ATP-INDUCED CHLOROPHYLL LUMINESCENCE IN ISOLATED SPINACH CHLOROPLASTS

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1. Introduction

Upon a light–dark transition chloroplasts emit post-illumination luminescence [1], which is commonly considered to result from the recombination of positive charges (Z^+) and negative charges (Q^-) at the photosystem II reaction centers [1,2]. Stimulation of post-illumination luminescence by a variety of treatments has been reported (for a recent review, see [3]) such as transthylakoidal ΔpH [4], ΔpH-reduced, reverse electron flow [5], salt addition [6], a rapid temperature increase [7], addition of DCMU [8] or dithionite [9]. These treatments have in common, that they either increase the supply of electrons at Q or provide an additional source of activation energy for the recombination reaction (see [3]).

After light-activation of the latent adenosine triphosphatase (ATPase), addition of ATP in the dark to isolated chloroplasts leads to formation of a transthylakoidal ΔpH (see [10]) and reduction of Q by reverse electron flow [11,12].

In this report we wish to describe a procedure by which ATP-induced luminescence can be readily measured. Such luminescence is dependent upon preactivation of the ATPase, a preilluminating flash, conditions which prevent a rapid post-illumination luminescence decay, and an active and well-coupled ATPase.

2. Materials and methods

Spinach chloroplasts were prepared as previously described [13]. The measuring apparatus and details of the measuring procedure were as reported before [12]. The standard reaction mixture contained; Tricine, pH 8.0, 15 mM; NaCl, 20 mM; $MgCl_2$, 5 mM; P_i, 1 mM; PMS, 5×10^{-8} M; DTT, 5 mM; and chloroplasts containing about 20 μg chlorophyll/ml. ATP was injected by a microsyringe into the thoroughly stirred reaction mixture, to a final concentration of 0.8 mM. Tentoxin was kindly provided by Dr J. Steele, University of Wisconsin, Madison. For saturating flashes of about 6 μs duration, a Stroboslave (General Radio, Type 1539-A) with manual triggering was employed. When more than one flash was given, flashes were spaced about 1 s apart.

3. Results and discussion

Figure 1 illustrates the procedure which was found optimal for high yields of ATP-induced luminescence. The chloroplast suspension was first illuminated with strong, heat-filtered white light for 3 min to activate the latent ATPase [10,12]. From about 5 s after the activating light was turned off, the decay of post-illumination luminescence was monitored for 90 s, by which time it approached zero. Two saturating flashes

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Abbreviations: Q, The primary electron acceptor of photosystem II; Z, The primary electron donor of photosystem II; PQ, Plastoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PMS, Phenazine methosulfate; DTT, Dithiothreitol; ADRY, acceleration of the deactivation reactions in the water-splitting enzyme Y

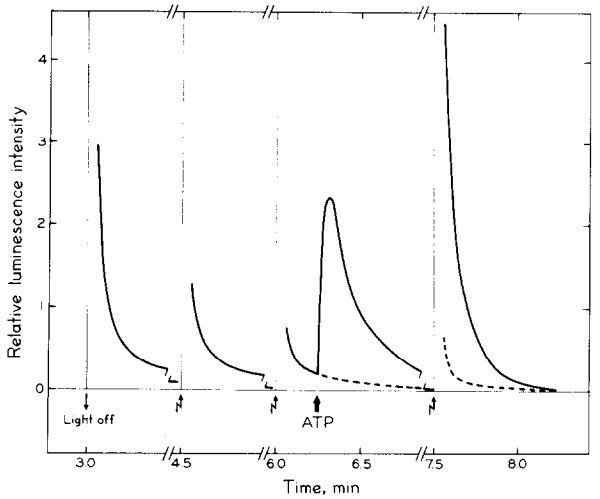

Fig.1. ATP-induced stimulation of luminescence. The light-off arrow indicates the end of the 3 min light-activation period. The application of flash pairs is indicated by zig-zag arrows. The broken lines represent the decay of flash-induced luminescence when no ATP was added. During illumination or flashing the photomultiplier was turned off; monitored of post-illumination luminescence was routinely initiated about 5 s after preillumination. Temperature, 10°C. For other conditions, see Materials and methods.

were given and the flash-induced luminescence was recorded for 90 s. Two more flashes were then given, and ATP was injected before the flash-induced luminescence had decayed completely. ATP induced a marked burst of luminescence which decayed with a half-time of about 12 s. ATP addition stimulated luminescence by about 10-fold, as compared with the post-illumination luminescence during the same time period (dashed line in figure). At 90 s after the second flash pair, a third flash pair was given and the luminescence monitored. The dashed lines in fig.1 illustrate the decays of flash-induced luminescence when no ATP was added.

