Biochimica et Biophysica Acta, 546 (1979) 448—454

BBA 47666

PROPERTIES OF ATP-INDUCED CHLOROPHYLL LUMINESCENCE IN CHLOROPLASTS *

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(Received August 30th, 1978)

Key words: Luminescence; Chlorophyll luminescence; ATP induced

Summary

1. The recently described reaction of ATP-induced luminescence is analyzed for its relation to other ATP-induced reactions such as ATP-driven transmembrane proton gradient formation and ATP-driven reverse electron flow.

2. In the absence of phenazine methosulfate ATP-induced luminescence is optimal while the main phase of ATP-driven reverse electron flow is eliminated.

3. DCMU which by itself causes a much smaller luminescence, inhibits the ATP-induced luminescence.

4. Nigericin plus valinomycin, but not each by itself, fully inhibit the ATP-induced luminescence.

5. The observations are interpreted as indicating that ATP stimulates luminescence by a 2-fold mechanism: (a) increasing the amount of the reduced primary electron acceptor of Photosystem II, Q, and (b) creating a transmembrane electrochemical potential which serves to decrease the activation energy required for the charge recombination reaction which leads to luminescence.

Introduction

Upon a light-dark transition chloroplasts emit post-illumination luminescence [1], which is commonly considered to result from the recombination of

* CIW-DPB Publication No. 646.

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Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Q, primary electron acceptor of Photosystem II; R, secondary electron acceptor of Photosystem II.

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 ref. 3) such as transthylakoidal ΔpH [4], ΔpH-induced 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 ref. 3).

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

We have recently briefly reported [13] on conditions under which a new type of reaction, ATP-induced chlorophyll luminescence can be observed. In this communication we describe the properties of this new reaction, its relation to the previously described ATP-induced ΔpH and reverse electron flow, and the effects of inhibitors and uncouplers on the reaction.

Materials and Methods

Spinach chloroplasts were prepared [14] and chlorophyll determined [15] essentially as previously described. The chloroplasts were finally suspended in a small volume of 0.4 M sucrose, 0.05 M NaCl at a concentration of about 2 mg chlorophyll per ml. The instrument used [12] and the methodology employed [13] were as previously described with variants indicated in the figure and table legends.

Results

Properties and relation to ATP-dependent reverse electron flow

Fig. 1 illustrates the phenomenon and its dependence on the concentration of ATP. 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 (light-off arrow), the decay of post-illumination luminescence was monitored (see Fig. 1) for 90 s, by which time it approached zero. Two saturating flashes were given (zig-zag arrow), and ATP was injected 20 s after the last flash. Clearly ATP induced a large burst of luminescence which decayed slowly. As previously indicated [13] preactivation of the latent ATPase in the presence of dithiothreitol and magnesium and preillumination with a flash or two were absolutely required for ATP to induce the extra luminescence. The figure illustrates that a half-maximal effect was observed with about 100 μM ATP, which is similar to values previously reported for ATP-induced reverse electron flow [11] and other light-triggered reactions [16,17].

In Fig. 2 the relation of the ATP-induced luminescence to the ATP-induced reverse electron flow is illustrated. We have recently shown [18] that the latter reaction is kinetically composed of at least two phases, a rapid small phase and a slow main phase. The former was found to be independent of the addition of

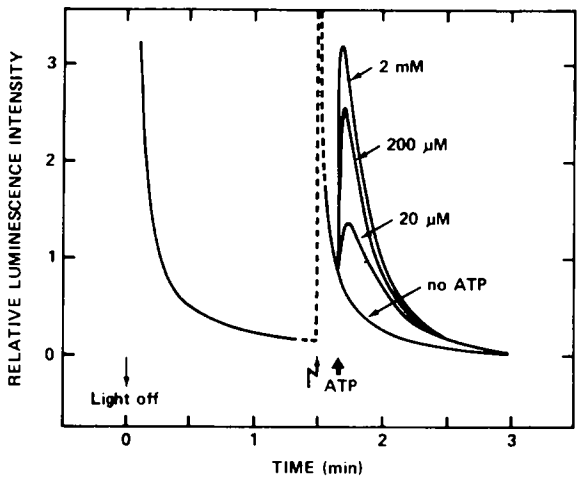

Fig. 1. ATP-induced luminescence. The reaction mixture contained: 15 mM tricine, pH 8.0, 20 mM NaCl, 5 mM $MgCl_2$, 1 mM inorganic phosphate, pH 8.0, 5 mM dithiothreitol, 0.1 μM phenazine methosulfate, and chloroplasts containing 20 μg of chlorophyll per ml. ATP was injected with a microsyringe into the thoroughly mixed reaction mixture in a volume not exceeding 10 μl. The light-off arrow indicates the end of the 3 min preillumination period. The zig-zag arrow indicates illumination with two saturating microsecond flashes, one second apart. During illumination or flashing the photomultiplier was turned off. The dashed lines indicate extrapolations into non-measured times. Temperature, 10°C. Other details in text.

