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Inhibitions of Photosystem II activity by proton gradient

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Abstract

Nigericin stimulates PS II turnover in thylakoids at low but not at high light intensity in a low-frequency regime (6 Hz) at pH 8. When the external pH is decreased, stimulation by the uncoupler is also observed at high light intensity. Neither phosphorylation of LHCII nor unstacking of the thylakoids changes the effect of nigericin at pH 8. The observations are interpreted to indicate that ΔpH regulates PS II activity at two levels: (a) the transfer of excitation energy to the reaction centre; (b) PS II turnover.

Key words: Photosystem II regulation; Localized proton; Light harvesting complex phosphorylation; Excitation energy; Energy transfer; Reaction center

1. Introduction

The influence of $\Delta\mu_{H^+}$ on photosynthetic electron transport has been studied extensively [1], as has its effect on chlorophyll fluorescence [2]. However, its influence on excitation energy transfer to reaction centres has received much less attention.

We have recently addressed this problem, showing that localized proton domains formed during electron transport can influence the efficiency of transfer of the energy absorbed by the antenna of PS II to the reaction centres [3,4]. The evidence for such a conclusion came from measurements of electron transport rate under conditions where the transfer of energy to PS II reaction centre was rate-limiting, i.e., single turnover flashes (λ_2) modulated at low frequency (6 Hz) supplemented with a far-red (730 nm) continuous beam (λ_1) of intensity such as to saturate the Emerson enhancement [3]. We observed a stimulation by the lipophilic uncouplers [4] of electron transport from water to NADP only at rather low light intesities. Under such conditions no effect of nigericin was detected at saturating intensity of the modulated light (λ_2), indicating that the low-frequency activation of the PS II primary photochemistry prevented any inhibition by $\Delta\mu_{H^+}$ of secondary electron transport to become apparent.The inhibition of excitation energy transfer to PS II reaction centre was not due to $\Delta\Psi$, as indicated by the lack of effect of $\Delta\Psi$ suppressing ionophores [3].

The observation that the stimulation by lipophilic uncouplers of PS II activity showed a spectrum with maxima at 475 and 650 nm and minima at 500 and 550 nm [4] further supported the conclusion that inhibition by membrane localized protons is related to excitation energy transfer.

We report here an extension of our previous results, showing that depending upon the pH of the medium the occurrence of another kind of electron transport inhibition by ΔpH is apparent, which is not related to the excitation energy transfer efficency but rather to the inhibition of the turnover of PS II by low iumenal pH. It is also reported that the inhibition of excitation energy transfer was observed in unstacked thylakoids and in phosphorylated thylakoids as well, suggesting that the mechanism of energy redistribution between PS II and PS I is not involved in the inhibition observed.

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Abbreviations: LHC II, light harvesting chlorophyll-protein complex II.

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2. Materials and methods

Stroma-free thylakoids were prepared as previously described [6] from spinach leaves just harvested and kept 2 h at room temperature in darkness. This procedure was adopted to allow dephosphorylation of LHC II by the endogenous phosphatase [7]. Thylakoids were resuspended in Tricine-NaOH buffer (pH 8) containing 0.4 M sucrose, 10 mM NaCl, 20 mM KCl and 5 mM $MgCl_2$. The same buffer with sucrose 0.1 M served as the reaction medium with 0.05 mM or 5 mM $MgCl_2$ as indicated in the captions of the figures. The unstacking of the thylakoids in the low $MgCl_2$ containing medium was monitored by the associated decrease of maximum fluorescence, F_m, in the presence of DCMU and was complete in approx. 90 seconds. Complete restoration of the F_m value was reached upon addition of 5 mM $MgCl_2$. Phosphorylation of LHC II was performed in the dark for 15 min as previously described [6], in the presence of 5 μM ferredoxin, 0.5 mM NADPH, 1 mM ATP and 10 mM NaF. The decrease of F_m and of the Emerson enhancement upon phosphorylation were taken as the indication of LHC-II phosphorylation [5]. The control was treated in the same way with the omission of ATP. All the samples were then diluted 1:50, cooled and stored at 0°C.