This sequence displays the ATP-induced luminescence in two days:

(i) The burst of luminescence upon injection of ATP.

(ii) The much enhanced luminescence following a flash-couple, when ATP is present and hydrolysed by the ATPase (compare the dashed and solid curves following the last flash couple).

The requirements for observing a significant ATP-induced luminescence are listed in table 1. Clearly,

Table 1
Requirements for ATP-induced luminescence

Conditions	ATP-induced luminescence (% control)
No light activation	1
DTT omitted	15
PMS omitted	94
$MgCl_2$ omitted	0
$MgCl_2$ omitted during activation only	49
No flashes	8
One flash	32

The values given represent the relative height of the luminescence peak obtained upon ATP injection (see fig.1).The complete reaction mixture and control procedure are described in Materials and methods and in the text. When $MgCl_2$ was omitted during activation only, it was added 15 s after the activating light was turned off. No flashes – ATP was added when the post-activation luminescence reached the same level as in the control.

the phenomenon requires preactivation of the latent ATPase, as indicated by the low activity observed when no activating preillumination was given or when DTT was omitted [10]. In contrast to the ATP-induced reduction of Q [11], PMS does not seem to be obligatory in this system. Actually the concentrations of PMS ($> 1\ \mu M$), which are optimal for ATP-induced reverse electron flow [11] or photophosphorylation [14], accelerate the decay of the flash-induced luminescence to a point, where ATP-induced luminescence cannot be observed. Magnesium ions, which are known to be required for ATPase activity are also necessary for stimulation of luminescence. When Mg^{2+} was omitted during activation only, a partial inhibition was observed, indicating that Mg^{2+} enhances the activation process per se [15].

An important observation was the fact that unless at least one preilluminating flash was given prior to ATP addition, almost no luminescence was stimulated by ATP. This result is in agreement with the postulated mechanism of luminescence by recombination of the positive and negative charges separated at the photosystem II reaction centers [2,3]. During the light-activation period a steady state is reached which is characterized by a fully-reduced acceptor pool and a partially-oxidised donor pool. When the activating light is switched off, the luminescence yield is limited by

the availability of the positive charges at the donor side rather than by the negative charges within the much bigger pool at the acceptor side. Therefore the ATP-induced luminescence, which is due to an effect on the acceptor side, is very small. A 90 s dark period following activation permits the acceptor pool to partially empty, and the much smaller pool of positive charges at the donor side to decay almost completely [16]. A saturating flash given at this point will create an equal number of positive and negative charges. While the negative charges will rapidly drain into the large, secondary acceptor pool (plastoquinone), the positive charges are known to possess a relatively long half-life, around 30 s [16]. Thus, a situation is created where the availability of electrons at the acceptor side becomes the limiting factor in the luminescence forming reaction. Accordingly, addition of ATP and the induced back-flow of electrons from the PQ-pool into Q, results in a marked stimulation of luminescence.

This rationale explains:

(i) The lack of ATP-induced luminescence when ATP is added after activation (no flashes in table 1).

(ii) The pronounced effect after a short flash (fig.1).

(iii) The much-enhanced, flash-induced luminescence observed in the presence of an active ATPase (right trace in fig.1).

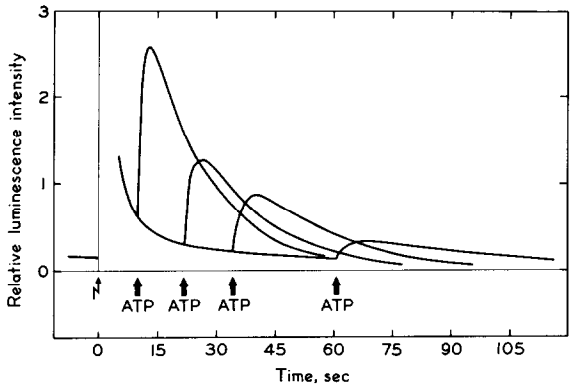

Fig.2. Dependence of the ATP-stimulated luminescence on the time elapsed between a preilluminating flash couple and ATP-injection. Experimental details as in fig.1.

