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QUENCHING OF CHLOROPHYLL FLUORESCENCE BY QUINONES IN ALGAE AND CHLOROPLASTS

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SUMMARY

1. A number of quinones and substituted quinones quenched strongly chlorophyll fluorescence in Swiss chard chloroplasts and in the intact algae, *Ulva lobata* and *Porphyra perforata*. Quenching occurred in the absence as well as in the presence of 3(3,4-dichlorophenyl)-1,1-dimethylurea.

2. Among the quinones found to have a high quenching activity were 2,3,5,6-tetramethylbenzoquinone, 2-methyl-1,4-naphthoquinone, 5-hydroxy-1,4-naphthoquinone, phenanthrenequinone, and 1,2-dihydroxyanthraquinone. Reduced quinones had no or little quenching activity.

3. The quinones tested quenched less strongly the initial fluorescence, observed immediately upon illumination, than the subsequent rise of fluorescence yield during illumination. For the most active compounds, the concentrations needed for 50% quenching of the initial and the subsequent increase of fluorescence were about 70 and 15 μM, respectively.

4. The kinetics of fluorescence quenching at different light intensities and concentrations of quencher and absence of stimulation of O_2 evolution indicate that the quenchers do not stimulate photosynthetic electron transport but interact directly with chlorophyll molecules of photosystem 2 by formation of traps for the excitation energy.

5. In agreement with the assumption that the site of action of the quinones is near system 2, a number of these compounds inhibited light-induced cytochrome reduction in Porphyra *in vivo*. However, for most compounds no quantitative relation was found between the extent of inhibition of cytochrome reduction or of the Hill reaction in chloroplasts and the activity in quenching chlorophyll fluorescence.

INTRODUCTION

Quinones have attracted much interest as possible intermediates in photosynthesis, especially since it became known that large amounts of these substances occur naturally in chloroplasts and photosynthetic microorganisms[1,2].

Abbreviations: DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea; DCIP, 2,6-dichlorophenolindophenol; DAD, 2,3,5,6-tetramethyl-*p*-phenylenediamine; diquat, 1,1'-ethylene-2,2'-dipyridylium dibromide; paraquat, 1,1'-dimethyl-4,4'-dipyridylium diiodide; simazin, 2-chloro-4,6-bis(ethylamino)-*s*-triazine.

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GAFFRON[3] found that the substituted naphthoquinones, menadione and phthiocol, inhibit photosynthesis, probably near the site of O_2 evolution[4]. These compounds also inhibit the Hill reaction in chloroplasts[5]. A large number of quinones themselves serve as oxidants in the Hill reaction[6–8]. Certain quinones are active as cofactors of photosynthetic phosphorylation[8]. Chloroplasts which have been inactivated photochemically by extraction with solvents can be reactivated upon addition of plastoquinones which are endogenous to the chloroplast, or by other substituted benzoquinones[2,8–10]. Light-induced oxidation–reduction reactions of endogenous and added plastoquinone by isolated chloroplasts have been observed[2,11–13] and measurements on intact algae[14] indicate that plastoquinone or a related quinone is an intermediate electron carrier between the two photochemical systems. It has also been proposed[5,15,16] that a quinone may serve as the primary photo-oxidant for system 2.

It has been known for a number of years that benzoquinone and benzoquinone derivatives quench fluorescence of chlorophyll in organic solution[17]. WESSELS[5] and, more recently, THOMAS *et al.*[18] and ARNON, TSUJIMOTO AND MCSWAIN[19] reported quenching of chloroplast fluorescence by naphthoquinones. GAINES, TWEET AND BELLAMY[20] observed quenching by vitamin K_1 of fluorescence of chlorophyll monolayers.

We investigated the quenching effect of quinones in chloroplasts and in intact algae and the relation between quenching and inhibition of the Hill reaction as well as the reduction of the *f*-type cytochrome in *Porphyra perforata*. A number of quinones were found to quench not only fluorescence but also to affect the kinetics of fluorescence, and to depress the steady state much more strongly than the initial yield of fluorescence upon illumination. The available evidence indicates that these effects are not caused by a chemical reaction, *e.g.* at the reaction center, but that the quenching is caused by formation of artificial traps by interaction of quinone and chlorophyll molecules.

MATERIALS AND METHODS

Ulva lobata and *Porphyra perforata* were collected locally and maintained in the laboratory as previously described[21].

