*[12] observed that the afterglow of chlorophyll-a generated by illumination of the dye in the presence of oxygen was quenched by addition of benzoquinone. Because of this contradiction to our results, the experiment of KRASNOVSKII *et al.* was repeated. We found that the high benzoquinone concentration employed by KRASNOVSKII *et al.* did indeed quench the light induced chlorophyll-a afterglow. However, if only very low concentrations of benzoquinone were employed an enhanced chemiluminescence could be observed.

In the case of the singlet oxygen/chlorophyll-a system, the dependence of the afterglow intensity upon the benzoquinone addition was determined also. The results are presented in Fig. 5. It can be seen that benzoquinone enhances the luminescence at low concentrations but reverses its effect at higher concentrations. An explanation for this behaviour might be given in the competition between the reactive character of the quinones with peroxides and its well known quenching ability for electronically excited states[11, 12].

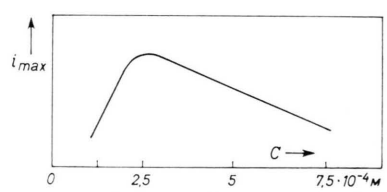


Fig. 5. Influence of benzoquinone on i_{max} in dependence of its concentration.

Analysis of kinetics

The analysis of the time course of the afterglow and its dependence upon different parameters utilizing an analog computer simulation technique can be used as a method to determine the minimal number of reaction steps necessary for the mathematical representation of the experimental results. In many cases a complicated mechanism consisting of several consecutive and competitive reaction steps can be approximated by some main consecutive steps and corrected afterwards by addition of appropriate competitive terms.

The method may be described briefly as follows: $a \rightarrow b \rightarrow c \rightarrow d$ shall be a sequence of first order reactions, $a^2 \rightarrow b^2 \rightarrow c^2 \rightarrow d^2$ a sequence of second order reactions. Combinations of first order and second order reactions can also occur, *e. g.* $a^2 \rightarrow b \rightarrow c^2 \rightarrow d$, there being 16 possible combinations. For each of the combinations, three rate constants determine the reaction course. Naturally this is valid only for a sequence of four reaction steps, for n steps there are n^2 combinations and n-1 constants. A hybrid-analog computer (or even better, a digital-analog computer) allows the design of a computing program, which can find the best combination of reaction steps and the best set of rate constants by comparing the computed values with the experimental data (optimization). In principle, it would be possible to give the computer the determination of the number of steps, n, as a task but this would require a digital device capable of setting analog programs by itself*.

Because such a computer was not at our disposition, the determination of n was performed by trial and error. Starting with $n = 2$ the necessary programming steps including their combined tasks were added with increasing numbers.

The kinetic problem on hand involves a complication which usually does not occur in kinetic measurements; the zero-concentrations are unknown. The parameters known are the concentration of chlorophyll-a, pressure of O_2, and duration of treatment τ. The problem is to be solved by introduction of a two computing-time program. During the first computing time the treatment of the chlorophyll solution with

* *e. g.* EAI A.S.P.E.

$^1\Delta_g$-O_2 is simulated and during the second, the reaction course after switching off the microwave discharge is computed. Because the observed time of the afterglow had always been about two minutes and the treatment time varied from five to 30 min, it is easy to simulate this variation by choosing scaling constants which correspond to the real time.

The results of the simulation are represented in Fig. 6 for the combination a → b^2 → c → d^2. The accord with the experimental results is better than expected for an unknown mechanism but shows a time lag which could not be simulated by an optimization method (curve 2, Fig. 6). To compensate for this deviation, one more assumption had to be made in the simulation program. It was derived from the observation that singlet oxygen quenched the afterglow which could only be due to a reaction of $^1\Delta_g$-O_2 with one of the intermediates. Naturally this reaction should also take place during the $^1O_2$-treatment period. If a reaction between $^1\Delta_g$-O_2 and the intermediate b during the time τ is part of the computer pogram, a function is derived which is in almost complete accord with the experimental curves (curve 3 of Fig. 6).

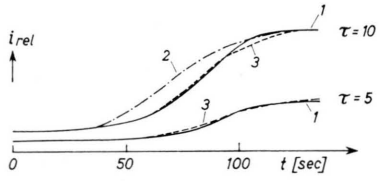


Fig. 6. Comparison of measured and computed chemiluminescence intensity-curves at $p_{O_2} = 4$ mm.
1. Experimental curves for $\tau = 5$ and $\tau = 10$ min,
2. computer simulation of simple scheme a→b^2→c→d^2,
3. the same simulation, corrected for the quenching influence of $^1\Delta_g$-O_2 during the treatment.

