Effect of pH on the Slow Phase Components of Delayed Luminescence in Chloroplasts

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The pH dependence of two, flash-induced slowly decaying delayed luminescence components – resulting from charge recombination of Q_A^- and Q_B^- with the S_2 and S_3 states ($S_2Q_A^-$ + $S_3Q_A^-$ and $S_2Q_B^-$ + $S_3Q_B^-$ recombinations) – in untreated and herbicide treated chloroplasts was investigated within a pH range of 5.5 to 8.5.

The component associated with Q_A^- showed the same pH-dependence in untreated and in DCMU-treated chloroplasts: its half-decay time increased with increasing pH reflecting an appr. +5 meV/pH change in the free energy of activation. Contrary to this the component associated with Q_B^- exhibited an opposite behaviour: its half-decay time decreased with increasing pH and the free energy of activation changed with about −5 meV/pH.

It is concluded that both acceptor and donor side effects are involved in the pH-dependence of slow phase delayed luminescence components.

Introduction

Delayed luminescence originates from a reversal of the light induced PS II electron transfer reactions: from the recombination of negative and positive charges stored on the acceptor and donor side of PS II [1]. As more than one component of electron transport is involved in the recombination process more than one delayed luminescence component appears in the μsec-minutes time range [1]. Since the donors and acceptors which are participating in the charge recombination are in equilibrium with the H_2O/O_2 and plastoquinone pools the potentials of which are pH-dependent [2, 3] it can be expected that this pH-dependence is reflected in the kinetics of delayed luminescence components.

The pH-dependence of the μsec [4] and msec delayed luminescence components [5, 6] have already been investigated. Vermaas *et al.* [7] observed a delayed luminescence component in the seconds time scale which became slightly accelerated by a pH change from 7.6 to 6.0 indicating the effect of pH on Q_A^- *via* the $Q_A^-Q_B \rightleftharpoons Q_AQ_B^-$ equilibrium.

Components decaying in the seconds to minutes time scale were demonstrated to originate from charge recombination between positive charges stored on the S_2 and S_3 states of the water-splitting enzyme system and electrons located on the reduced primary PS II acceptor Q_A^- (Q_A-component with $t_{1/2} \sim$ 3 s at pH 7.5) and the reduced secondary acceptor Q_B^- (Q_B-component with $t_{1/2} \sim 44$ s at pH 7.5) [8, 9].

Thermoluminescence bands originating from the same redox couples have recently been shown to depend on pH by Demeter and Sallai [10] who attributed the phenomenon to both donor and acceptor side effects in PS II. On the basis of thermoluminescence results it can be expected that the delayed luminescence components decaying in the seconds to minutes time region will also exhibit a pH dependency.

In the experiments reported here we have examined the behavior of the Q_A- and Q_B-delayed luminescence components over the pH range 5.5–8.5 in untreated and in PS II-herbicide (DCMU and dinoseb) treated chloroplasts.

The pH-dependence for the Q_A- and Q_B-components were experimentally measured. The free energies of activation required for the charge recombination process were calculated, based on the known half-decay times.

Materials and Methods

Chloroplasts were isolated from spinach as described in [8] and suspended in a medium containing

Abbreviations: PS II, photosystem II; Q_A, primary acceptor of photosystem II; Q_B, secondary acceptor of photosystem II; Hepes, N-2-hydroxy-ethylpiperazine-N′-ethane sulfonic acid; DCMU; 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; dinoseb, 2,4-dinitro-6-(2-butyl)phenol; $t_{1/2}$, halftime; ΔF, free energy of activation; Chl, chlorophyll.

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0.4 M sorbitol, 10 mM NaCl, 5 mM $MgCl_2$, 2 mM EDTA and 50 mM Hepes (pH 7.5) to give a final concentration of about 1000 μg/ml. This suspension was further diluted with the suspension medium at appropriate pH to 30 μg Chl/ml and incubated in the dark for 5 min. Herbicides were added to the samples in the dark and were incubated for 10 min before delayed luminescence measurements were recorded.

