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Regulation by localized protons of Photosystem II photochemical efficiency

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In a previous report (Ehrenheim et al. (1991) Biochim. Biophys. Acta 1059, 106-110) it was shown that membrane-localized protons inhibit energy utilization at the Photosystem II reaction centre. We report here that the stimulation of PS II energy utilization due to the removal of such protons by nigericin is dependent on the wavelength of the actinic modulated light. Maximal fluorescence is not affected by nigericin. The effectiveness of the different uncouplers is related to their lipophilicity. These observations led us to conclude that electron-transport-dependent localization of protons in the membrane lowers the efficiency of energy transfer to the PS II reaction centre. It is also shown that the effect of uncouplers on PS II is not related to inhibition of electron recycling around PS II or cation-dependent energy spillover.

Introduction

The primary photochemical reactions of PS I and PS II are known to generate an electric potential difference ($\Delta\psi$), negative outside, across the thylakoid membrane [1]. A gradient of protons across the membrane (ΔpH) is generated during electron transport at the steps of water oxidation and of plastoquinol oxidation [1], and the proton electrochemical potential is utilized in the process of ATP synthesis. More recently, evidence has been provided that localized proton domains within the thylakoid may be formed during electron transport and may be relevant to the synthesis of ATP (see Ref. 2 for review). The role of ΔpH and $\Delta\psi$ in regulating the rate of electron transport has been extensively studied (see Ref. 3 for review), whilst much less is known about their influence on the utilization of excitation energy at the reaction centres.

Braun and Malkin reported that uncouplers and $\Delta\psi$ inhibiting ionophores increase the imbalance in favor of PS II of the energy distribution between the two photosystems [4], and more recently a report from the same laboratory indicated that ADP plus P_i under conditions where the turnover of the ATP synthase is not inhibited, enhance the imbalance in favor of PS II [5]. In a previous paper [6] we have shown that PS-II-limited electron transport is stimulated at pH 8 by nigericin but not by NH_4Cl, while valinomycin and nonactin stimulate only at the high concentrations (in the micromolar range) needed to supress 9-aminoacridine fluorescence quenching, but not at the low concentrations (in the nanomolar range) sufficient to suppress $\Delta\psi$. Futhermore, it was shown that nigericin enhanced the $\Delta\psi$, indicating 518 nm absorbance change while increasing PS II turnover, whilst NH_4Cl had no such effect [6]. It was concluded that, due to localized proton domains generated within the membrane during electron transport, the efficiency of PS II photochemistry is lowered.

The observations reported here extend those conclusions. A number of amines are here reported to stimulate PS-II-limited electron transport from H_2O to NADP. We report that the effectiveness of amines is related to their lipophilicity. The action spectrum of this effect shows peaks at 475 and 650 nm. Furthermore, the PS II steady-state fluorescence was stimulated by the lipophilic uncouplers. Data are discussed in terms of membrane-localized protons controlling the efficiency of energy transfer to the PS II reaction centre.

Abbreviations: DCMU: 3-(3,4-dichlorophenyl)-1,1 dimethylurea; Q_a, the quinone primary acceptor of PS II; HA, hydroxylamine, $\Delta\psi$, electrical potential difference across the thylakoid membrane.

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Materials and Methods

Stroma-free thylakoids were prepared as previously described (7) from freshly harvested spinach leaves. The leaves were kept in darkness 2 h before extracting the thylakoids, to allow for the dephosphorylation of LHC-II by the endogenous phosphatase [8]. All buffers used contained 5 mM $MgCl_2$, to ensure the preservation of the granal structure. Steady-state NADP reduction was measured as previously described [9]. The reaction medium contained 5 mM $MgCl_2$, 10 mM NaCl, 10 mM NaF, 20 mM KCl, 0.1 M sucrose, 30 mM tricine, adjusted to pH 8 by adding NaOH. Chlorophyll concentration was 15 μg/ml unless otherwise stated. Actinic light, filtered through the appropriate filters, was provided by a xenon flashlamp producing single turnover flashes at the rate of 6/s (defined as λ_2) and was supplemented with a continuous beam of 722 nm (defined as λ_1). The intensity of the 722 nm beam was such as to saturate the PS I activity needed to keep Q_a oxidized (see Ref. 9). The rate of NADP reduction was measured in the steady state and was linear for several minutes. Emerson enhancement was calculated according to Myers [10] (see also Table I). Light intensity was attenuated by means of neutral density filters. All reactions were carried out at 20–22°C.