Experiments with marine algae were done in natural sea water. Chloroplasts (once washed), obtained from Swiss chard (*Beta vulgaris* var. *cicla*) grown in the garden, were prepared and used in a medium containing 0.4 M sucrose, 0.05 M phosphate buffer (pH 6.8) and 0.01 M NaCl.

When necessary, quinones were further purified by resublimation.

Measurements of changes of absorbance were made with an apparatus described previously[21]. Fluorescence was measured with the same apparatus except that an EMI 9558B photomultiplier was substituted for RCA 6217. The apparatus was slightly modified such that the fluorescence excitation light was incident on the bottom of the cuvette, which faced the photomultiplier. In order to avoid absorption of the exciting light by some of the more colored quinone solutions, the thalli of Ulva and Porphyra were held firmly against the bottom of the cuvette by a plastic grid and in experiments with chloroplast suspensions the optical path length was kept short (0.5 mm). The absorbance of the chloroplast suspensions was less than 0.3 at 680 mμ. A combination of interference and colored glass filters with a peak trans-

mission at 684 mμ, half-band width 15 mμ, was placed in front of the photomultiplier to transmit the fluorescent and absorb the actinic light. Fluorescence was excited by a broad band of green or blue light. The blue light had a peak near 420 mμ and half-band of 40 mμ; the green peak was at 545 mμ, half-band 30 mμ.

Emission spectra of Porphyra fluorescence were measured with the apparatus designed[22] and recently modified by FRENCH. Fluorescence was excited by 540-mμ light obtained from a Bausch and Lomb 'high intensity' monochromator used with a 150-W xenon lamp.

The Hill reaction with 2,6-dichlorophenolindophenol (DCIP) was measured as the rate of decolorization at 620 mμ. In some experiments O_2 evolution was measured simultaneously with a teflon-covered platinum electrode.

Algal photosynthesis and the Hill reaction with $K_3Fe(CN)_6$ were measured with the electrode assembly for measuring rate of O_2 production described earlier[23].

All experiments were done at room temperature (approx. 22°).

RESULTS

The effect of 3(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and quinones on fluorescence of Swiss chard chloroplasts and intact algae

Fig. 1 shows the fluorescence kinetics of Swiss chard chloroplasts suspended in sucrose–phosphate buffer upon illumination with green or blue light. As has been noted by others[24,25] the fluorescence yield upon illumination is relatively low initially, and rises to a higher level, which is nearly steady after a few seconds. The fluorescence intensity immediately upon onset of illumination after a long dark period will be called the 'initial' fluorescence, the final level reached will be called the 'total' fluorescence, and the difference between the two will be called the 'variable' fluorescence. Approximately the same initial and variable fluorescence was observed in the presence of 10 μM DCMU, but the fluorescence increase was considerably faster. In some chloroplast preparations the initial level was higher with than without DCMU.

There is evidence[15,26,27] that chlorophyll fluorescence in algae and higher plants originates mainly from system 2 and that the variable fluorescence reflects the oxidation state of the primary photo-oxidant (Q) of system 2 which in the oxidized

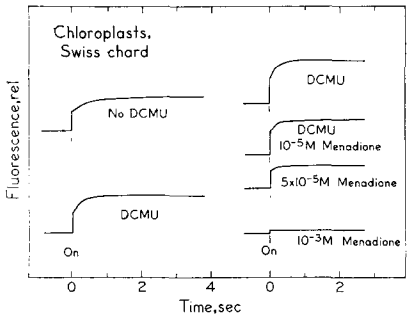


Fig. 1. The effect of DCMU (10 μM) and of DCMU with three different menadione concentrations on the kinetics of fluorescence in Swiss chard chloroplasts. The excitation light was green ($6 \cdot 10^3$ ergs·cm^{-2}·sec^{-1}) for the traces on the left and blue ($1.4 \cdot 10^3$ ergs·cm^{-2}·sec^{-1}) for the traces on the right. The vertical scales for the traces, made with different samples, on the left and right are different. A 24-sec dark period preceded each illumination.

state quenches chlorophyll fluorescence[15]. The slow rise of fluorescence in the absence, as compared to that in the presence of DCMU, may be caused by re-oxidation of reduced Q (QH) by a relatively large pool, possibly plastoquinone. Reduction of this pool may also give rise to the O_2 burst of chloroplasts without added Hill oxidants[23].

