Volume 258, number 2, 339–342 FEB 07907 December 1989

Correlation between dissipative fluorescence quenching at photosystem II and 50 μs recombination luminescence

Ulrich Schreiber and Christian Neubauer

Lehrstuhl Botanik I, Universitat Wurzburg, Mittlerer Dallenbergweg 64, D-8700 Wurzburg, FRG

Received 2 October 1989

Charge recombination luminescence in the 50 μs time range was measured in isolated chloroplasts under identical conditions as modulated chlorophyll fluorescence In this way the relationship between fluorescence quenching, as analysed by the saturation pulse method, and charge recombination was investigated Various treatments known to slow down the donation rate of water splitting, were found to induce correlated increases in 50 μs recombination luminescence and fluorescence quenching, with both photochemical and non-photochemical quenching components being enhanced It is concluded that charge recombination at photosystem II provides a mechanism for rapid dissipation of excitation energy

Chlorophyll fluorescence, Luminescence, Photosystem II, Fluorescence quenching, Water splitting

1. INTRODUCTION

The fluorescence yield of chlorophyll in vivo is determined by photochemical and non-photochemical quenching mechanisms (see review in [1]). Recently, the so-called 'saturation pulse method' was developed, which allows one to determine the coefficients of photochemical and non-photochemical quenching, q_P and q_N, respectively [2]. The application of this method has yielded results suggesting that part of q_N reflects dissipation of excess energy in PS II [3], while another part is related to the presence of zeaxanthin in the antenna system [4]. So far, the actual mechanisms of both types of non-photochemical quenching are not known.

We have previously shown that maximal fluorescence yield, F_m, is preferentially suppressed when effective electron donation from water splitting is disturbed [5]. Several mechanisms have been considered to cause this 'donor-side related quenching': formation of $P680^+$ and $Pheo^-$, or a population of PS II centers displaying exceptionally fast reoxidation of the primary acceptor via a dissipative cycle around PS II, either by direct charge recombination or involving some additional component, like cyt b-559.

Delayed chlorophyll fluorescence, more briefly called 'luminescence', is an indicator for charge recombination at PS II centers (for a recent review see [6]). Only rapid recombination occurring within about 100 μs or faster should have the potential of preventing full reduction of Q_A in a saturating pulse, whereas slower recombination may well increase q_P without effecting q_N.

Recently, we have introduced a luminometer based on pulsed light-emitting diodes [7]. Here, we report on the use of a modified version of this luminometer to obtain for the first time continuous recordings of 50 μs recombination luminescence in parallel with modulated prompt fluorescence. It will be shown that a strong stimulation of 50-μs luminescence accompanies the development of donor-side related quenching.

Correspondence address U Schreiber, Lehrstuhl Botanik I, Universitat Wurzburg, Mittlerer Dallenbergweg 64, D-8700 Wurzburg, FRG

Abbreviations PS I and PS II, photosystem I and II, Q_A, primary stable acceptor of photosystem II, NH_2OH, hydroxylamine, Tris, tris(hydroxymethyl)-aminomethane, q_P, coefficient of photochemical quenching; q_N, coefficient of non-photochemical quenching

2. MATERIALS AND METHODS

Intact chloroplasts were isolated in dim light Leaves from greenhouse-grown spinach were harvested in the morning following a 10-h dark period If not stated otherwise, they were suspended at a concentration of 80 μg Chl ml^{-1} in a reaction medium containing the following additions 0 3 M sorbitol, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM EDTA, 30 mM KCl, 0.5 mM KH_2PO_4, 50 mM Hepes/KOH, pH 7 6 For the experiments in which the pH was rapidly changed, essentially the same medium was used, except that only 2 5 mM Hepes/KOH was present and the KCl concentration was raised to 40 mM. The pH was lowered by appropriate additions of a 0 5 M Mes/2 M glycine buffer adjusted to pH 3 For Tris-treatment intact chloroplasts were osmotically ruptured in water, resuspended at 400 μg Chl ml^{-1} in the reaction medium with varying amounts of Tris added and adjusted to pH 8. After 35 min, Tris-treatment chloroplasts were diluted by a factor of 5 in the normal reaction medium and induction curves of fluorescence and luminescence measured immediately.

