Chemiluminescence from the Action of Singlet Oxygen ($^1\Delta_g$) on Chlorophyll a and some other Luminescing Substances

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In systems containing singlet-oxygen and aromatic fluorescers energy transfer from singlet-oxygen dimers to the dye should be observable by emission of the fluorescer. In order to prove this hypothesis, externally generated singlet-oxygen ($^1\Delta_g$) was bubbled through the solutions of dyes (chlorophyll a, eosin y, rhodamine b, luminol, rubrene and acridine orange) in organic solvents.

Luminescence could be observed and its spectral distribution analyzed by sharp cut-off filters and interference filters (rubrene). Spectra, rates of oxidation, addition of quenchers and the long lasting time dependence of the reported reactions lead to the conclusion that the observed afterglow is due to chemical oxidation mechanisms producing a chemiluminescence. Therefore an excitation of the substances investigated in these experiments by simple physical energy transfer seems not to be predominant.

Until recently energy transfer from excited oxygen dimers ($^1\Delta_g$, $^1\Delta_g$) to luminescing dyes had been reported in three cases only the acceptors being violanthrene [1], rubrene [2] and methyleneblue [3]. A new example was presented by KHAN [4] that the dye should be excited by O_2-dimers ($^1\Delta_g$, $^1\Sigma_g^+$) the singlet oxygen species originating from a reaction of KO_2. However in none of the cases the spectrum of the emitted light had been presented.

In connection with an investigation concerning the role of excited oxygen possibly produced during the photosynthesis of green plants the energy transfer of $O_2(^1\Delta_g)$ chlorophyll as has been studied extensively. For comparison also reactions of O_2 ($^1\Delta_g$) with several other fluorescing substances have been investigated.

The substances were dissolved (concentration $10^{-4}-10^{-5}$ M) in DBP or DMA *. Oxygen was excited by a microwave device, O atoms were removed by HgO and the excited O_2 bubbled through the solutions at pressures of 10 – 20 Torr. Traces of H_2O could be added to the gas stream to quench O_2 ($^1\Sigma_g^+$). The light emission of the reaction was measured by a red sensitive photomultiplier (RCA 7265) cooled by boiling N_2 as described recently [3]. Since the intensity of the emitted light is too weak for measurement of the spectrum with a spectrometer the spectral distribution had been determined in raw outlines using a series of sharp edge cut off filters.

In regard to the energy level of the O_2 ($^1\Delta_g$, $^1\Delta_g$) dimers at 1.58 μm^{-1} the luminescing substances under investigation can be divided into three groups. The first group consists of substances where $S_1 > T_1 > 1.58\ \mu m^{-1}$, no energy transfer should be possible in this case. In the second group is $S_1 > 1.58 > T_1$, energy could be transferred to the triplet state only. In the third is $1.58 > S_1 > T_1$, energy transfer to both states is possible. The following substances were investigated: Chlorophyll a (group 3), rubrene, eosine y, acridine orange (group 2) and luminol (group 1).

The emission spectra during the treatment with excited O_2 are presented in Fig. 1 (details see legend).

With the exception of chlorophyll a all spectra show the same band structure, one band is situated in the region of the ($^1\Delta_g$, $^1\Delta_g$) $\rightarrow$ ($2\ ^3\Sigma_g^-$, $v=0$) transition of O_2 dimers (at 634 nm) and is well to be recognized. The transition ($^1\Delta_g$, $^1\Delta_g$) $\rightarrow$ ($2\ ^3\Sigma_g^-$, $v=1$) at 706 nm is also quite intensive and easily to be seen. The $^1\Sigma_g^+ \rightarrow\ ^3\Sigma_g^-$ transition at 760 nm could not been separated by the applied methods. (Its presence is only detectable by addition of H_2O vapour to the gas stream – see Fig. 1 a, dotted lines – the region of higher wavelength is then

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* Abbreviations: DBP = Dibutylphthalate, DMA = *N,N'*-dimethylacetamide.