Fig. 1 also shows the effect of 2-methyl-1,4-naphthoquinone (vitamin K_3, menadione) in quenching fluorescence in the presence of DCMU. Menadione was more effective in quenching the variable than the initial fluorescence.

Fig. 2 shows the effect of concentration of menadione in quenching the initial, the variable, and the total fluorescence. The variable fluorescence yield was already reduced to half of the original level at 18 μM; the initial fluorescence required 0.1 mM menadione for 50 % quenching. Fig. 3 shows the quenching by 1,4-naphthoquinone.

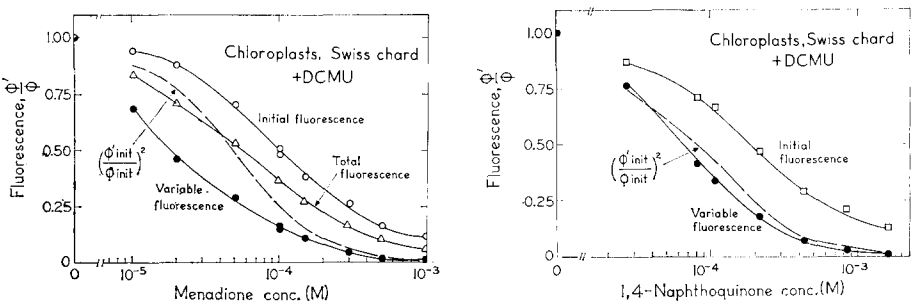


Fig. 2. Quenching of the initial, variable, and total fluorescence of Swiss chard chloroplasts as function of menadione concentration in 10 μM DCMU. ϕ is the relative fluorescence yield in the absence and ϕ' in the presence of quencher. The broken line is explained in the DISCUSSION. The experiments were done with the same sample and under the same conditions as for traces shown on the right of Fig. 1.

Fig. 3. Quenching of the initial and variable fluorescence of Swiss chard chloroplasts in 10 μM DCMU by 1,4-naphthoquinone. The experimental conditions were the same as for traces on the right of Fig. 1.

Not all quinones were equally effective in quenching chloroplast fluorescence. Juglone and phenanthrenequinone were very effective but lawsone and benzoquinone had little quenching effect even at 1 mM. Table I gives the quenching activity of a number of compounds. Other compounds not mentioned in the table which were inactive as quenchers in the presence of DCMU included DCIP, 2,3,5,6-tetramethyl-*p*-phenylenediamine (DAD), 1,1'-ethylene-2,2'-dipyridylium dibromide (diquat) and 1,1'-dimethyl-4,4'-dipyridilium diiodide (paraquat).

Quenchers of chloroplast fluorescence were also found to be active in quenching the fluorescence of the green and red algae *U. lobata* and *P. perforata*. Fig. 4 shows the effect of 1,4-naphthoquinone on the fluorescence kinetics of Porphyra. As with chloroplasts, the variable fluorescence was much more strongly quenched than the initial fluorescence, by 1,4-naphthoquinone as well as by other quenchers. Fig. 5 shows the yield of fluorescence as a function of concentration of 1,4-naphthoquinone and lawsone. Table II summarizes the quenching effect of a number of compounds. Comparison with Table I shows that for most substances a higher concentration was necessary to bring about the same quenching as in chloroplasts. Lawsone and phthiocol, which showed little activity with chloroplasts, showed little or no activity with algae also.

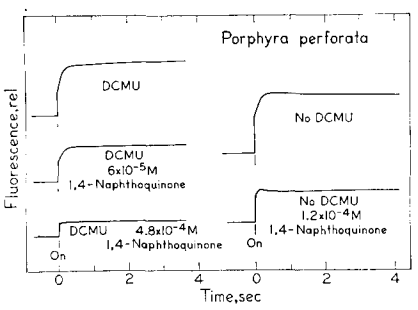
The experiments described above on the quenching of chlorophyll fluorescence were performed in the presence of air. Menadione was equally effective in quenching chloroplast fluorescence when the gas phase was helium. Menadione and 1,4-naphthoquinone showed the same activity in quenching the fluorescence of Porphyra and chloroplasts in the absence as in the presence of DCMU. The fluorescence quenching by menadione in Ulva was approximately the same in the presence of 40 μM 2-chloro-4,6-bis(ethylamino)-*s*-triazine (simazin) as in the presence of DCMU. The quenching of chlorophyll fluorescence was much weaker in ethanol solution than *in vivo*. A concen-