Fluorescence was measured simultaneously with electron transport using a PAM fluorimeter (Walz, Effeltrich, Germany). The low intensity measuring beam was provided by 1 μs LED flashes of 650 nm, modulated at 1.6 kHz, and produced no measurable NADP reduction. Chlorophyll concentration was measured according to Arnon [11].

Results

NADP reduction was measured at low intensity actinic light modulated at 6 flashes/s (λ_2) in the presence of a continuous beam of 722 nm (λ_1). Under these conditions nigericin, but not NH_4Cl, stimulated electron transport and Emerson enhancement (Table I). PS II fluorescence (indicated as F_{λ_2} in Table I) was stimulated by nigericin in the absence of the 722 nm beam.

The effect of nigericin as a function of light intensity is shown in Fig. 1. A rise to a maximum was observed, followed by a decline at higher energy flux, in agreement with our previous observations [6].

NH_4Cl and amines are known uncouplers of photosynthetic electron transport, and their effect is understood in terms of the free diffusion across the membrane of the unprotonated species, which then equilibrate with protons, so effectively eliminating the proton gradient [12]. McCarty and Coleman reported that the effectiveness of amines in inhibiting photosynthetic phosphorylation under saturating light intensity is related to their carbon chain length and their lipophilicity [13]. We have tested a number of amines for their capacity to stimulate the PS-II-limited NADP reduction. All amines were tested at the concentration needed to give the maximum rate of electron transport under saturating continuous light (Table II, last column), and were compared with nigericin 200 nM for their stimulation of PS II photochemistry and the fluorescence of PS II (F_{λ_2}). It is shown that the effect was related to the lipophilicity of the amines [14], the most effective being benzylamine (Table II). These observations are consistent with the previously reported idea [6] that proton domain(s) in the membrane control the availability of excitation energy to PS II.

Hydroxylamine, which is a known inhibitor of cyclic electron transfer around PS II [15], had no influence on the stimulation by nigericin (Table III). Fig. 2 shows the ratio of the velocity of PS II photochemistry in the presence and absence of nigericin as a function of the wavelength of the modulated light (λ_2) with constant background light of 722 nm. The intensities of modulated light were adjusted to give nearly equal electron transport rate before the addition of nigericin. The low rate of electron transport observed with the 722 nm beam only was subtracted to calculate the ratios of the reaction rates in the presence and absence of nigericin, represented on the ordinate. The spectrum of this effect (Fig. 2) shows peaks at 475 and 650 nm and a

TABLE I

Effect of uncouplers on NADP reduction and fluorescence parameters.

The modulated light (λ_2, 475 nm) intensity was 1.2 W/m^2. The 722 beam was 2.8 W/m^2. V_{λ_1}, $V_{\lambda_1+\lambda_2}$, V_{λ_2} reaction rates (reported as 10^{-3} absorbance change at 340–390 nm/min) under illumination with the 722 nm, 722 + 475 nm and 475 nm respectively. PS II was calculated as in Fig. 1. E_2 is equal to PS II/V_{λ_2}. F_0, minimal fluorescence (before actinic illumination); F_{λ_2}, steady state fluorescence measured under λ_2 excitation; F_m, maximal fluorescence obtained with DCMU 20 μM added with a saturating flash. (a) Control; (b) uncoupler added.

Additions	V_{λ_1}	$V_{\lambda_1+\lambda_2}$	V_{λ_2}	E_2	PS II	F_0	F_{λ_2}	F_m	PS II(b) / PS II (a)
None	4.35	13.2	6.2	1.42	8.8	11.2	19.8	45	–
Nigericin, 200 nM	4	15.8	6	1.96	11.8	11.2	22.8	45.3	1.33
NH_4Cl, 4 mM	3.6	12.6	6.2	1.44	9	11.5	19	45	1

TABLE II

Effect of different uncouplers on electron transport and fluorescence parameters

In columns 2 and 3, conditions and symbols were as in Table I. In column 4, high intensity continuous red light illumination-saturated electron transport, and ferredoxin concentration was 9 μM.

