Activation of Alternative Electron Transfer in PS II by Inhibition of Proton Transfer at the Acceptor Side

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Abstract: Cd^{2+}, Zn^{2+} and Cu^{2+} have expressed effect on Q_B protonation and reduction in bacterial reaction centers (BRC) (Utschig *et al.*, 2001). This work is dedicated to investigating influence of Cd^{2+}, Pb^{2+}, Hg^{2+}, Zn^{2+}, and Cu^{2+} at the level of acceptor side of photosystem II (PS II). Light-dependent proton uptake decreased in the presence of 80 μM Cu^{2+} by 90%, and of 200 μM Zn^{2+}—by 70%. At that, considerable reductions of electron transport rate were not observed. In the concentration range of 50–200 μM Cu^{2+} and Cd^{2+} caused an insignificant growth of $[\text{PSII-}Q_B\text{-non-reducing}]_{rel}$, whereas in the presence of Zn^{2+} and Pb^{2+} this value grew more than by 25%. At all concentrations of ions of heavy metals the $[\text{PSII-}Q_B\text{-non-reducing}]_{rel}$ value did not exceed 60%. By their effect on dark relaxation of variable chlorophyll fluorescence, heavy metals used might be divided in two groups: (1) Cu^{2+} Hg^{2+}, accelerating the fast and medium components of fluorescence decay; (2) Zn^{2+}, Pb^{2+} and Cd^{2+}, slowing down medium and fast components. An assumption was made that blocking the reaction of Q_B protonation by ions of heavy metals activates the alternative acceptor site in PS II.

Keywords: *Spinacia oleracea*; Electron transport; Proton transport; Photosystem II; Q_B-non-reducing PS II centers

Introduction

By the latest data, regulatory contribution of the mechanism regulating the input of the energy of light into the photosynthetic apparatus at photosystem II (PS II) level often reaches the maximum long before reaching the saturating light intensity (Oxborough and Baker, 1997). Thus, at the saturating light intensity, the regulation of plastoquinone reduction is decisive for protecting PS II from photodamage, which can be provided by the induction of cyclic and non-cyclic electron-transport chains in PS II. Whereas the importance of the cyclic electron transport in PS II is beyond any doubts (Arnon and Tang, 1988), and even its stimulating conditions were characterized (Miyake and Yokota, 2001; Laisk *et al.*, 2006), non-cyclic way, in which, instead of plastoquinone, an alternative acceptor participates, and, probably, an alternative PS II active center, was poorly investigated. In some works its existence is proven (Barr and Crane, 1981; Arnon, 1995), but the conditions of its induction are not specified.

The aim of this work: to track the connection between suppression of proton transport in isolated chloroplasts of spinach and activation of alternative non-cyclic electron transport in photosystem II, using ions of cuprum and zinc as inhibitors.

Materials and Methods

Class “C” chloroplasts were isolated by modified method of Avron. Reaction medium contained sorbitol (100 mmol), NaCl (10 mmol), tris-HCl (10 mmol, pH 7.5) and chloroplasts equivalent to chlorophyll 10 μg/ml. Samples were preincubated for 5 min in dark. Actinic light was 1,000 μmol quanta/m^2s. Q_B reduction/protonation was followed by light-dependent

T. Kuang et al., *Photosynthesis Research for Food, Fuel and the Future*

proton uptake in unbuffered medium by glass pH electrode. Linear electron flow rate was estimated by light-dependent oxygen uptake in Mehler reaction with Clark type electrode. Estimation of ΔpH was conducted as in (Schuldiner *et al.*, 1972) by 9-aminoacridine fluorescence quenching analysis with the help of Xe-PAM fluorometer (“Walz”, HmbH). $[\text{PSII-Q}_B\text{-non-reducing}]_{rel}$ was determined as in (Tomek *et al.*, 2003). Analysis of Q_A^- dark reoxidation was conducted according to (Bukhov *et al.*, 2001).