We attempted to determine the dependence of the ATP-induced luminescence on the number of pre-illuminating flashes. As shown in table 1 two flashes were always far more effective than a single flash, but no consistent pattern was observed with a longer sequence of flashes. We confirmed that the intensity of the flashes was indeed saturating, and therefore tend to interpret the enhanced effect by the second

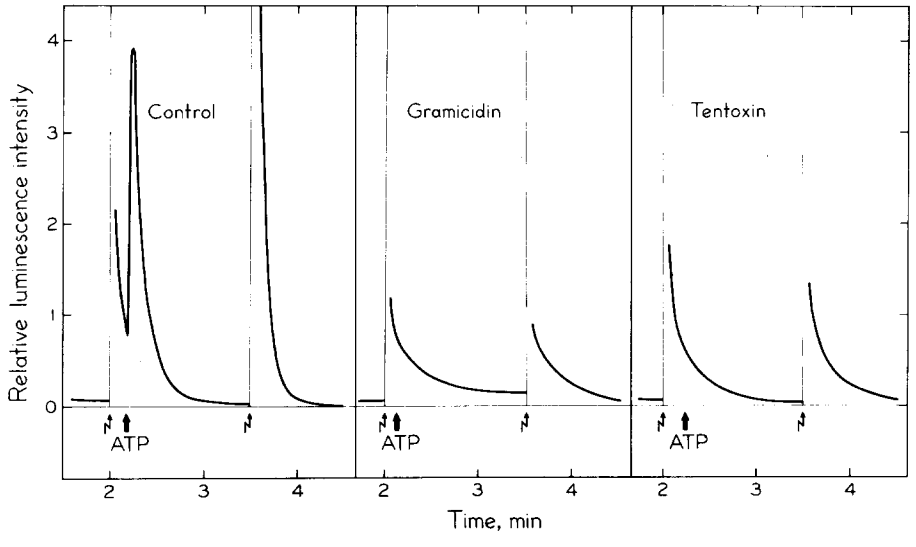

Fig.3. Inhibition of ATP-stimulated luminescence by gramicidin and tentoxin. Two minutes after the activating light was turned off, one preilluminating flash couple was given before ATP addition. Note: The time scale refers to time after activation. The inhibitors were added 15 s after activation. Concentrations: gramicidin, 5×10^{-6} M; tentoxin, 10^{-5} M. Temperature, 15°C. Other conditions as in fig.1.

flash in terms of the properties of the Q–R equilibrium [17,18] and/or of the S-states [16].

The concentration of ATP required for a half-maximal effect is around 50 μM. An important parameter for the yield of ATP-induced luminescence is the time elapsed between the flashes and ATP addition (fig.2). The phenomenon is more pronounced the earlier the ATP is added, but a significant stimulation can be observed even 1 min after the flashes, when the flash-induced luminescence itself is almost completely decayed.

If the phenomenon of ATP-induced luminescence is indeed due to the ATP-synthase system working in reverse, it should be sensitive to uncouplers and ATPase inhibitors. As can be seen in fig.3, gramicidin, which is a potent uncoupler (but not an ADRY agent [19]) did not completely abolish the effect. Note that in this experiment gramicidin was added after the activation stage. Tentoxin, a recently described potent inhibitor of the ATPase [20], also completely abolished the ATP-induced effect. Parallel measurements of fluorescence yield confirmed that tentoxin also inhibited the ATP-induced reduction of Q (not shown in the figures).

In conclusion these experiments demonstrate that after proper activation the ATPase system can affect the electron-transport system all the way to the photosystem II reaction center. This extends and complements the previous demonstrations of an ATP-induced proton gradient (see [10]), ATP-induced reduction of Q [11,12], acid–base-induced Q-reduction [21] and acid–base-induced luminescence [4,5].

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