Delayed luminescence was excited in a 1 cm cell with a Xenon flash (Stroboslave, model 1539, General Radio, Concord, Mass. 01742 USA, 3 μs, 0.5 J). The emitted delayed light was detected by a photomultiplier (EMI 9558B) situated at right angle to the exciting light source. The photomultiplier signal was amplified by a home-made differential amplifier, stored in a multichannel analyser (model ICA 70, Central Res. Inst. Phys., Budapest, Hungary) and plotted on an X-Y recorder (EMG-200).

Delayed luminescence decays were resolved into exponentials from semilogarithmic plots and the free energies of activation were calculated from decay half-times by a formula derived specially for this case from the theory of thermoluminescence [11] as:

$$\Delta F = k \cdot T \cdot \ln(t_{1/2} \cdot k \cdot T/(\ln 2 \cdot h))$$

where k is Boltzmann's constant, T is absolute temperature, $t_{1/2}$ is half-decay time of delayed luminescence and h is Planck's constant.

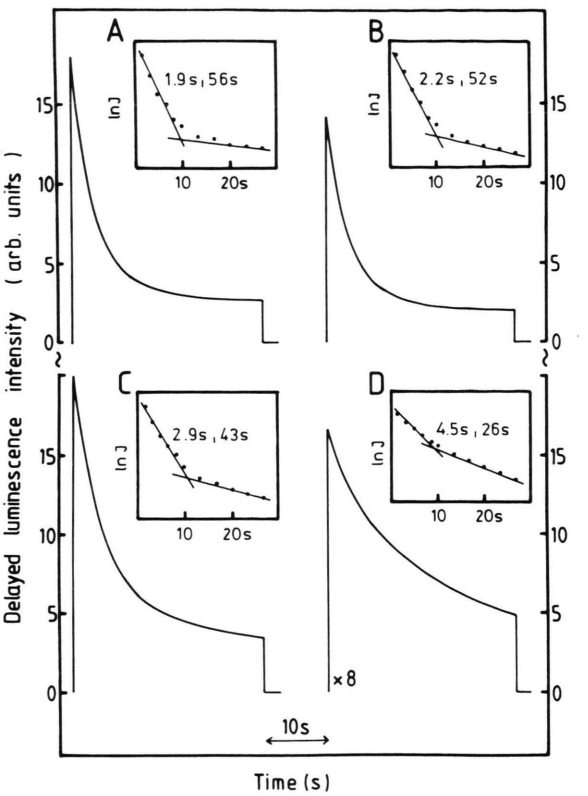

Fig. 1. Decay of delayed luminescence intensity after an exciting flash in chloroplasts at (A): pH 5.5; (B): pH 6.5; (C): pH 7.5 and at pH 8.5 (D). For better comparison the delayed luminescence intensity at (D) is magnified eight-times. Insets: curve resolutions into exponentials with half-decay times.

Results and Discussion

In the seconds to minutes time region, as it has been previously reported [8, 9], the flash induced delayed luminescence decay of isolated chloroplasts consists of two components: a relatively faster one with $t_{1/2} \sim 3$ s (pH 7.5) originating from $S_2Q_A^-$ and $S_3Q_A^-$ recombination (Q_A-component) and a slower one with $t_{1/2} \sim 44$ s (pH 7.5) arising from $S_2Q_B^-$ and $S_3Q_B^-$ recombination (Q_B-component).

Fig. 1 shows these delayed luminescence components at pH values 5.5, 6.5, 7.5 and 8.5 (Fig. 1A, 1B, 1C and 1D, respectively). On the insets decay curve-resolutions into exponentials demonstrate that the two components are present at every pH-value but their half-times are changing with pH. The half-time of the Q_A-component increased with increasing pH while the decay of the Q_B-component performed an opposite behavior: its $t_{1/2}$ decreased with increasing pH. At pH 8.5 both components showed a marked decrease in intensity which can be attributed to the gradual inactivation of the water-splitting enzyme system above pH 8.0 [12, 13].

