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Luminescence of isolated chloroplasts induced by organic solvents

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SUMMARY

Preilluminated lettuce chloroplasts emit light when injected with pure organic solvents. A positive linear correlation was found between the luminescence intensity and the dielectric constant of the solvent. It is suggested that the dissolution of the solvent in the chloroplast membrane increases its dielectric constant. This modifies the energy levels of charged trapped photoproducts and enables their recombination leading to luminescence.

There are various triggering processes which cause isolated chloroplasts to emit a brief pulse of light. The reported ones to date are: pH transition[1,2], salt addition[2,3], temperature increase[4] and external electric field changes[5]. These emissions require prior preillumination[6,7,2]. The action and emission spectra confine the emission process to the excited singlet state of chlorophyll *a* in Photosystem II[6]. The absolute requirement for preillumination indicates that relatively stable photoproducts are formed. These products, affected by the triggering, backreact to yield the chlorophyll excited state[6].

In this report a new type of triggering process is described: the injection of pure organic solvents to chloroplasts suspension. This operation causes light emission in a pulse form. The spectrum of this emission was not analyzed, but it was detected after passing through a far-red cut-off filter ($\lambda > 665$ nm) and presumably it is also due to the chlorophyll *a* singlet excited state.

In a typical experiment 1 ml of a chloroplast suspension [200 μg chlorophyll/ml, in either 0.2 M sucrose, 0.125 M KCl, 0.1 mM Tris (pH 7.5) or in 1 mM Tris (pH 7.5) alone] was preilluminated by a broad spectral band (420–600 nm, intensity approx. 140

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; FCCP, carbonyl cyanide *p*-trifluoromethoxy phenylhydrazone; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

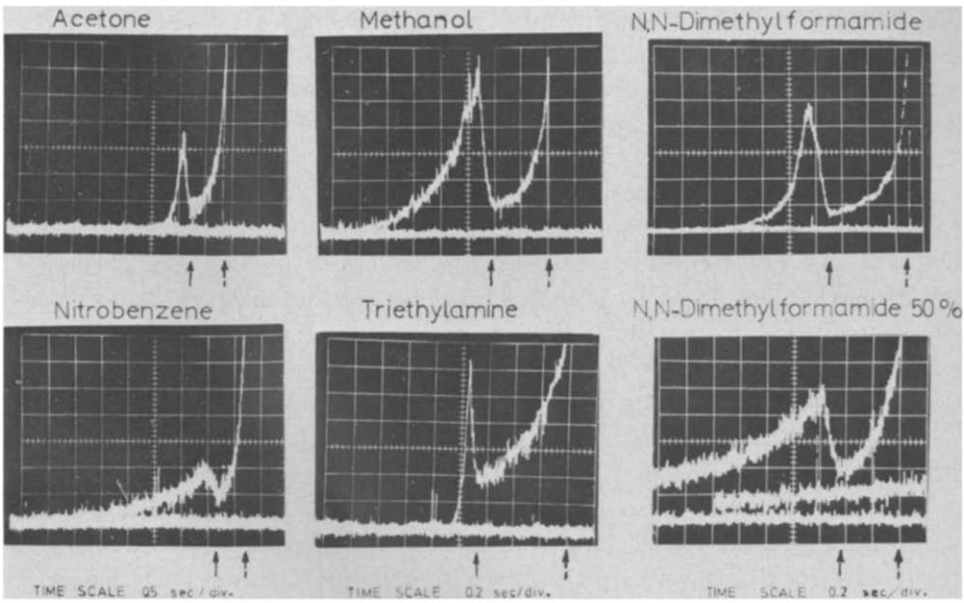
nEinsteins$\cdot cm^{-2} \cdot s^{-1}$) for 1/8 s. After a dark period of approx. 0.5 s, during which the delayed light was observed, 1 ml of organic solvent was rapidly injected, while the suspension was vigorously stirred. The injection caused an emission of a light pulse which was detected by a photomultiplier and recorded on the oscilloscope. Other experimental details were as described before[7,8].