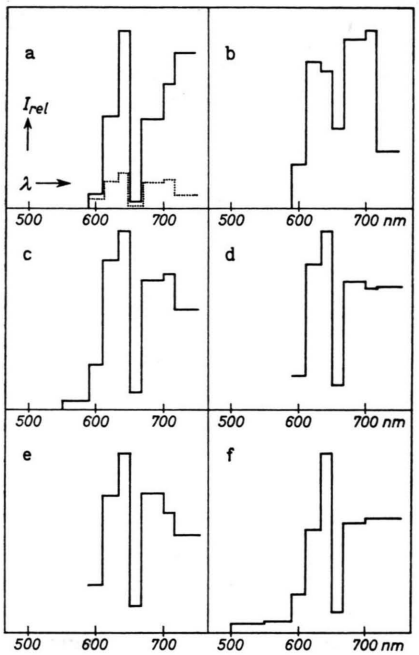

Fig. 1. Outlines of the emission spectra of solutions during the treatment with $O_2(^1\Delta_g)$, obtained with cut-off-filters. a) solvent DBP (Puncted lines: with H_2O-vapor), b) Chlorophyll a in DBP, c) eosine y in DBP, d) rubrene in DBP, e) luminol in DMA, f) acridine orange in DMA. Concentrations $1 \cdot 10^{-4}$ M.

quenched more than the other regions.) However, the spectra of chlorophyll a solutions (Fig. 1 b) have a different structure. The largest peak of the luminescence spectrum lies in the region of the fluorescence maximum (~ 685 nm) although a still higher peak appears between 700 and 715 nm which is not detectable in the fluorescence spectrum of chlorophyll a. In the case of acridine orange a weak luminescence band could be observed between 500 and 600 nm (Fig. 1 f).

In solutions of rubrene in DBP the existence of a luminescence could be proved by the use of an interference filter (560 nm) (see also Fig. 4). Thus the observation of WILSON [2] could be confirmed, who related this emission to a fluorescence of rubrene produced by direct energy transfer from O_2 ($^1\Delta_g$, $^1\Delta_g$) dimers.

However, the excitation of molecules by energy transfer is not the only possible way of activation performed by singlet oxygen. There seems to exist another way which has its origin in its chemical reactivity having been established in many cases [5]. It was found that in all investigated cases — also in the cases of substances of group 1 and 2 — a long-lasting afterglow appears when the stream of excited oxygen is interrupted. With chlorophyll a the afterglow is still detectable after hours. Its intensity increases with the duration of treatment with singlet oxygen. Fig. 2 shows the time dependence of the total luminescence intensity after reaction with excited O_2. The emission of most of the substances

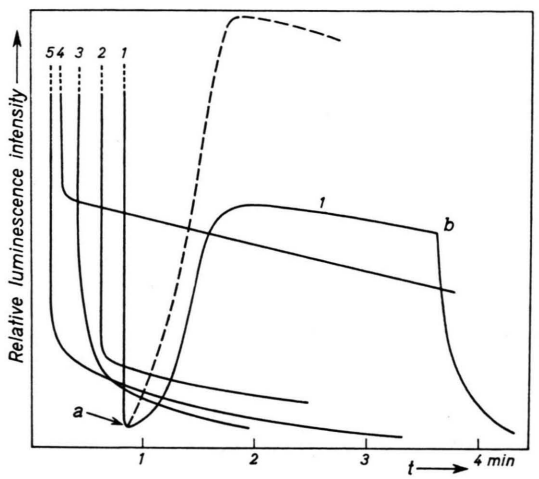

Fig. 2. Decay curves of luminescence after interruption of excited O_2. 1: chlorophyll a in DBP, drawn-out line: undisturbed decay dotted line: addition of p-benzoquinone at a. At b addition of H_2O, hydroquinone or t-tributylphthalate. 2: acridine orange in DBP, 3: rhodamine B in DBP, 4: eosine y in DMA, 5: eosine y in DBP.