TABLE I

QUENCHING OF INITIAL AND VARIABLE FLUORESCENCE IN SWISS CHARD CHLOROPLASTS

Fluorescence was excited by green light (2 to $6 \cdot 10^3$ ergs·cm^{-2}·sec^{-1}) after a preceding 24-sec dark period. The figures for menadione represent experiments with two different preparations; for the first one blue actinic light ($1.4 \cdot 10^3$ ergs·cm^{-2}·sec^{-1}) was used. The DCMU concentration was 10 μM.

Compound	*Concentration (μM) for 50% quenching of*	
	Initial fluorescence	*Variable fluorescence*
p-Benzoquinone	1200	1500
2,3-Dimethyl-*p*-benzoquinone	800	120
2,3,5,6-Tetramethylbenzoquinone	450	83
1,4-Naphthoquinone	200	63
2-Methyl-1,4-naphthoquinone (menadione)	100; 160	18; 28
2-Hydroxy-1,4-naphthoquinone (lawsone)	>2000	>2000
2-Hydroxy-3-methyl-1,4-naphthoquinone (phthiocol)	>1000	500
5-Hydroxy-1,4-naphthoquinone (juglone)	70	13
1,2-Naphthoquinone	580	340
1,2-Naphthoquinone-4-sulfonic acid	>2000	>2000
Phenanthrenequinone	68	16
1,2-Dihydroxyanthraquinone (alizarin)	66	21
m-Dinitrobenzene	930	130



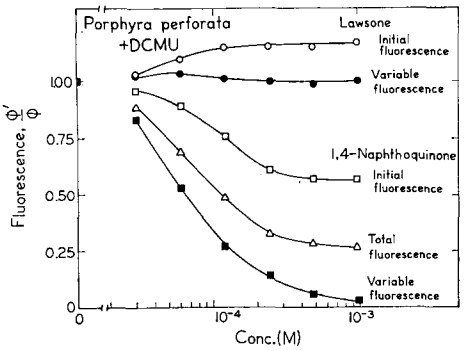


Fig. 4. Fluorescence kinetics of *P. perforata* in the presence of DCMU (50 μM) and 1,4-naphthoquinone. Fluorescence was excited by green light ($3.9 \cdot 10^2$ ergs·cm^{-2}·sec^{-1}, traces on left, and $9.1 \cdot 10^3$ ergs·cm^{-2}·sec^{-1}, traces on right). The preceding dark intervals were 18 and 30 sec, respectively.

Fig. 5. The effect of concentration of lawsone and of 1,4-naphthoquinone on the initial, variable, and the total fluorescence in *P. perforata* in 50 μM DCMU. The conditions were the same as for the left side of Fig. 4.

tration of approx. 16 mM menadione caused 50 % quenching of chlorophyll *a* fluorescence in 95 % ethanol. Thus it appears that in organic solution menadione is even a less effective quencher than benzoquinone[17].

Menadione and 2,3-dimethylbenzoquinone when reduced by potassium borohydride and sodium ascorbate respectively did not quench fluorescence. This suggests that the quinones act only in their oxidized state.

There are several indications that the strong quenching of the variable fluorescence in the presence of DCMU by quinones is not caused by reoxidation of QH. The extent of quenching was the same for different intensities of exciting light and the kinetics of the variable fluorescence were the same for different concentrations of menadione, contrary to what would be expected if the fluorescence level were the result of a photochemical and an opposing chemical reaction. Moreover, for Ulva and chloroplasts the total fluorescence yield at high concentration of quencher was considerably lower than the lowest level observed without DCMU with and without Hill oxidants. We estimated the rate of re-oxidation of QH in the dark by measuring the fluorescence level immediately upon illumination after increasing dark intervals. Fig. 6 shows that this level was approximately half way down to the lowest value

TABLE II

QUENCHING OF INITIAL AND VARIABLE FLUORESCENCE IN ALGAE

Fluorescence was excited by green light ($4 \cdot 10^2$ ergs·cm^{-2}·sec^{-1}). The DCMU concentration was $5 \cdot 10^{-5}$ M.