NADP reduction was measured as previously described [3] at room temperature (22–23°C), as the change of absorbance at 340 minus 390 nm measured in a dual-wavelength spectrophotometer; actinic light was provided at 90° with respect to the measuring light, and the photomultiplier was protected from the actinic beam by the appropriate filters [3,5]. Actinic light was produced by a xenon flashlamp (EG and G) providing single turn-over flashes modulated at 6 Hz [5]. The flashes (λ_2) were filtered through a heat filter and a RG-630 long-pass filter. The intensity of the modulated light was regulated by means of neutral filters. A 730 nm continuous beam (λ_1) was provided, of intensity sufficient to saturate PS I activity in order to keep Q_A oxidized and to saturate Emerson enhancement [5]. Under these conditions, light capture by PS II antenna and PS II photochemistry were strictly limiting the electron transport from water to NADP.

3. Results

Fig. 1 shows that the stimulation by the uncoupler nigericin of PS II activity at rate-limiting flashed light (modulated at 6 Hz) intensity was not affected by phosphorylation of LHC II (compare curve c to curve a). Phosphorylation decreased maximal fluorescence of 14%. At saturating intensity of the modulated light, nigericin stimulated PS II activity only in the phosphorylated sample. Such a stimulation at saturating light

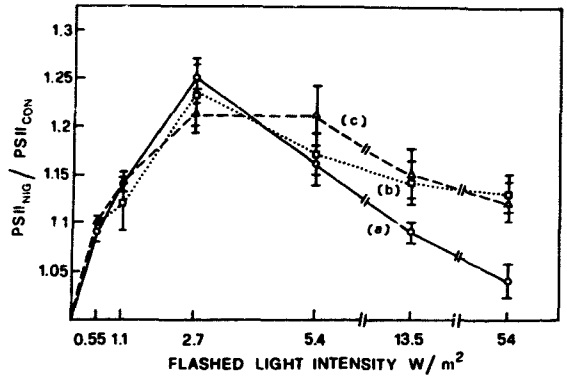

Fig. 1. Effect of nigericin on phosphorylated and non phosphorylated thylakoids. Conditions as described in the text. pH 8, Chl 15 μg ml^{-1}. PS II was calculated as $V_{\lambda 2+\lambda 1} - V_{\lambda 1}$ (Ref. 4). Symbols: ○, control; △, phosphorylated; □, control + ATP 12 μM. λ_2 intensity was 4.2 W m^{-2}.

intensity was observed, however, in the control sample as well (Fig. 1, curve b) when ATP was added at the same concentration (12 μM) as that present in the phosphorylated samples as a result of the phosphorylating treatment.

The data reported in Fig. 2 show that at saturating light intensity, the stimulation of PS II limited electron transport by nigericin increased upon increasing ATP concentration (inset) because of the inhibition by ATP of the reaction rates in the control (Fig. 2, triangles). The possibility that phosphorylation of LHC II occurred during the rate measurements (1–2 min) caused by the low ATP concentration present has been ruled

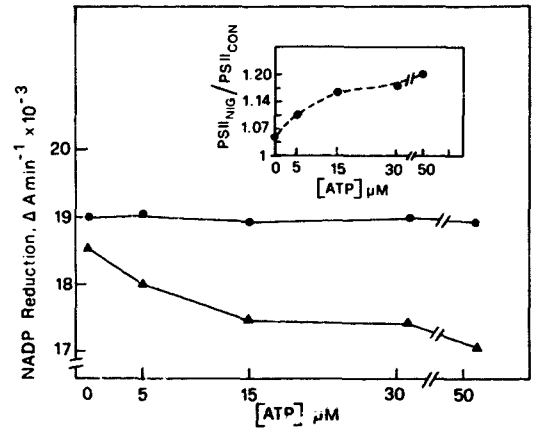

Fig. 2. ATP concentration dependence of the effect of nigericin on PS II at saturating λ_2 intensity. Conditions as in Fig. 1. λ_2 intensity was 54 W m^{-2}. ▲, control; ●, nigericin, 200 nM. Inset: ratio (PS II + nig)/(PS II cont) as a function of ATP concentration.