decreases in a monotone way but chlorophyll a produces a curve which is going through a maximum the position of which depends on substance concentration and duration of treatment with excited O_2. Since the lifetimes of the excited states of the molecules have only the order of seconds the afterglow cannot be the result of a purely physical process. It is very likely to be a chemiluminescence induced by chemical reactions of singlet O_2 with the organic molecules. Moreover, the curve for chlorophyll a has a course which is typical for intermediary products of chemical reactions and which also can be observed in a considerable number of chemiluminescence processes [6]. The chemical nature of the afterglow can be ascertained by some experiments with additives. The afterglow of chlorophyll is a quench-

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[6] K. D. GUNDERMANN, Chemilumineszenz organischer Verbindungen, Springer-Verlag, Berlin-Heidelberg-New York 1968.

ed by water, benzohydroquinone and the radical scavenger tri-*t*-butylphenole as seen Fig. 2. Tetraethylethylene, a scavenger for O_2 ($^1\Delta_g$) has no influence at all. H_2O enhances the afterglow of chlorophyll a solutions after a period of quenching (not shown in Fig. 2). Most remarkably,, a short flush of singlet O_2 applied during the decay period causes a sharp decrease of intensity followed by a somewhat slower increase up to about the same intensity as before the treatment.

The spectra of the afterglow are presented in Fig. 3. While the yield of light emission of chlorophyll (measured in the maximum) in comparison to the yield of the 634 nm band of O_2 ($^1\Delta_g$, $^1\Delta_g$) dimers is about 5 per cent the yields of all other substances have values between 0.04 – 1 per cent at

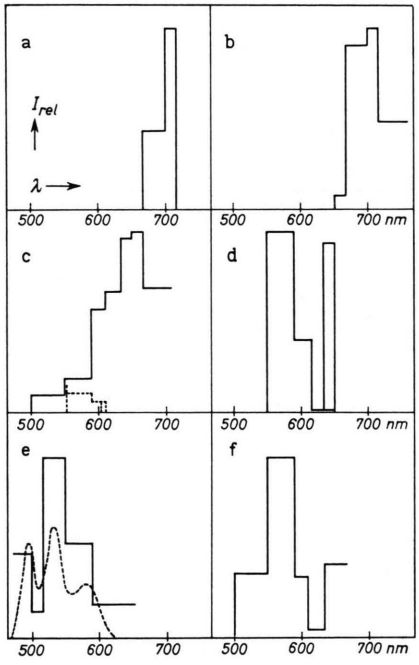

Fig. 3. Outlines of the emission spectra of the afterglow of solutions after the treatment with $O_2(^1\Delta_g)$. a) chlorophyll a in DMA ($2\cdot10^{-5}$ M), relative intensity: 0,2, b) chlorophyll a in DBP ($1\cdot10^{-4}$ M), rel. int.: $5\cdot10^{-2}$, c) eosine in DMA ($1\cdot10^{-4}$ M), rel. int.: $8{,}1\cdot10^{-3}$, – – – eosine y in DBP ($1\cdot10^{-4}$ M), rel. int.: $1{,}1\cdot10^{-3}$, d) rubrene in DBP ($1\cdot10^{-4}$ M), rel. int.: $4{,}1\cdot10^{-4}$, e) luminol ($1\cdot10^{-3}$ M), rel. int.: $1\cdot10^{-3}$ (the dotted line presents the phosphorescence spectrum of luminol at 77 °K).

a concentration of $1\cdot10^4$ M. The spectrum of chlorophyll a is located in the same region as its fluorescence spectrum but has different outlines in different solvents. In DMA the solution peak at 700 – 715 nm which could not been indentified until now is quite enlarged compared with the same peak registered in DBP solution, moreover, in the latter case the spectrum is extended to the far red region.

More drastic differences could be observed with the substances of group 2. Eosine y in DMA produces a spectrum with a maximum at 640 – 660 nm (1.51 – 1.56 μm^{-1}) which does not coincide with any excited state of the dye (T_1: 1.42, S_1: 1.76 μm^{-1} [7]). Only a minor band is to be seen at the fluorescence region (565 nm). The same band remains observable if a solution of eosine y in DBP is treated with singlet O_2 (Fig. 3 c) whereas the 650 nm band disappears completely. A similar behaviour shows acridine orange (Fig. 3 f), it emits neither at its fluorescence nor its phosphorescence bands, the band at 560 – 580 nm must be caused by an unknown reaction product of the dye with singlet O_2.