These delayed luminescence observations correlate well with recent thermoluminescence results [10]: When the pH was increased from 5.5 to 9.0, a shift was observed in the peak position of the TL_{+10} thermoluminescence band (attributed to $S_2Q_A^- + S_3Q_A^-$ recombination and peaking at +10 °C, at pH 7.5 [9, 14]) from −5 °C to +25 °C.

Contrary to the TL_{+10} band the reverse situation was found for the TL_{+30} band (peaking at +30 °C at pH 7.5 and attributed to $S_2Q_B^- + S_3Q_B^-$ recombination) [9, 14]: upon changing the pH from 5.5 to 9.0 the peak position of the band shifted from +40 to +20 °C.

It is of note that at high pH values a third, very fast decaying component could also be distinguished in the msec time region of the decay course of delayed

luminescence (data not shown). It was observed that the half-time of this component increased with increasing pH ($t_{1/2} \sim 0.7$ s at pH 8.0; $t_{1/2} \sim 0.82$ s at pH 8.5 and $t_{1/2} \sim 0.9$ at pH 9.0).

Fig. 2A shows the pH-dependence of half-times of the Q_A (−○−) and Q_B (−△−) luminescence components estimated from delayed luminescence decay curves of untreated chloroplasts. If the electron transport inhibitor, DCMU (which blocks the electron transport between Q_A and Q_B [15]) was added to the samples the same effect was observed for every pH-value as we have reported for pH 7.5 [8]: the Q_B-component disappeared and only the Q_A-component remained, showing the same pH-dependence as in untreated chloroplasts: its decay became slower with increasing pH (−▲− on Fig. 2A).

The pH dependence of slow delayed luminescence was also measured in the presence of the phenolic type inhibitor, dinoseb, which has an action site in the acceptor side of PS II as well as an additional one at the donor side [15]. In dinoseb-treated chloroplasts at pH 7.5 we have already reported the observation of a delayed luminescence component with a decay faster than that in DCMU-treated samples. This fast component was attributed to the recombination of Q_A^- with positive charges stored on an unidentified donor of PS II localized at a shorter redox distance from the reaction center than the water-splitting enzyme system [16]. The decay half-time of this component increased with increasing pH from 5.5–8.5 as demonstrated in Fig. 2A (−●−). The rate of the luminescence reaction depends on the redox span between positive and negative charges involved in the charge recombination process resulting in luminescence [14, 17]. Thus the pH-dependence of the observed delayed luminescence half-times as well as the pH-dependence of the thermoluminescence peak temperatures [10] reflect the pH-dependence of positively charged donor and negatively charged acceptor molecules of the electron transport chain. Theoretically, the pH-dependence of the slow phase delayed luminescence components might be caused by the pH-dependence of any of the participating electron transport components: Q_A, Q_B, S_2 and S_3.

The redox properties of the S_2 state are known to be pH-independent, since the $S_1 \rightarrow S_2$ transition of the water-splitting enzyme system is not associated with proton release [5, 18], while the $S_2 \rightarrow S_3$-transition is pH-dependent [5, 18]. However, it is difficult to give the exact pH-dependence of the S-states because of their interaction with other unidentified donors [3, 19]. The midpoint redox potential of Q_A (appr. +40 mV at pH 8.0 [20]) and Q_B (appr. +106 mV at pH 7.2 [21, 22]) has been demonstrated to depend on pH with ~ −60 mV/pH [20, 21].