Fluorescence and luminescence were measured simultaneously with a fiberoptics system combining a PAM fluorometer (H Walz, Effeltrich, FRG) with a laboratory-built luminometer [7]. Details on the technical progress rendering the instrument capable of monitoring

Published by Elsevier Science Publishers B V. (Biomedical Division)
00145793/89/$3 50

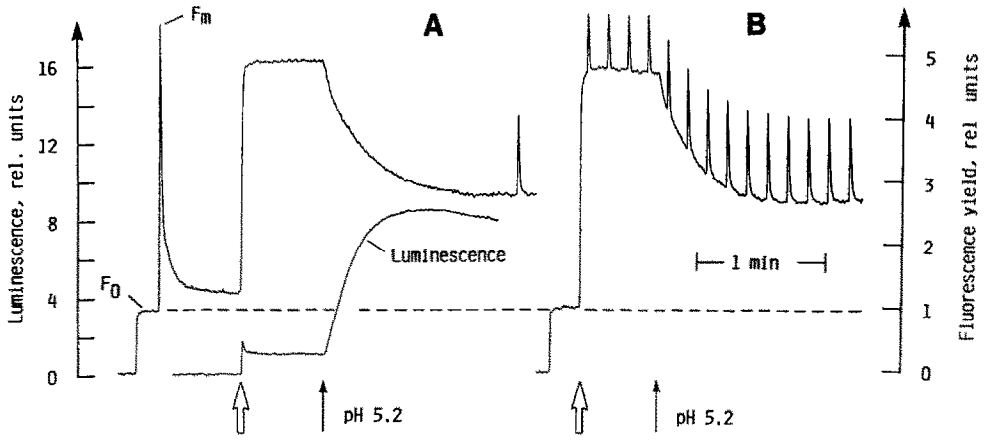

Fig 1 Parallel stimulation of 50-μs luminescence and fluorescence quenching in spinach chloroplasts by pH-lowering (A) Simultaneous measurement of modulated fluorescence yield and luminescence After onset of the weak fluorescence measuring beam the quasi-dark level, F_o, is monitored; a pulse of saturating light induces the maximal yield, F_m At the open arrow actinic illumination is started, allowing measurement of 50-μs luminescence in intermittent dark-periods (see section 2) At the end of the recording another saturation pulse is applied to assess photochemical and non-photochemical quenching (B) Experiment identical to the one in (A) except that saturation pulses were applied every 10 s in order to follow the kinetics of development of acid-induced non-photochemical quenching For technical reasons luminescence could not be recorded when saturation pulses were applied

50-μs luminescence will be published elsewhere (Schreiber and Schliwa, manuscript in preparation) Luminescence was integrated between 25 and 75 μs following 125 μs light pulses which were applied repetitively every 250 μs The overall integrated light intensity was 85 μE m^{-2} s^{-1}, with the LED emission peaking at 655 nm

3. RESULTS

Fig.1A shows a simultaneous recording of modulated fluorescence and 50-μs luminescence. First, the dark-level fluorescence, F_o, is monitored with weak modulated measuring light. A pulse of saturating light is applied to determine maximal fluorescence yield, F_m. The pulsed light used for producing luminescence also serves as actinic light for inducing a rise of variable fluorescence. Due to the presence of the protonophore, nigericin, light-driven acidification of the thylakoid lumen and consequent 'membrane energization' are prevented. Therefore, no 'energy-dependent' fluorescence quenching is observed. When the pH of the weakly buffered medium is rapidly lowered from pH 7.5 to pH 5.2 by addition of Mes/glycine (pH 3), a decay of fluorescence yield is induced, which for the first 45 s following acidification is kinetically closely correlated with a 7-fold stimulation of 50-μs luminescence. In a separate experiment, but under identical conditions, fluorescence was also recorded with repetitive application of saturating light pulses (fig.1B). It is apparent that the acid-induced quenching is dominated by an increase of q_N (from 0 to 0.36). At the same time there is an increase of q_P from 0.12 to 0.28.