Fig. 1 demonstrates oscilloscopic photographs of the emission events for some typical experiments. A large variety of solvents, some of which mix poorly or negligibly with water, showed this effect to a varying degree. The highest emissions were obtained with solvents like dimethylformamide, dimethylsulfoxide and methanol. Note the effect of injecting different concentrations; while the peak luminescence decreased with decreasing concentration the total luminescence did not. This is shown for *N*,*N*-dimethylformamide in Fig. 1 and was also observed with methanol.

It has to be proven that the emission events observed are indeed due to a genuine triggering process rather than to a pH change, a possible temperature increase or a dilution caused by the injection of the solvents. pH readings were taken before and after injection. In most experiments only small changes in the pH-meter reading were observed (maximum 0.5 pH unit). The meaning of the pH reading is not well defined for water–organic solvent mixtures[9]. However, the fact that independently of the solvents the pH reading was changed only slightly, indicated that the actual pH change was insignificant. To further prove this point we checked the pH reading in 50% methanol, where the pH can be calibrated by buffers of known p*K* values[9]. For this case the actual pH shift caused by the injection of pure methanol to the chloroplast suspension was negligible (0.1 pH unit). Control experiments were run in which the pH was changed by injecting appropriate Tris-succinate or HCl buffers in lieu of the organic solvent. For a pH change up to approx. 1.5 pH units no emission was observed. Another control experiment, for a possible dilution effect, was done by injecting pure water. This also resulted in no emission.

In several particular cases there was indeed a large pH shift, which was undoubtedly responsible for the appearance of emission, as shown by control experiments with NaOH and HCl. These were the cases with the typical bases like the aliphatic amines triethylamine and isopropylamine (final pH approx. 12) or pyridine (final pH approx. 10.8), and with acetaldehyde and dioxane (final pH approx. 4.5 and approx. 3.2, respectively), which were undoubtedly contaminated with acid.

The injection of a few solvents did cause some temperature increase. This was the case notably with dimethylsulfoxide ($\Delta T = 20^\circ C$), *N*,*N*-dimethylformamide ($\Delta T = 14^\circ C$) and methanol ($\Delta T = 10^\circ C$). Some solvents caused temperature increase below $\Delta T = 8^\circ C$, 14 solvents did not cause any measurable ($\Delta T < 1^\circ C$) temperature increase. In all cases we made a parallel control experiment to allow for the *T*-jump luminescence by injecting hot water so that the final temperature was the same. In many cases the control luminescence was negligible compared to the solvent induced luminescence. In most cases where a *T*-jump is observed the *T*-jump luminescence can account for about 1/10–1/6 of the solvent-induced luminescence. In the less favorable case



Figs 1 and 2. Oscilloscopic photographs of luminescence signals induced by injection of organic solvents. The arrows point to the opening of the photomultiplier shutter and the start of the delayed light recording (dashed arrow, right) and to the injection of the organic solvent (solid arrow, left). Time scale, as indicated, from right to left. Sensitivity is the same for all the photographs except for pure *N*,*N*-dimethylformamide where it is smaller by a factor of 2.5.

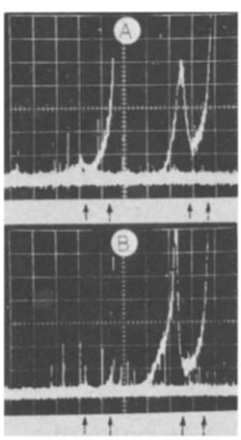


Fig. 2. A. Effect of $3 \cdot 10^{-3}$M hydroxylamine with $5 \cdot 10^{-6}$ M DCMU (left) compared to a control experiment (right) without the inhibitors. Addition of each inhibitor alone (picture not shown) gave a close result to the control experiment. B. Effect of 10^{-6} M CCCP (left) compared to a control experiment (right). The inhibitors were added before the preillumination. Time scale, 0.5 s/division. Both solvent-induced and -delayed light are inhibited.