phenazine methosulfate, while the latter was optimal in the presence of 1 μM phenazine methosulfate. The right side of the figure illustrates these observations. The ATP-induced reduction of the primary electron acceptor of Photosystem II, Q, is followed by monitoring the increase in chlorophyll fluorescence yield which accompanies its reduction [11,12]. The left side of the figure

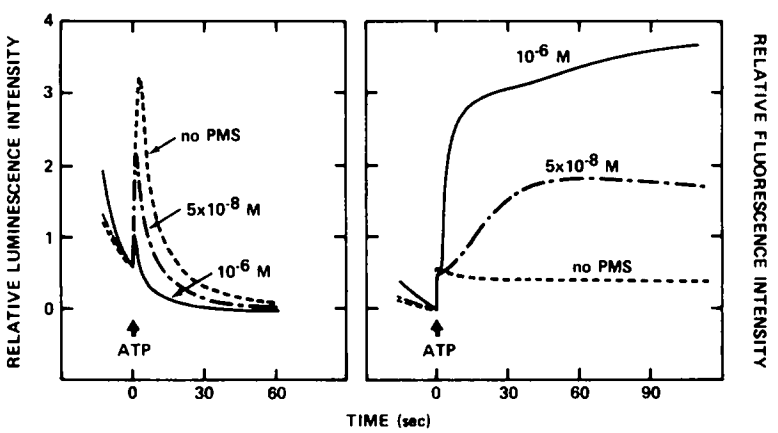

Fig. 2. The effect of phenazine methosulfate (PMS) on ATP-induced reverse electron flow and luminescence. Reaction conditions as described under Fig. 1, except that the phenazine methosulfate final concentration was as indicated. ATP to 0.8 mM was injected 2 min after the termination of the preillumination (time zero in figure) in the fluorescence measurements, and 15 s after the two flashes in the luminescence experiments. The intensity of the fluorescence measuring beam [12] was 10 ergs · cm^{-2} · s^{-1}, yielding a fluorescence intensity about 100-fold higher than the luminescence intensity.

illustrates a similar experiment in which the ATP-induced luminescence was monitored. Clearly, phenazine methosulfate is not required for the luminescence reaction. Furthermore, phenazine methosulfate concentrations which are optimal for Q reduction, severely inhibit the luminescence reaction. Thus, we have here a new type of ATP-induced reaction, which correlates with the small rapid phase of the ATP-induced rise in chlorophyll fluorescence yield, but not with the main phase, and is too rapid to correlate with the slow ATP-dependent buildup of the transmembrane proton gradient [12]. Phenazine methosulfate was previously suggested to be required in ATP-induced reverse electron flow as a catalyst for electron flow from dithiothreitol to the electron transport chain [11,18]. Its inhibition of the ATP-induced luminescence may relate to the acceleration of the reduction of Z^+ by dithiothreitol, by Q^-, or by both.

The effect of inhibitors

DCMU, the potent electron flow inhibitor, is considered to inhibit electron transport after Q. It also caused a rapid reduction of Q by R [19,18], and thus stimulates luminescence [8,20,21]. In Fig. 3 the DCMU- and dithionite [19] -induced luminescences are compared with that elicited by ATP. The ATP-induced luminescence is by far the largest in extent and in the total number of emitted quanta. Fig. 4 shows that when DCMU is added before ATP, the ATP-induced luminescence is severely inhibited.

The effect of ionophores

In Fig. 5 the effects of the K^+ ionophore valinomycin, and the $K^+ \rightleftharpoons H^+$ exchange ionophore nigericin, are shown. Neither ionophore by itself had a marked effect on the ATP-induced luminescence, but the combination of both had synergistically fully inhibited the phenomenon. Such behaviour is typical of reactions whose driving force is an electrochemical potential of protons com-