Uncoupler	PS II	$F(\lambda_2)$	Phot. control reaction rate uncoupled/cont.
None	11.5	18	–
NH_4Cl, 2 mM	11.3	18	3.6
Methylamine, 8 mM	13.1	20	3.9
Propylamine, 1 mM	13.9	–	3.9
Dibutylamine, 1 mM	15.1	21.5	3.7
Benzylamine, 1 mM	15	21.5	3.9
Nigericin, 200 nM	16.1	22	3.9

minor peak at 520 nm. Less effect was observed from 550 to 600 nm, while stimulation was seen up to 681 nm.

Finally, we observed that the increase of Mg^{2+} concentration (up to 25 mM), had no influence on the effect of uncouplers on PS II photochemistry and fluorescence $F\lambda_2$ (Table IV).

Discussion

The observations reported here extend our previous report [6], in which we showed that 'membrane-localized' protons inhibit energy utilization at the level of PS II in stroma-free thylakoids as indicated by the stimulation of NADP reduction by lipophilic uncouplers. All our experiments were performed in the presence of 5 mM $MgCl_2$ and a total monovalent cation (Na^+, K^+) concentration of 55 mM: a cation concentration sufficient to ensure the stability of grana and

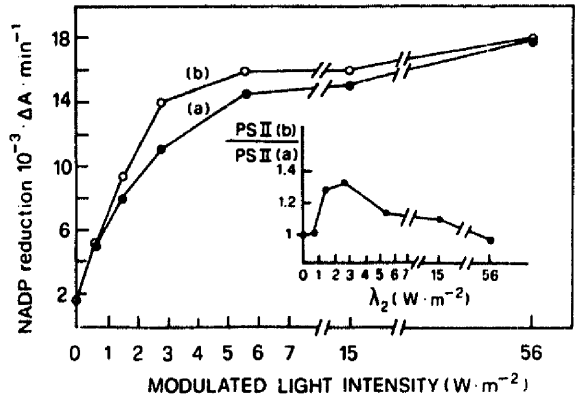

Fig. 1. Influence of nigericin on NADP photoreduction as a function of modulated light intensity. Conditions: as in Materials and Methods. The 722 nm (λ_1) beam was 2.8 W/m^2; λ_2 was 420–700 nm. Curve (a) control; curve (b) nigericin 200 nM. Inset: PS II activity was calculated as: $V_{\lambda_1+\lambda_2} - V_{\lambda_1}$.

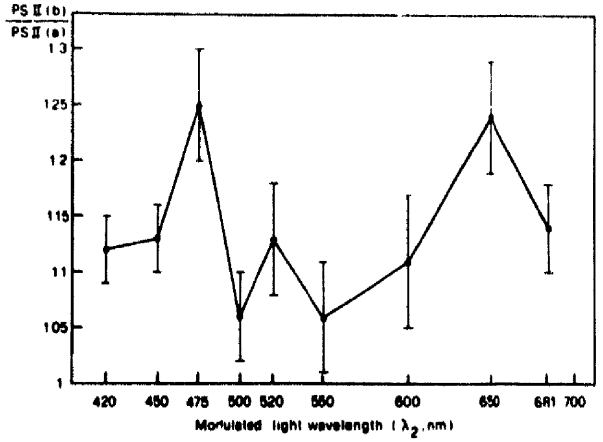

Fig. 2. Action spectrum of the stimulation by nigericin of PS II photochemistry. Conditions as in Fig. 1. The light intensity at the different wavelengths was chosen to give the same reaction rate in the control sample. The wavelength of the actinic light was selected by means of interference filters of 8–10 nm FWHM. The bars indicate the standard deviation. Average of data from ten thylakoid preparations.