Compound	*Organism*	*Concentration (μM) for 50 % quenching of*	
		Initial fluorescence	*Variable fluorescence*
1,4-Naphthoquinone	Porphyra	>1000	65
Menadione	Porphyra	>1000	350
m-Dinitrobenzene	Porphyra	>2000	1300
Menadione	Ulva	180	50

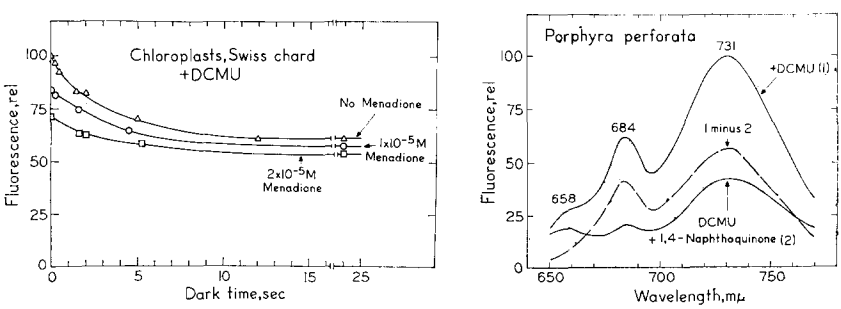


Fig. 6. Fluorescence produced immediately upon illumination of Swiss chard chloroplasts in 10 μM DCMU as a function of dark interval between exposures to blue actinic light ($6.6 \cdot 10^3$ ergs·cm^{-2}·sec^{-1}). The same chloroplast preparation was used as for Fig. 2.

Fig. 7. Fluorescence emission spectra plotted as relative number of quanta per frequency interval of *P. perforata* in DCMU (50 μM) and DCMU with 1,4-naphthoquinone (90 μM), excited by light of 540 mμ. The broken line gives the difference between the two spectra.

after about 2 sec dark time and that menadione did not accelerate the rate of this decrease, which indicates that a reaction of QH with quinone did not occur.

Emission spectra

Fluorescence emission spectra of *P. perforata* in green light, which excites mainly phycoerythrin, are shown in Fig. 7. The spectra are composed of at least 3 emission bands, at about 658, 684 and 730 mμ. The bands at 658 and 684 mμ belong to phycocyanin and chlorophyll *a*, respectively[22,28]. The strong emission band at 730 mμ was first observed by DUYSENS[28] in *Porphyra lacineata*.

Comparison of the two spectra with and without quinone shows that 1,4-naphthoquinone quenched the emission band at 684 mμ and, somewhat less strongly, that at 730 mμ, but had little or no effect on phycocyanin fluorescence. This indicates that there is no interaction with the biliprotein chromophore and that the transfer of energy between the phycobilins and between phycocyanin and chlorophyll is not affected. In the absence of quinone, DCMU enhanced approx. 3-fold the emission band at 684 mμ and about 2.5-fold that at 730 mμ, but not the phycocyanin band at 658 mμ.

If it is assumed, according to current theories, that DCMU enhances the fluorescence yield of system 2 only, the relative heights of the bands in the emission spectra with and without DCMU indicate that energy transfer from a chlorophyll *a* type fluorescing at about 684 mμ to the pigment fluorescing at 730 mμ occurs not only in system 1 (refs. 28 and 29), but also in system 2. The somewhat stronger quenching by naphthoquinone of the band at 684 mμ is then in agreement with the assumption that this compound quenches system 2 fluorescence, possibly by shortening the lifetime of the excited state of chlorophyll *a* fluorescing at 684 mμ, and thus decreasing both fluorescence at 684 mμ and the efficiency of energy transfer to the 730-mμ pigment.

O_2 evolution and cytochrome reduction

As discussed in the previous section, fluorescence kinetics in the presence of DCMU make it unlikely that the quenching of fluorescence is caused by a reaction of QH with menadione or the other quinones. Further evidence that such a reaction does not take place was obtained by experiments on O_2 evolution. Experiments to determine if quenchers like menadione and juglone were able to reverse the inhibition by DCMU of light-induced O_2 evolution gave negative results. Menadione, 2,3-dimethylbenzoquinone, and juglone (0.01 to 1 mM) did not regenerate O_2 evolution of Ulva or Porphyra in 0.01 to 0.1 mM DCMU. The same applied to O_2 evolution by Swiss chard chloroplasts with and without $K_3Fe(CN)_6$ and with 10 μM DCMU. All experiments were performed in N_2 because experiments in air indicated that quinones enhanced both O_2 uptake in the dark and the light-induced changes of O_2 uptake, which made an interpretation of the results more difficult.