(dimethylsulfoxide) the *T*-jump luminescence was only about 1/4 of the solvent-induced luminescence. The contribution of the *T*-jump luminescence is shown in Table I. Furthermore, in each case the *T*-jump luminescence decay kinetics was much more slower than the solvent-induced luminescence. Also, note in Table that while the decay time of the *T*-jump luminescence increased considerably as the ΔT decreased, which is according to expectation, the decay time of the solvent-induced luminescence stays approximately constant.

TABLE 1

COMPARISON BETWEEN THE SOLVENT-INDUCED LUMINESCENCE AND THE *T*-JUMP CONTROL LUMINESCENCE

Solvent:	*Dimethyl-sulfoxide*	*N,N-Dimethyl-formamide*	*Methanol*	*Acetone*	*Ethanol*	*All the rest*
ΔT, temperature increase (°C)	20	14	8	7	6	< 4
Ratio *T*-jump luminescence/solvent-induced luminescence (peak/peak)	1/4	1/6.5	1/7	1/7.5	1/6	< 1/10
Half time of decay *T*-jump luminescence (s)	0.65	0.8	1	1.2	1.5	
Half time of decay solvent luminescence (s)	0.15	0.15	0.1	0.2	0.15	

We may conclude that the solvent-induced luminescence is a genuine effect, not caused by secondary effects resulting from the addition of the solvent.

The methanol-induced luminescence was investigated with respect to the effects of photosynthetic inhibitors and uncouplers to show the relevancy to the photosynthetic process. It was inhibited by a mixture of $3 \cdot 10^{-3}$ M hydroxylamine and $5 \cdot 10^{-6}$ M 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) (Fig. 2A) but not by anyone of these reagents alone (*cf.* also refs 4, 10–12). The methanol-induced luminescence was totally inhibited by 10^{-6} M carbonyl cyanide *p*-trifluoromethoxy phenylhydrazone (FCCP) or carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) (Fig. 2B) but not inhibited by other uncouplers like 10^{-4} M gramicidin D, 10^{-2} M NH_4Cl, 10^{-4} M atebrine or $3 \cdot 10^{-5}$ M valinomycin.

In the other types of luminescence the triggering is thought to give rise to trans membrane gradients. For example, a model was suggested for the salt-induced luminescence, in which the difference in salt concentrations together with the different permeabilities of anions and cations rapidly induce an electric potential difference across the thylakoid membrane[3,13,14]. The establishment of an electric field inside the membrane renders a recombination of trapped charges[5], initially separated by the photosynthetic photoact.

In the present case it is evident that it is not a trans membrane effect but a direct

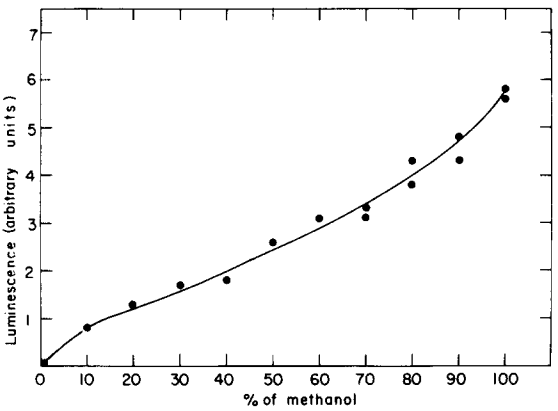


Fig. 3. Luminescence peak obtained by injection of methanol at different concentrations *vs* concentration.

interaction of the solvents with the membrane, since the luminescence is also induced by solvents not miscible with water. Presumably their contact with the chloroplasts is rendered by the vigorous injection and stirring. It is likely that there is a dissolution of the solvent into the membrane phase, which brings about a change in the membrane properties. Such a change is probably not total destruction; Fig. 3 shows that injection of methanol–water in decreasing concentrations caused approximately proportional decrease in the luminescence peak. The photochemical activities of chloroplasts (electron transfer and photophosphorylation) were not seriously affected by low (10%) methanol concentration[15].