the inhibition of PS II to PS I energy spillover [16]. Indeed, we observed that a further addition of Mg^{2+} (maximum total concentration up to 25 mM) did not enhance PS II activity, calculated at $V_{\lambda_1+\lambda_2} - V_{\lambda_1}$ (PS II), while increasing Emerson effect (see Table IV). Moreover, Mg^{2+} addition did not inhibit the effect of uncouplers on PS II energy utilization. We conclude, therefore, that the nigericin stimulation of PS II turnover under our conditions is unrelated to any modification of the PS II–PS I energy spillover. Our experimental conditions for the measurement of H_2O to NADP electron transport (low-frequency actinic illumination in the presence of a continuous 722 nm light) ruled out any effect of the uncouplers on secondary electron transport. The existence of a spectrum of the effect of nigericin (Fig. 2), its light intensity dependence (which is maximal at low and disappears at high light intensity, see Fig. 1), and the absence of effect on F_m (Table I) led us to think that we are dealing with some properties of PS II antenna. Furthermore, we did

TABLE III

Lack of influence of hydroxylamine on the stimulation of PS II by nigericin

Conditions as in Table I.

Addition	PS II	
None	8.5	$\frac{\text{PS II nigericin}}{\text{PS II control}} = 1.29$
Nigericin	11	
HA, 1 mM	7.8	$\frac{\text{PS II HA + nig}}{\text{PS II HA}} = 1.31$
HA, 1 mM + nig 200 nM	10.2	

TABLE IV

Influence of $MgCl_2$ on nigericin effect on PS II photochemistry

Conditions as in Table I.

Additions	F_{λ_1}	$F_{\lambda_1+\lambda_2}$	F_{λ_2}	F_{λ_2}	PS II	F_0	F_{λ_2}	F_m
None	4.5	15.3	7.7	1.39	10.8	16	26.5	63
Nigericin, 200 nM	4.5	17.3	7.2	1.77	12.8	16.2	30.2	63.5
$MgCl_2$, 20 mM	3.7	13.9	5.7	1.79	10.2	16	33.5	64.5
$MgCl_2$, 20 mM + nigericin, 200 nM	3.5	15.7	5.2	2.3	12.2	16.5	36.7	63

not observe any non-photochemical quenching, as currently experimentally defined [17,18], in the absence or presence of nigericin under our conditions (not shown).

As shown in Table II, the effect of uncouplers on PS II antenna is dependent upon their lipophilicity, while all uncouplers at the reported concentration were equally effective in stimulating electron transport under saturating continuous illumination. This observation and those reported above are interpreted to indicate that the stimulation of PS II photochemical activity is due to the removal of membrane localized protons accessible only to lipophilic uncouplers. Indeed, we show here that the stimulation of PS II by nigericin (and by the other lipophilic uncouplers) is accompanied by an increase of the steady state fluorescence excited by the actinic modulated light in the absence of PS I-absorbed background light. This would be expected if more energy were utilized by PS II and the redox state of Q_a were shifted towards a more reduced one with respect to the control (Table I and Table II). The energy flux being the same in both conditions, one can argue that some inhibiting mechanism has been removed by removing the localized proton gradient.

However, we cannot exclude other interpretations of the fluorescence data, since the omission of λ_1 has created a different condition respect to the electron transport measurement experiments. The possibility was considered that the observed effects of the lipophilic uncouplers might be due to inhibition of a cyclic electron transport around PS II which would compete for Q_a with NADP reduction. Two observations are inconsistent with such hypothesis. Firstly, the stimulation by nigericin of $NADP^+$ reduction was unaffected by the presence of hydroxylamine (Table III), a reagent known [15] to suppress the cyclic electron transport around PS II. Furthermore, according to such hypothesis, the stimulation by nigericin should be wavelength-independent, while the opposite was observed (Fig. 2). The data reported here, besides extending our previous results [6], confirm that the effect of localized protons does not concern the PS II primary photochemistry. Moreover, they suggest a modulation of the energy transfer efficiency either per se or in terms of different populations of PS II with different antenna properties, as the possible cause of the observed effect.

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