It has been reported that menadione and phthiocol inhibit O_2 evolution[3,5]. Photoreduction is not inhibited[4], which indicates that the site of action is near system 2. This was also apparent from the effect of quinones on cytochrome reactions in Porphyra. Fig. 8 shows kinetics of absorbance changes at 420 mμ, reflecting the oxidation and reduction of an *f*-type cytochrome. The difference spectrum for the oxidation showed a maximum at 402 mμ and minima at 419 and 553 mμ, similar to

those observed in other red algae[30–32]. Traces e and f (see Fig. 8) indicate that 1,4-naphthoquinone, like DCMU[31], inhibits the reduction of the cytochrome by system 2. Similar results were obtained with other quinones.

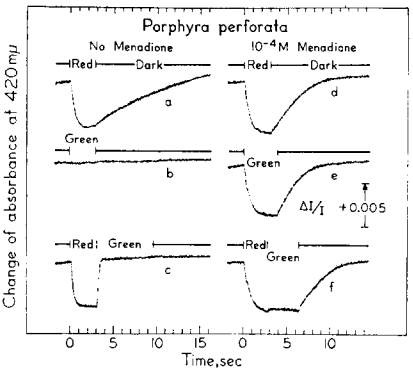


Fig. 8. The effect of menadione on the cytochrome absorbance changes in *P. perforata*. Red actinic light, to excite mainly system 1, had a half-band between 670 and 780 mμ ($3.8 \cdot 10^3$ ergs·cm^{-2}·sec^{-1}), and green actinic light (system 2) from 550 to 570 mμ ($3.9 \cdot 10^3$ ergs·cm^{-2}·sec^{-1}). A downward deflection corresponds to oxidation of the cytochrome.

A number of quinones were also tested for their inhibitory effect on DCIP reduction by Swiss chard chloroplasts. In general strong quenchers were more powerful inhibitors of cytochrome and DCIP reduction than weak quenchers. However, a quantitative relation was not found. Menadione gave 50 % inhibition of DCIP reduction at 0.2 mM, about the same concentration as needed for 50 % quenching of the initial fluorescence, but with 1,4-naphthoquinone, lawsone, and phthiocol the effect on cytochrome and DCIP reduction was stronger than that on fluorescence.

DISCUSSION

The experiments described above leave little doubt that the quenching of chlorophyll fluorescence by substances such as menadione and juglone is due to an interaction of quencher and chlorophyll molecules rather than to an indirect effect caused by stimulation of the rate of photochemical reactions as suggested by ARNON, TSUJIMOTO AND MCSWAIN[19]. The latter mechanism is also improbable in view of the E'_0 of the various quenchers, which varied between −0.40 V (as estimated for alizarin from the E_0 relative to that of anthraquinone and other quinones[33,34]) and +0.18 V (2,3-dimethylbenzoquinone). Quenching by stimulation of photochemistry probably occurs in chloroplasts in the presence of Hill oxidants such as ferricyanide and DCIP (see refs. 29 and 35) but only in the absence of DCMU and other system-2 inhibitors.

The lowering of the yield of initial fluorescence in algae and chloroplasts, when presumably the natural traps are open, is probably due to quenching of chlorophyll molecules belonging to system 2 which thus act as artificial traps for the excitation energy (see refs. 5 and 36). The much stronger quenching of the variable than of the initial fluorescence does not necessarily indicate that the quenchers act in two different ways and that at low concentration they act specifically at the reaction center by quenching the excited state of the chlorophyll associated with QH. In Porphyra part

of the effect is due to the fact that phycocyanin fluorescence, which is not quenched, contributes only to the initial fluorescence. Further, since the fluorescence lifetime of chlorophyll is longer when Q is in the reduced state than when it is oxidized[37] a stronger quenching can be expected for the variable than for the initial chlorophyll fluorescence, even if the quencher acts only on the bulk of the pigment. An exact calculation of the effect is not possible as long as a quantitative understanding of the process determining the fluorescence yield under various conditions is lacking. A rough estimate of the effect may be made on the basis of the equation given by TEALE (ref. 36, Eqn. 4):