Some variations were made on the assumption that possible competitive reactions might take place, *e. g.* a+b, a+c or b+d, or that back reactions lead to equilibria, such as b → a or c → d. None of these reactions improved the computed curves. This result has to be interpreted with caution if one intends to draw conclusions for the sequence of the reaction steps of the mechanism. The combination of consecutive steps found by the computer is only to be regarded as a set of rate determining steps, which has to be included in every mechanism whatever the degree of complication might be. There might be other steps which are too fast to be rate determining. Possibly occurring side reactions cannot be detected by this method, but their influence is taken into account in the absolute values of the corresponding rate constants. As mentioned above, competitive reactions do not improve the computed curves. However, their possible participation in the overall reaction mechanism cannot be excluded assuming that they do not alter the results.

The complicated nature of the reaction mechanism is revealed by analyzing the i_{max} as a function of p_{O_2}. The plots representing these functions in Fig. 3 were analyzed by a simple linear analog program and resulted in the expression

$$i_{max} = a(\tau)\, p^3_{O_2} - b(\tau)\, p^2_{O_2} + c(\tau)\, p_{O_2}. \qquad (1)$$

The coefficients of this cubic equation are functions of τ altogether, With small values of τ an almost pure cubic function could be observed (curve 1, Fig. 3) indicating that only the first term of Equ. 1 is large.

The computer simulations in Fig. 6 correspond to curves measured at low pressure. Therefore, the simulated functions and the sequence of reactions derived therefrom are only valid in this low pressure region. At higher pressure of O_2 two other reactions or sequences of reactions ought to be considered, one of which is light producing and represented by the term c (τ) p_{O_2}. The dark reaction term is given by the expression b (τ) $p^2_{O_2}$.

Discussion

Though the measurement of the kinetical course of the afterglow and its analysis supplied many clues and some of the experiments with inhibitors revealed valuable facts, it is not possible to develop a complete scheme of the events which occur during and after the treatment of chlorophyll-a solutions with externally generated singlet oxygen. Particularly, it is difficult to determine the reacting center of the chlorophyll-a molecule, the nature of the intermediate and even the end products. Nevertheless, a tentative mechanism has been developed which explains the experimental results so far as possible.

There can be not doubt that gaseous singlet oxygen, bubbled through a solution of chlorophyll-a in DBPh excites the chlorophyll-a molecule into its first singlet state. This was proved by the emission spectrum of the luminescence accompanying this process. The

excitation of chlorophyll-a to its first excited singlet state requires 41.5 kcal/mol which corresponds well with the $^1\Delta^1\Delta$-dimole energy of 44.8 kcal/mole. This energy transfer is a well known process for several other dyes with low lying singlet states (OGRYZLO and PEARSON[13], WILSON[14], STAUFF and FUHR[15]).

In addition to this process, there are at least three other processes leading to energy rich intermediates, two of which are capable of producing light during the afterglow phase. The spectrum of the afterglow shows two peaks around 700 nm. One corresponds to the fluorescence maximum of chlorophyll at 685 nm and the other at 715 nm is attributed to a still unknown substance. Utilizing the reaction sequence found by the computer simulation and Equ. (1), the following scheme for low p_{O_2} can be formulated:

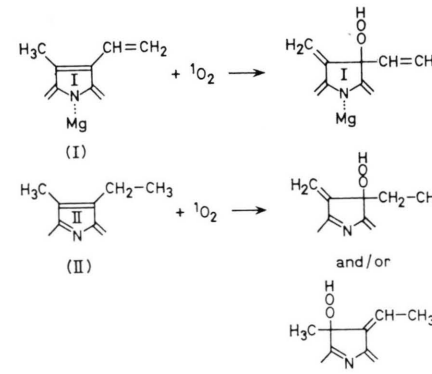
($^1O_2 = {}^1\Delta_g\text{-}O_2$,
Chl = chlorophyll-a,
^{1}Chl* = chlorophyll-a, (excited to its first singlet state)).

1. $\quad Chl + {}^1O_2 \rightarrow (ChlO_2)_a$
2. $\quad Chl + {}^1(O_2O_2) \rightarrow (ChlO_2)_b + O_2$
3. $\quad Chl + {}^1(O_2O_2) \rightarrow {}^1Chl^* + 2O_2$
4. $\quad {}^1Chl^* + {}^1O_2 \rightarrow (ChlO_2)_c$

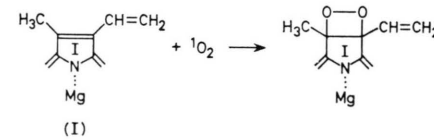
The above four reactions take place only during the treatment of the solution with $^1\Delta_g$-O_2 (treatment time = τ). The following steps can occur at all times, during and after the treatment with $^1\Delta_g$-O_2. The assumption of the formation of three different kinds of oxidation products is derived from the results represented by Fig. 3 and Equ. (1). The generation of $(ChlO_2)_a$ needs only one molecule of $^1\Delta_g$-O_2; it corresponds, therefore, to the linear term of Equ. (1) and should produce light. $(ChlO_2)_b$ which is not light producing, might not exist at all because the verification of Equ. (1) is already fulfilled if reaction 2 is a quenching process of $^1(O_2O_2)$ by chlorophyll. $(ChlO_2)_c$ should be regarded as the intermediate which produces chemiluminescence in every case, it corresponds to the cubic term of Equ. (1) and needs three molecules of $^1\Delta_g$-O_2 for its formation. One of the intermediates seems to form a stable peroxide, which is not destroyed during the first 30 min of the chemiluminescent reaction and which could still be detected after three months. It is possibly responsible for the light reaction induced by benzoquinone. Because $(ChlO_2)_a$ is surely involved in the light reactions at higher p_{O_2}, the stable peroxide could only be identified with $(ChlO_2)_b$.