In view of the electric field theory of the pH-shift and salt luminescences we tried to find a correlation between the polarity of the solvents, expressed by their dielectric constant ϵ, and the luminescence pulse peak. Such a correlation is shown for 23 solvents in Fig. 4. Most solvents agree roughly with a linear positive dependence between the luminescence and ϵ. Since the process is undoubtedly complex, other factors influence to scatter the results. The few marked exceptions to the above correlation can be explained by considering the following factors: (a) The solvent rate of solubility in the membrane. This factor is approached by inspecting permeability constants for some biological membranes[16]. While many solvents have permeabilities in the range of a few thousands$\cdot 10^{-7}$ cm/s, formamide, glycol and 1,3-propandiol have permeabilities two orders of magnitude less[16]. These solvents also have negligible tendency to dissolve in the lipid part of the membrane[16] and thus cannot interact with the membrane and give rise to luminescence. (b) If the solvent is immiscible with water, its penetration of the membrane is done by arbitrary mechanical contact; such is the case probably with nitromethane and nitrobenzene. (c) The quantum yield of the ^{1}chlorophyll a^* emission is influenced by the state of the membrane in an uncontrolled way.

We thus propose, as a first step to approach the problem, the following model: solvent molecules enter the lipid part of the membrane thereby increasing the dielectric

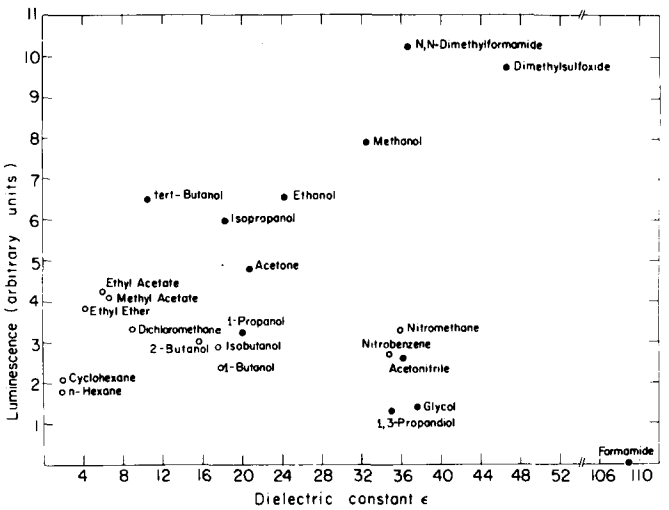


Fig. 4. Correlation between the luminescence peak and the dielectric constant of the solvents. All solvents were of analytical grade. Solvents fully miscible with water are represented by •.

constant of the membrane ϵ from its initial low value[17]. Trapped electrons and holes need less energy for detrapping as the dielectric constant increases. The detrapping energy is then easily supplied by the thermal energy of the environment, electrons and holes are released and recombine to give the chlorophyll excited state[5].

The energy of electrostatic interaction of the trapped electron with the surrounding pigment molecules (charge dipole, charge multipole interaction) which holds it in the trap is inversely proportional to ϵ, and thus the rate of detrapping might be written as $k = k_0 \exp(-H/\epsilon)$, where H contains the vacuum ($\epsilon = 1$) electrostatic interaction divided by kT (k = Boltzmann constant, T = absolute temperature). The relation above predicts that k increases with ϵ, but not in a linear manner; there is a "lag" for small ϵ and a tendency to saturation at high ϵ. However, the kinetics of the whole process is undoubtedly complex and may be controlled by the penetration of the membrane and the rate of change of ϵ inside the membrane rather than by a constant new ϵ value. Also, there may be several traps of various values of H so that the detrapping rate will be given by a sum $k = \Sigma\, k_{0i} \exp(-H_i/\epsilon)$. The rate of recombination is probably given by the rate of diffusion of the mobile electrons (after detrapping) and holes in the pigment matrix, and is little influenced by the decrease of the electrostatic attraction caused by the increase in ϵ. The rate-limiting step in the luminescence process, is thus probably the rate of detrapping.

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