$$\frac{\phi_0}{\phi} = \frac{1 + K\bar{n}T}{1 - T} \tag{1}$$

where T is the fraction of chlorophyll molecules which act as traps, K is the relative probability of transfer from a fluorescent molecule to the trap compared to that between fluorescent molecules, $\bar{n}$ is the average number of transfers between different molecules in the absence of traps, ϕ is the yield of fluorescence and ϕ_0 is the 'intrinsic' yield of fluorescence when there are no traps available. Assuming a homogeneous system with only chlorophyll *a* molecules, neglecting system-1 fluorescence and assuming that $K = 1$, $\phi_0/\phi \gg 1$, and $T \ll 1$, we obtain:

$$\frac{\phi}{\phi'} = \frac{T + T'}{T} \tag{2}$$

where ϕ' and T' are the yield and the number of additional traps divided by the total number of chlorophyll molecules in the presence of quencher. According to this equation when the initial fluorescence is 50 % quenched the number of artificial traps equals that of the active reaction centers. Thus, if the efficiency of transfer to the reaction centers approaches 100 % in the absence of quencher, the amount of excitation energy arriving at the reaction center would be about 50 % at this concentration of quencher. In Swiss chard chloroplasts the variable fluorescence in the absence of quencher was approximately equal to the initial fluorescence. This suggests that about an equal average number of random transfers are performed by the excitation energy before and after a visit to the closed trap of a reaction center if it is assumed that the variable fluorescence is produced after such a visit. When only, 50 % of the excitation energy reaches the reaction center in the presence of quencher the variable fluorescence would then be reduced to $0.5 \times 0.5 = 0.25$ of its original value, since the same amount of quenching would occur before and after a visit to the reaction center.

In general:

$$\frac{\phi'_{\mathrm{var}}}{\phi_{\mathrm{var}}} = \left(\frac{\phi'_{\mathrm{init}}}{\phi_{\mathrm{init}}}\right)^2 \tag{3}$$

where ϕ'_{var} and ϕ'_{init} and ϕ_{var} and ϕ_{init} are the variable and initial fluorescence yield in the presence and in the absence of quencher, respectively. As Fig. 3 shows, the equation describes the relation between variable and initial fluorescence reasonably well for 1,4-naphthoquinone but for most other quenchers the quenching of the variable fluorescence was stronger than predicted, especially at low concentration (see Fig. 2). It is not clear if this discrepancy is due to the approximations and un-

certainties involved or to a special affinity of the quencher to the chlorophyll belonging to, or in close proximity to, the reaction center*.

It is possible that the actual mechanism[41] of the quenching is the same in chloroplasts and algae as in chlorophyll solution. The list of quenchers given by LIVINGSTON AND KE[17] includes benzoquinone and tetramethylbenzoquinone and *m*-dinitrobenzene. Menadione also quenches the fluorescence of chlorophyll in solution. The stronger quenching by many of these substances *in vivo* may be caused by a concentration in the lipoid part of the chloroplast lamellae, and by the fact that quenching of the same fraction of chlorophyll molecules, by the formation of traps, could produce a stronger quenching in the chloroplast than in a dilute chlorophyll solution. The low activity of phthiocol and 1,2-naphthoquinone-4-sulfonic acid may be due to salt formation in the water phase.

There is no direct spectrophotometric evidence[14] for the hypothesis[5,15,16] that a quinone would be the primary photo-oxidant for system 2. However, the strong quenching of chlorophyll fluorescence *in vivo* indicates that association of a quinone with a chlorophyll molecule can produce an efficient trap for the excitation energy. It is possible that a reaction center consists of a similar trap, supplemented with the necessary enzymatic and structural arrangement for the formation of stable photochemical products.

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* The fact that the fluorescence only doubles during illumination contradicts, as discussed by others for analogous observations, the assumption that the efficiency of energy transfer to the reaction centers approaches 100%, unless an additional hypothesis is made (see also refs. 38 and 39). Such an hypothesis might be that a 'closed' reaction center can still trap some energy, or that the chlorophyll *a* of system 2 does not constitute a homogeneous system and that energy transfer is partly unidirectional within this system, as has been proposed for system 1 (see refs. 29 and 40). The latter hypothesis would invalidate equation (3) to some extent.

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