Table 1
Effect of nigericin on PS II in unstacked thylakoids as a function of modulated light intensity

Light intensity Wm^{-2}	1.1	4.3	8.6	13.6	54
PS II nig/PS II con	1	1.07	1.16	1.15	1.04

Conditions as in Fig. 1 except for the $MgCl_2$ content of the medium, which was 0.05 mM. 2 min were allowed before starting the measurement to permit complete unstacking of the thylakoids.

Table 3
Dependence of PS II activity on flash frequency at pH 7.5

Flash frequency (Hz)	Control			Nigericin 200 nM		
	V_{A1-A2}	V_{A1}	PS II	V_{A1-A2}	V_{A1}	PS II
6	15.3	2	13.3	21	2	19
3	8.6	2	6.6	11.9	2	9.9

Conditions as in Fig. 1. pH 7.5. Modulated light intensity was 54 Wm^{-2}. Electron transport rates are expressed in μeq 10^{-3}/min.

out because no change in the rate of NADP reduction was observed during the measurements at any light intensity. No difference between the activity of PS II in the presence of nigericin was detectable between the phosphorylated and the non-phosphorylated samples at saturating light intensity (data not shown).

Table 1 shows that the light intensity dependence of the effect of nigericin on PS II activity was observed also in the case of unstacked thylakoids. Comparing the nigericin effect vs. light intensity of Table 1 with that of Fig. 1 (curve a), one can see that in the case of unstacked thylakoids the maximum effect was displaced towards higher light intensity. This was expected because of the decrease of energy available to PS II due to spillover in unstacked thylakoids.

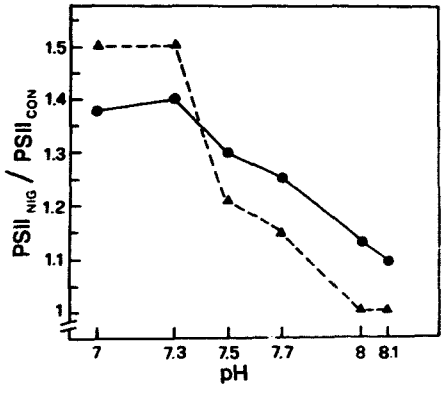

Fig. 3. pH dependence of the effect of nigericin on PS II at saturating and limiting light intensity. ▲, λ_2 intensity was 54 Wm^{-2} (saturating); ●, λ_2 intensity was 2.7 Wm^{-2} (limiting); Other conditions as in Fig. 1 The control NADP reduction rates at pH 8 were 20 mA/min (saturating λ_2) and 14 mA/min (limiting λ_2).

The dependence upon the pH of the medium of the effect of nigericin on PS II activity at low and high intensity of the modulated light is reported in Fig. 3. Stimulation upon nigericin addition at saturating light intensity appeared at pH 7.7 and increased at more acidic pH values. The effect of nigericin at low light intensity increased as well but a significant difference between low and high intensity was observed upon decreasing the pH value down to pH 7.5 (Table 2). The magnitude of this difference is almost constant in the pH range from 8.1 to 7.5. The cross-over of the low light and high light effect was located between pH 7 and 7.5 and was rather variable in different thylakoids preparations (Fig. 3, Table 2).

It must be emphasized (see Table 3) that at pH 7.5 the electron transport activity was halved upon halving the frequency of the high light intensity flashes, indicating that there was no influence of 'photosynthetic control' on electron transport rate at the level of plastoquinol reoxidation, in agreement with previous observations at pH 8 [3,5].

4. Discussion

Braun and Malkin reported that in stacked thylakoids increase of cations as well as the addition of uncouplers are effective in controlling the imbalance of excitation between the two photosystems, in favour of PS II [8].