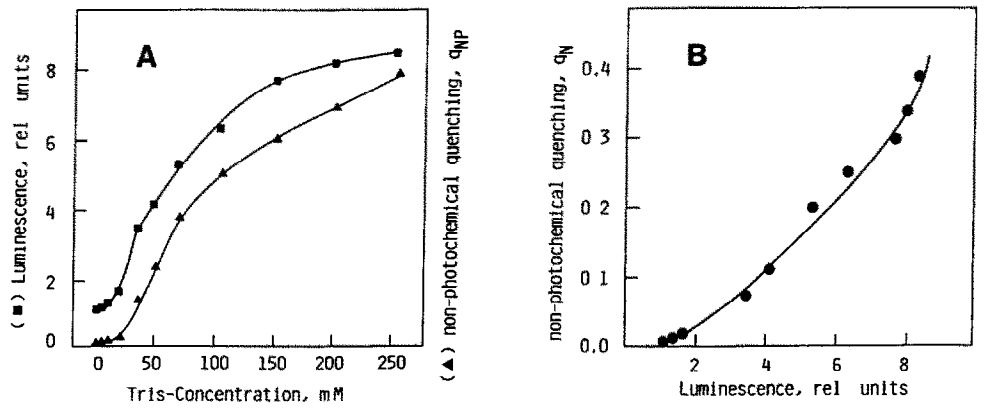

Fig 2 Relationship between the stimulation of 50-μs luminescence and non-photochemical quenching induced by Tris-treatment (A) Tris-concentration dependency of luminescence intensity and non-photochemical quenching, q_N, measured 1 min following onset of actinic illumination For Tris-treatment conditions, see section 2 (B) Plot of q_N versus luminescence intensity, using the data displayed in panel A

Table 1

Changes of photochemical and non-photochemical quenching and of 50-μs luminescence induced by a variety of treatments which effect the PSII donor side

Treatment	q_P	q_N	50-μs luminescence, rel units
Control	0 16	0 0	1 00
5 min 42°C	0 10	0 25	2 70
5 min 46°C	0 09	0 40	3 40
5 min 48°C	0 12	0 60	3 10
1 mM NH_2OH	0 18	0 14	5 40
2 5 mM NH_2OH	0 29	0 40	10 90
5 mM NH_2OH	0 27	0 64	11 40
10 mM NH_2OH	0 35	0 72	11 00
100 mM Tris	0.25	0 26	6 10
200 mM Tris	0 31	0 34	7 90
300 mM Tris	0 42	0.56	7 20
pH 5 6 30 s	0 21	0 07	6 50
2 min	0 25	0 14	7 90
pH 5 2 30 s	0 26	0 23	7 20
2 min	0 29	0 28	8 20
pH 4 8 30 s	0 30	0 29	7 00
2 min	0 32	0 70	6 20
5×10^{-6} M TPB	0 20	0 22	0 73
10^{-5} M TPB	0 24	0 45	0 59
2×10^{-5} M TPB	0 29	0 64	0 36
10^{-5} M FCCP	0.29	0 10	0 50
2×10^{-5} M FCCP	0 38	0 15	0 45
4×10^{-5} M FCCP	0 49	0 26	0 41
5×10^{-7} M ANT-2p	0 27	0 10	0 68
10^{-7} M ANT-2p	0 35	0 21	0 55
3×10^{-7} M ANT-2p	0 70	0 71	0 27

A relatively mild and selective means for deactivation of the natural PSII donor reaction is Tris-treatment. As shown in fig.2A and B with increasing Tris-concentration there is a more than 8-fold stimulation of 50-μs luminescence paralleled by an increase of non-photochemical fluorescence quenching, q_N. The correlation between q_N and luminescence is curvilinear over a wide range of Tris-concentrations. Above 150 mM Tris the stimulation of luminescence becomes somewhat curbed while q_N continues to increase.

Other treatments and chemicals which have been previously shown to cause 'donor-side related' quenching of fluorescence yield [5] are heat-pretreatment, hydroxylamine and ADRY reagents. In table 1 the effects of some donor-side treatments on fluorescence yield and 50-μs luminescence are summarized. Heat-pretreatment and hydroxylamine act in a similar way to Tris-treatment and acidification, i.e. increases in fluorescence quenching (q_P and q_N) are correlated with stimulation of 50-μs luminescence. However, it may be noted that at the highest applied doses (e.g. 5 min, 48°C pretreatment or 2 min at pH 4.8) there is the tendency of a reversal of luminescence stimulation, while fluorescence quenching is further promoted. The ADRY reagents ANT-2p, TPB and FCCP all cause suppression of both fluorescence and luminescence yield.

4. DISCUSSION AND CONCLUSION

The presented data show that there is a type of 'donor-side related' fluorescence quenching which is induced in parallel with strong stimulation of recombination luminescence in the 50 μs time range. As only part of recombination produces singlet excited states, it is representative of photochemical dissipation of excitation energy at the level of PSII reaction centers. Rapid recombination offers a pathway of Q_A-reoxidation which is dinstinctly faster than the forward reaction leading to Q_B-reduction. However, normally it must compete with the even faster donor reaction of water splitting for the substrate $P680^+$ [8]. Treatments which cause deactivation of water splitting will favor recombination and, hence, can be expected to induce an increase in photochemical quenching, q_P. Indeed, it is a common feature of all treatments affecting the PSII donor side that q_P is distinctly increased (see table 1). Furthermore, if recombination is strongly enhanced in the 50 μs time range, as demonstrated by the above results, it may become difficult to fully reduce the acceptor Q_A by saturating light pulses, i.e. an apparent increase of non-photochemical quenching, q_N, would in fact partially be caused by a type of non-suppressible, dissipative photochemical quenching. In this way, the lowering of PSII donor side activity will be paralleled by increases of q_P as well as of q_N (as determined by the saturation pulse method).