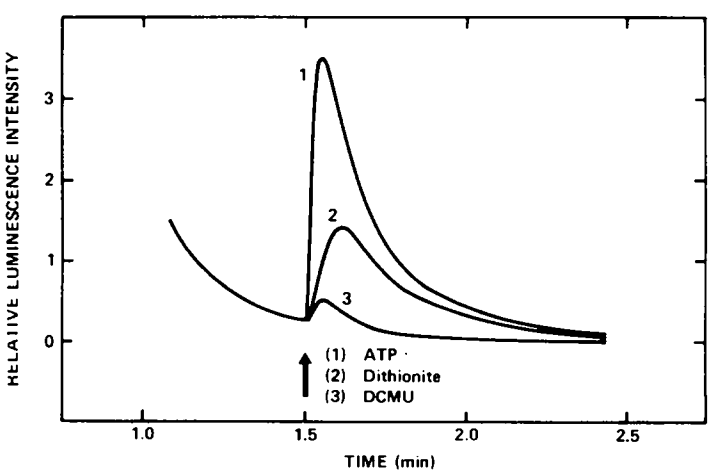

Fig. 3. Comparison of ATP-, dithionite- and DCMU-induced luminescence signals. Reaction conditions as described under Fig. 1, except that no phenazine methosulfate was present. The two saturating flashes were given 1 min after the light activation period (1.0 on figure). Where indicated DCMU to a final concentration of 1 μM; $Na_2S_2O_4$, to 10 mM; or ATP to 0.5 mM were injected.

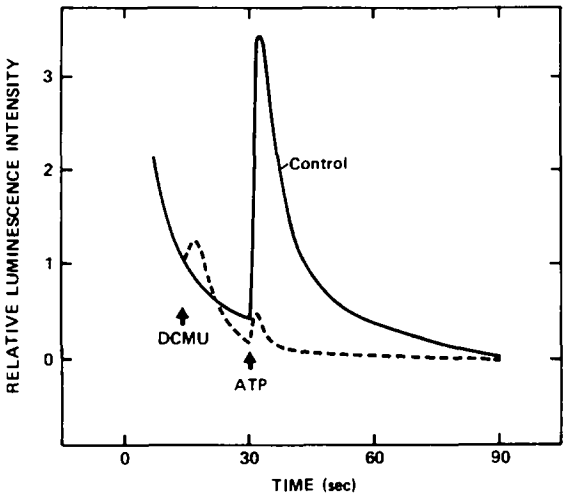

Fig. 4. Inhibition of ATP-induced luminescence by DCMU. Reaction conditions as described under Fig. 1, except that no phenazine methosulfate was present. The two flashes were given 90 s after the light activation period (zero time on figure). Where indicated DCMU to a final concentration of 1 μM and ATP to 1 mM were injected.

posed of both a membrane potential and a transmembrane proton concentration gradient [22]. Fig. 6 shows data, taken under similar conditions, of ATP-dependent proton gradient formation and Q reduction [12]. As expected, nigericin completely abolished the ATP-dependent proton gradient formation (the remaining small quenching of 9-aminoacridine fluorescence was previously established to be artifactual, due to chemical quenching by ATP [10,18]), and the main phase of Q reduction. However, the rapid phase of the ATP-induced

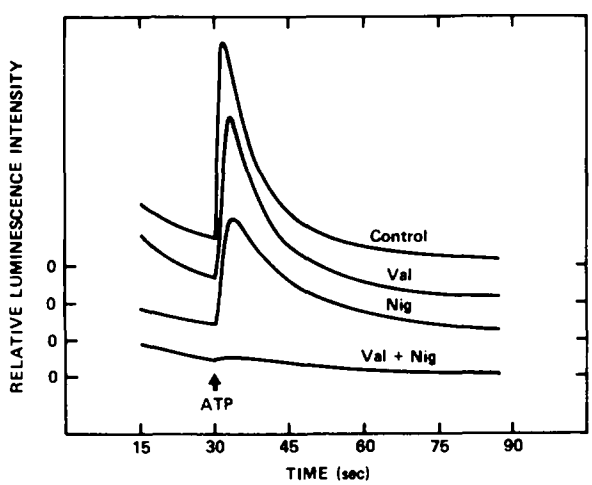

Fig. 5. Synergism between nigericin (Nig) and valinomycin (Val) in inhibiting ATP-induced luminescence. Reaction conditions as described under Fig. 1, except that no phenazine methosulfate was present, and half the NaCl was replaced by KCl. Valinomycin to a final concentration of 0.5 μM and nigericin to 0.5 μM were added immediately after the light activation period. Time after the application of the flash couple is indicated.