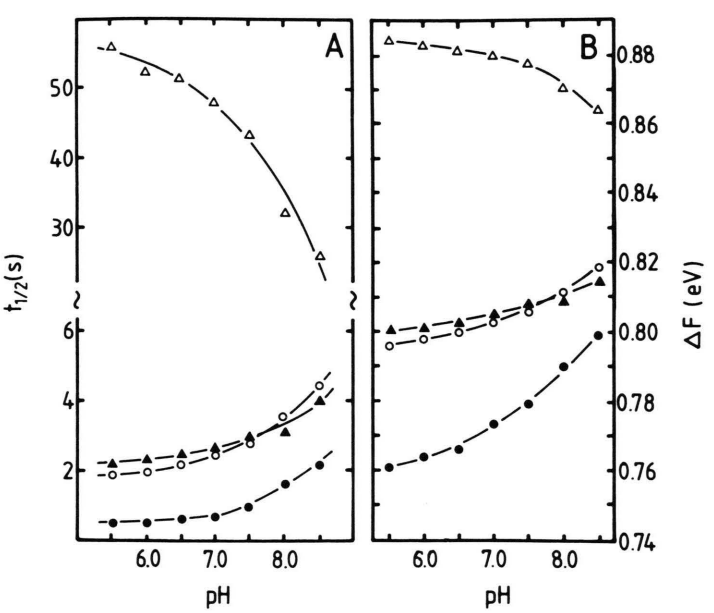

Fig. 2. pH-dependence of the half-decay times (A) and calculated free energies of activation (B) of the Q_A- and Q_B-delayed luminescence components in untreated chloroplasts (−○− and −△−); as well as the same characteristics of delayed luminescence in DCMU-treated (−▲−) and in dinoseb-treated (−●−) samples after an exciting flash.

To investigate in detail the pH-dependence of slow phase delayed luminescence components, we calculated the free energy of activation from the half-times of these components. The free energy of activation (ΔF) represents the energy deficit between the absorbed photon and the conserved energy during photosynthesis and corresponds to the redox potential difference between the electron transport components involved in charge recombination resulting in delayed luminescence.

Both in untreated (−○−) and DCMU-treated (−▲−) chloroplasts (Fig. 2B) the value of ΔF required for generation of the Q_A-component increased approximately linearly in the pH 5.5−8.0 region with a value of appr. +5 meV/pH (+5 mV/pH redox potential difference). Above pH 8.0 the pH-dependence of ΔF was more pronounced which might be attributed to the influence of high pH on the redox and charge stabilizing properties of the primary and secondary PS II acceptors [12].

The free energy of activation of the Q_B-component (−△− on Fig. 2B) depends on the pH to the same extent as that of the Q_A-component but with an opposite tendency: ΔF decreased in the pH 5.5−8.0 region with appr. −5 meV/pH. The value of ΔF for the Q_B-component was found to change more rapidly with increasing pH (similar to the behaviour of the Q_A-component above pH 8.0).

This symmetry in the pH-dependence of the Q_A- and Q_B-components might lead to the conclusion that this effect is connected with the pH-dependence of the $Q_A^- Q_B \rightleftharpoons Q_A Q_B^-$ equilibrium [7, 17]. On the other hand the changes in the value of the free energy of activation per pH units (~ 5 meV/pH) are about ten-times smaller than the corresponding changes in the redox potentials of the Q_A and Q_B electron acceptors [20, 21]. In addition while the redox potentials of both the Q_A and Q_B acceptors decreased with increasing pH the ΔF of the Q_A-delayed luminescence component increased with increasing pH values. These differences would indicate that not only acceptor side effects alone are involved in the process, but the donor side of PS II may also contribute.

In dinoseb-treated samples, where the delayed luminescence component is not associated with the water-splitting enzyme system (since the negative charges from Q_A^- recombine with positive charges from an unidentified donor located at a shorter redox distance from the reaction center than the water-splitting enzyme system [17]) the pH-dependence of the free energy of activation is stronger than that of the Q_A- and Q_B-components. It increases non-linearly with pH (−●− in Fig. 2B). This observation demonstrate that although the negatively charged acceptor undergoing charge recombination is the same (Q_A^-) both in DCMU- and dinoseb-treated chloroplasts the pH dependencies of the corresponding ΔF values are very different. Consequently the comparision of the pH-effects on dinoseb-treated (−●−) and on DCMU-treated (−▲−) samples indicates that pH-induced changes in the redox states of positively charged donors which are participating in the charge recombination are also reflected in the pH-dependence of delayed luminescence.

Summarizing our results on the basis of activational free energy calculations we suggest that the pH-dependence of the Q_A- and Q_B-components of delayed luminescence results from both acceptor and donor side pH-effects in PS II. This observation is in good agreement with recent thermoluminescence results on the pH-dependence of the same charge recombination process [10].

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