The data reported here show that the stimulation of energy transfer to PS II reaction centres by nigericin under low light intensity was unaffected by unstacking the thylakoids (Table 1). It was also unaffected by the

Table 2
Difference between the stimulation by nigericin of PS II at low and high intensity of the modulated light

	Medium pH					
	7	7.3	7.5	7.7	8	8.1
Ratio Difference	-0.14 ± 0.14	-0.10 ± 0.06	0.09 ± 0.06	0.14 ± 0.04	0.16 ± 0.03	0.1 ± 0.02

The 'Ratio difference' is defined as:

$$\frac{(\text{PS II nig})}{(\text{PS II con})}_{\text{low light}} - \frac{(\text{PS II nig})}{(\text{PS II con})}_{\text{high light}}$$

Other conditions as in Fig. 3. The average values and standard deviations were obtained from ten different preparations.

phosphorylation of LHC II (Fig. 1), a condition which decreases the antenna of PS II through the transfer of a fraction of the phosphorylated LHC II to the antenna of PS I [5]. So, we can conclude that the stimulation of energy transfer to PS II reaction centres by nigericin shown here (Fig. 1, Table 1), depends upon the removal of membrane-localized protons [3,4] and is not related to the energy distribution between the photosystems, but concerns the inhibition by H^+ of excitation energy transfer within PS II antenna.

The fact that the stimulation by nigericin in phosphorylated as well as non-phosphorylated thylakoids was observed also at high light intensity in the presence of low ATP concentrations (Figs. 1 and 2) can be satisfactorily explained on the basis of the well-known inhibition by ATP of proton leakage through the ATPase [9]. Such inhibition would decrease the lumenal pH, thus inhibiting $P680^+$ reduction [10] and PS II turnover. Nigericin prevents such inhibition (see Fig. 2). The inhibition of PS II turnover caused by low lumenal pH and its prevention by uncouplers can also be observed if the pH value of the medium is lowered [11]. Under such conditions the inhibition of PS II activity does not concern the excitation energy transfer to the reaction centre but rather the reduction of $P680^+$ [10]. Therefore, we measured the effect of nigericin addition on PS II activity at low as well as at high modulated light intensity in the rather narrow pH range between 7 and 8.1.

In the typical experiment represented in Fig. 3, stimulation of PS II activity upon nigericin addition at saturating light intensity was observed only below pH 8 and was larger at more acidic values.

This observation seems in agreement with the hypothesis used above to explain the effect of ATP, namely that when the lumen became acidic enough PS II turnover was inhibited, probably at the level of H_2O oxidation [10]. While the magnitude of the difference between the effect of nigericin at low and high light intensity seemed constant at pH values down to 7.5, below this value a larger effect of nigericin on PS II turnover was observed at saturating light intensity (Fig. 3 and Table 2).

The cross-over point (Fig. 3) was rather variable in different thylakoids preparations (Table 1), and its existence can be explained by thinking that the inhibition of PS II turnover rate by low lumenal pH is prevailing on the inhibition of excitation energy transfer by membrane localized protons (low light effect) at low pH values of the medium.

The absence under our conditions (low-frequency flashes of λ_2 and continuous beam of saturating intensity on PS I) of the photosynthetic control at the level of plastoquinol reoxidation (Table 3) confirmed that the inhibition by low lumenal pH was at the level of PS II turnover.

5. Conclusions

We interpret our observations to indicate that PS II electron transport activity can be regulated (inhibited) by proton translocation coupled to electron transport in two ways: (a), at the level of excitation energy transfer to the reaction centres, as previously reported [3,4]; (b), at the level of PS II photochemistry. The former inhibitory mechanism which we have indicated to be due to localized protons on the basis of the requirement for lipophilic uncouplers to remove it [4], could involve components of the antenna more closely linked to the reaction centre than 'mobile' LHC II [12] as shown in Fig. 1. Furthermore, it is not dependent on the cation regulated excitation energy distribution between the two photosystems, as shown here by the lack of influence of thylakoid stacking (Table 1). The latter mechanism of inhibition, (b), is suggested to be the effect of the acidification of the lumen which is known to inhibit P680 reduction [10].

It is relevant to note that the inhibition at the level of PS II photochemistry seems to require lower pH values in the lumen than the inhibition of the energy transfer to the reaction centre. It could then be suggested that after the onset of illumination the progressive accumulation of H^+ within the membranes and into the lumen causes firstly a change at the level of PS II antenna leading to an inhibition of the efficiency of excitation energy transfer to the reaction centre (see Refs. 3 and 4), followed by an inhibition of $P680^+$ reduction when the lumen is allowed to become excessively acidic [10].

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