In seeking the position in which the chlorophyll-a molecule is attacked by singlet oxygen, the known reactivity of singlet oxygen towards C-C-double bonds must be considered. The phytole-rest as a reactive center can be ruled out because chlorophilline-a showed the same effect as chlorophyll-a. There are three potential reactions of chlorophyll-a with singlet oxygen which have to be considered:[16]

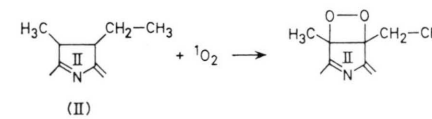
a. "ene"-reaction at ring I or ring II of chlorophyll-a.



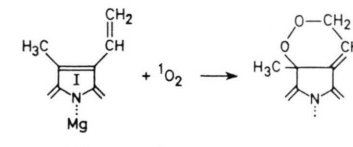
b. Dioxetane formation at ring I or II.



(Direct dioxetane formation at the ethylene group of ring I seems unlikely and should lead after bond cleavage to chlorophyll-d, which is definitely not the oxidation product).



c. Possible 1.4 addition with ring closure at ring I[17].



Thus, the assumption that several different products are formed in the reaction of chlorophyll-a with singlet O_2 seems not to be an improbable hypothesis.

The light producing reactions at low p_{O_2} should begin with the formation of a radical intermediate by a first order decomposition of $(ChlO_2)_c$.

5. $$(ChlO_2)_c \rightarrow \text{Rad}$$

To fulfill the requirements of the kinetic analysis, this step should be followed by a second order reaction. It is reasonable to choose the recombination of the radicals, Rad

6. $$2\ \text{Rad} \rightarrow X_2$$

The presence of the unknown product, X_2, is necessary for the construction of the reaction sequence. X_2 is an intermediate which decomposes to a species which by a bimolecular reaction is able to produce an excited singlet state of chlorophyll-a or an oxidized chlorophyll-a derivative. The most reasonable assumption is the generation of chlorophyll-a in its triplet state (^{3}[Chl]*).

7. $$X_2 \rightarrow 2\ {}^3(\text{Chl})^* + n\ O_2\ (n = 2\ ?)$$

The second order reaction at the end of the sequence in this case is a triplet-triplet annihilation.

8. $$2\ {}^3(\text{Chl})^* \rightarrow {}^1(\text{Chl})^* + \text{Chl}$$

and

9. $$^1(\text{Chl})^* \rightarrow \text{Chl} + h\nu.$$

The reaction mechanism at higher p_{O_2} must take into account the oxidation products $(ChlO_2)_a$ and $(ChlO_2)_b$. The first could be a dioxetane which decomposes to an excited carbonyl compound of unknown structure. The observed new absorption band should be caused by this decomposition and not by the stable peroxide, because the band at 697 nm still increases in the afterglow phase when the formation of the peroxide should be finished. Because chlorophyll-d only could have been formed by a reaction of the vinyl group of chlorophyll-a, $(ChlO_2)_a$ cannot be identified with a dioxetane of the vinyl group (I). So, a light producing step would be

10. $$(ChlO_2)_a \rightarrow {}^1(ChlO_2)^*_n + Z$$

11. $$^1(ChlO_2)^*_n \rightarrow (ChlO_2)_n + h\nu$$

The emitted light may be responsible for the peak at 715 nm in the luminescence spectrum.

The formation of $(ChlO_2)_b$ might lead to the stable peroxide which appears as a quenching term in Equ. (1), because it does not lead to a chemiluminescence.

The proposed mechanism is consistent with the results of the experiments with radical scavengers (see Table I) but no information could be obtained on the nature of the radical intermediate itself. Endoperoxides and dioxetanes could possibly form diradicals, discussed by RICHARDSON *et al.*[18] to explain the mechanism of the thermal decomposition, but no experimental evidence for their real existence has been presented until now. The first order decay of the afterglow should be controlled by X_2 at low values of p_{O_2} and by X_2 and $(ChlO_2)_a$ together at higher p_{O_2}; this explains the dependence of the decay constant on p_{O_2} and τ.

No explanation can be offered for the quenching effects of water and chlorophyll-a. Water may destroy one of the intermediates but nothing can be said about the reversible influence of the chlorophyll molecule.

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