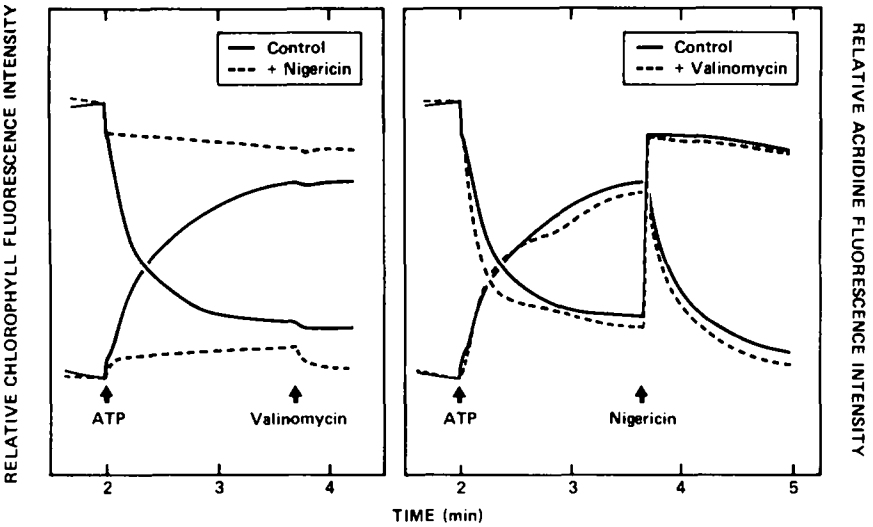

Fig. 6. The effect of nigericin and valinomycin on the ATP-induced transmembrane pH gradient and reverse electron flow. Reaction conditions as described under Fig. 1, except for the presence of 1 μM phenazine methosulfate, 2 μM 9-aminoacridine, replacement of half the NaCl by KCl, and measuring fluorescence as previously described [12]. ATP, nigericin and valinomycin were added, where indicated to final concentrations of 0.1 mM, 0.5 μM and 0.5 μM, respectively.

increase in chlorophyll fluorescence yield, which correlates with the ATP-induced luminescence (Fig. 2) was unaffected. It was fully inhibited only when both nigericin and valinomycin were present. The right side of the figure illustrates that valinomycin by itself had small effects on the ATP-induced proton gradient formation and the ATP-induced reverse electron flow.

The $Mg^{2+} \rightleftharpoons H^+$ exchange inducing ionophore A23187 [23], behaved similarly to nigericin in all responses tested in Figs. 5 and 6 (not shown).

Discussion

It was previously shown [13] that ATP-induced luminescence is dependent upon preactivation of the latent ATPase of chloroplasts in a manner which maintains it in a well coupled state, and upon preillumination with at least one light flash shortly before ATP addition. These results were interpreted in view of the generally accepted working hypothesis for chlorophyll luminescence [2,3] which postulates it to result from a back reaction of the primary stable charge separation products of the photoact in Photosystem II, Z^+ and Q^-. The function of the flash was suggested to be production of Z^+, which decays with a half-life of about 30 s under conditions where Q^- decays much more rapidly, and the function of ATP was envisaged to produce Q^- via the previously demonstrated [11] reverse electron flow reaction.

Several observations reported in this manuscript suggest that this latter conclusion must be extended: (a) The data of Fig. 2, which demonstrates that ATP-induced luminescence occurs best under conditions where the main phase of ATP-induced Q reduction does not occur. (b) The observation reported in Fig. 3 that the ATP-induced luminescence is considerably larger than the

DCMU induced one, while both produce equal increments of Q reduction [18]. (c) The fact that the initial increase in the ATP-induced luminescence is considerably faster than the ATP-induced development of either the transmembrane pH gradient or the main phase of Q reduction (compare Figs. 1–6, and refs. 12, 13 and 18).

It was previously suggested that part of the activation energy for the back reaction which produces the luminescence is provided by the creation of a transmembrane electrochemical potential (see ref. 3 for review) and indeed the luminescence has been used to measure the electrochemical potential and the activation energy [24].

It seems reasonable to assume, therefore, that ATP stimulates luminescence not only by producing Q^- via reverse electron flow, but in the early phases mostly by rapidly producing a transmembrane electrochemical potential which stimulates luminescence by lowering the activation energy needed for the back reaction.

This interpretation would also be in accord with the data of Figs. 5 and 6, where valinomycin by lowering the membrane potential and stimulating the transmembrane pH gradient (see Fig. 6 and ref. 25) has little effect on the ATP-induced luminescence; nigericin, which markedly lowers the pH gradient while stimulating the membrane potential (not shown, but see ref. 25) also had a marginal effect. However, the combination of both reagents in detrimental to the ATP-induced luminescence since in the presence of both neither component of the transmembrane electrochemical gradient is maintained.

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