Weis and Berry [3] have proposed that two populations of PSII exist, non-energized and energized, with the energized centers displaying low quantum efficiency and almost no variable fluorescence. Thylakoid energization is closely associated with membrane acidification [9]. We have shown above that membrane acidification at pH 5.2 causes significant increases in q_P and q_N, which are well correlated with a strong stimulation of 50 μs recombination. Therefore, it could be suggested that the inefficient, 'energized centers' in the Weis and Berry model are centers with a high rate of charge recombination. Further work should clarify whether the tacit assumption is justified that acidification of uncoupled thylakoid membranes is equivalent to the internal thylakoidal acidification in vivo, as far as non-photochemical quenching is concerned.

Besides direct charge recombination, leading to singlet or triplet excited states, non-radiative recombination of the separated charges via intrinsic or added reactants is possible. We have previously considered a dissipative cycle around PSII with the low potential form of cyt b-559 as electron carrier feeding into $P680^+$ and being re-reduced by the PSII acceptor side [5,10]. Such a mechanism may explain the induction of strong photochemical and non-photochemical quenching by

ADRY reagents (see table 1), while at the same time 50-μs luminescence is suppressed. Also with donor side treatments (acidification, Tris, heat, NH_2OH, etc.) which at low doses strongly stimulate 50-μs luminescence, at higher concentrations and longer incubation times a reversal of stimulation was noticed, which is correlated with further stimulation of fluorescence quenching (table 1). All of these treatments are known to cause lowering of the cyt b-559 redox potential [11, 12].

Demmig and co-workers [4] have characterized a type of non-photochemical quenching which is closely correlated with the content of zeaxanthin. It may be excluded that zeaxanthin is involved in the donor side related quenching dealt with in the present communication, as the chloroplasts were harvested from spinach leaves dark-adapted overnight, containing no zeaxanthin.

In conclusion, with the presented evidence for correlated stimulation of recombination luminescence at PSII and quenching of fluorescence (q_P and q_N), a possible mechanism of energy dissipation in PSII has been demonstrated. Further work must clarify what is the actual contribution of recombination-quenching to overall dissipative quenching in vivo. It may be predicted that this contribution is most significant when the quantum yield of PSII is lowered as a consequence of stress-induced damage of the water-splitting system.

Acknowledgements Ulrich Schliwa is thanked for skillful electronic engineering. Christof Klughammer, Jorg Kolbowski and Heinz Reising are thanked for helpful discussions Financial support by the Deutsche Forschungsgemeinschaft (SFB176 and 251) and by the German-Israeli Foundation is gratefully acknowledged

REFERENCES

[1] Briantais, J -M , Vernotte, C , Krause, G H and Weis, E (1986) in. Light Emission by Plants and Photosynthetic Bacteria (Govindjee, Amesz, J and Fork, D.C eds) pp 539-577, Academic Press, New York
[2] Schreiber, U , Bilger, W and Schliwa, U (1986) Photosynth Res 10, 51-62
[3] Weis, E and Berry, J (1987) Biochim. Biophys Acta 894, 198-208
[4] Demmig, B , Winter, K , Kruger, A and Czygan, F -C (1987) Plant Physiol 84, 218-224
[5] Schreiber, U. and Neubauer, C (1987) Z Naturforsch 42c, 1255-1264
[6] Jursinic, P A (1986) in· Light Emission by Plants and Bacteria (Govindjee, Amesz, J and Fork, D C eds) pp 291-328, Academic Press, Orlando, FL
[7] Schreiber, U and Schliwa, U (1987) Photosynth Res 11, 173-182
[8] Schlodder, E , Brettel, K , Schatz, G H and Wit, H T (1984) Biochim Biophys Acta 765, 178-185
[9] Briantais, J -M , Vernott, C , Picaud, M and Krause, G H (1979) Biochim. Biophys Acta 548, 128-138.
[10] Schreiber, U and Rienits, K G (1987) FEBS Lett 211, 99-104
[11] Horton, P and Cramer, W A (1975) FEBS Lett 56, 244-247
[12] Butler, W L (1978) FEBS Lett 95, 19-24