Only rubrene in DBP produces an afterglow with two distinct bands (Fig. 3 d) which coincide with its fluorescence band at 580 nm and with the emission band of O_2 ($^1\Delta_g$, $^1\Delta_g$) dimers at 634 nm.

A strange result was obtained with luminol in DMA (Fig. 3 e) which should not be excited at all as substance of group 1. Against our expectations its afterglow spectrum is very similar to its phosphorescence spectrum (measured at 77 °K [8]) and not with the fluorescence spectrum of aminophthalic acid which mostly is observed at its oxidative chemiluminescence [9].

For control the absorption spectra of the solutions after treatment with singlet O_2 were compared with those before treatment. The extinction of the characteristic absorption bands were diminished in all cases. Chlorophyll a showed a new absorption band at 698 nm which also has been observed after its illumination in the presence of O_2 [10]. Rubrene, acridine orange and eosine were bleached drastically, the latter much more in DBP than in DMA.

[7] C. A. PARKER, Photoluminescence of Solutions, Elsevier, Amsterdam 1968.

[8] M. BAACK, Diplomarbeit, Frankfurt a. M. 1967.

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Further evidence for the participation of chemical processes at the luminescence mechanism delivers the registration of decay curves of the rubrene afterglow at 560 nm.

Fig. 4 presents two of them, the first one (4 a) is a decay where the stream of oxygen is stopped suddenly, the second one (4 b) is a decay curve

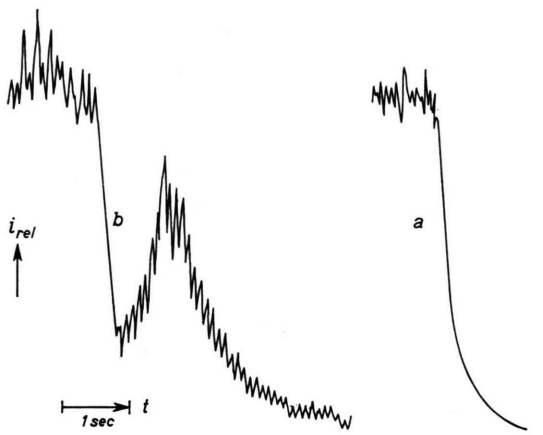

Fig. 4. Decay curve of rubrene in DBP. Interference filter at 460 nm (for details see text).

obtained by switching off the microwave generator and bubbling a stream of not-excited O_2 through the solution. It can be seen that the heavy deflections caused by each bubble continue after a short period of decrease and a new maximum appears. This indicates that light can be produced by a step at which a reaction with O_2 in the ground state(!) plays a dominant role and which can by no means be an energy transfer step in the physical sense.

These observations lead us to the conclusion that the luminescence of substances in organic solvents generated by treatment with gaseous singlet oxygen is not to be caused by an energy transfer in every case. Though this excitation mechanism cannot be excluded in the case of chlorophyll a (group 3) it cannot be valid for the substances of group 1 and 2. In these cases a chemical mechanism is to be the cause of the luminescence. Since the afterglow of chlorophyll a is doubtless of the same nature it is possible that this might be the case also for the whole phenomenon.

The theory of KHAN and KASHA [11] relates chemiluminescence which can be observed in many systems developing O_2 in the course of a redox reacto a energy transfer from excited O_2 dimers ($^1\Delta_g$, $^1\Delta_g$) to the substance in question. DOUZOU et al. [12] tried to verify these conception with systems of luminescing dyes at low temperatures. As result of the experiments communicated here it seems to be very doubtful wether the theory is applicable on every case. Even in the cases where the energy relations meet the requirements of the theory the real mechanisms seem to be much more complicated and need further investigation.

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