Results and Discussion

To research electron transport in PS II under conditions of proton transfer suppression, we investigated the effect of Cu^{2+} and Zn^{2+} on: (1) light-dependent reaction of proton uptake; (2) light-induced transmembrane proton gradient (ΔpH), and (3) electron transport. It was established that light-induced proton uptake in thylakoid suspension is characterized by a high sensitivity to Cu^{2+} and Zn^{2+} (Fig. 1).

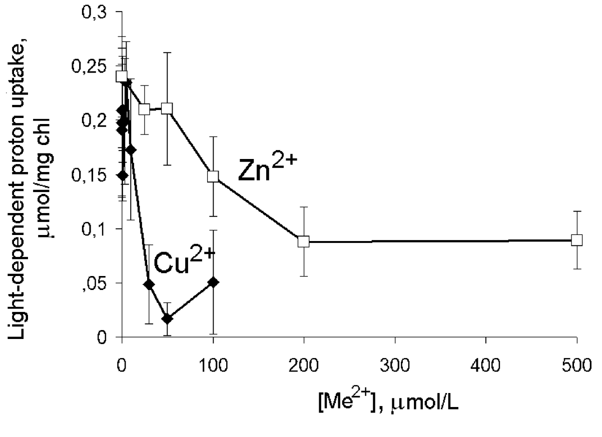


Fig. 1 The effect of Cu^{2+} and Zn^{2+} on light-dependent proton uptake.

Light-dependent proton uptake (ΔH^+) is associated with proton binding to the primary bound quinone Q_B, subsequent proton transfer into thylakoid membrane and protonation of membrane buffer groups with suitable pK values. Thus, the maximum value of light-induced proton uptake is determined by a number of intramembrane buffer groups (Walz *et al.*, 1974). Fig. 1 demonstrates that Cu^{2+} or Zn^{2+} addition results in ΔH^+ inhibition.

Zn^{2+} addition did not cause total suppression of proton uptake, which can be connected with the fact that a part of protons in Mehler’s reaction is bound at reduction of molecules of oxygen, and not Q_B. In practice, almost total suppression of light-dependent proton uptake by Cu^{2+} can be explained, to our mind, by their competing with methyl viologen and/or oxygen for the PS II acceptor site. In the presence of ions of cuprum in the concentration of 50 µM and of zinc—in the concentration of 200 µM their inhibiting action was reaching maximum, saturating value. Cuprum maximally suppressed this reaction in average by 91%, and zinc—by 63% (Fig. 1).

The nature of ΔpH concentration dependence substantially differed from the previous value (Fig. 2). The calculated ΔpH value lowered at the increase of the concentration of Cu^{2+}, as of Zn^{2+}; at that, similar to light-dependent proton uptake, it was inhibited in a greater extent by Cu^{2+}, as compared to Zn^{2+}. The dependence was approaching the linear one. Relative changes of ΔpH value remained in much narrower limits than light-dependent proton uptake under similar conditions. Thus, adding of 100 µM Cu^{2+} caused the decrease of ΔpH approximately by 14%, and of 100 µM Zn^{2+} caused the reductions by only about 3% from the control value. Whereas 200 µM Zn^{2+}caused the saturating inhibition of light-dependent proton uptake value by 63%, reductions of ΔpH value did not exceed 8%.

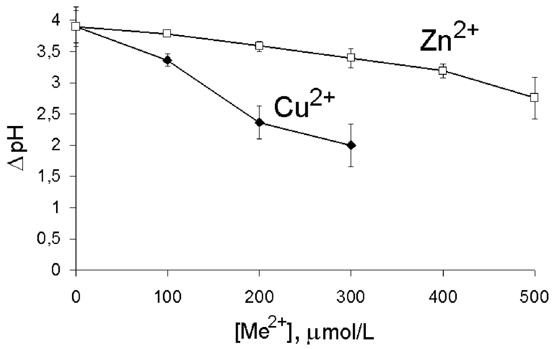


Fig. 2 The effect of Cu^{2+} and Zn^{2+} on light-induced ΔpH value.

Investigation results regarding the influence of growing concentrations of Zn^{2+} and Cu^{2+} on light-dependent oxygen uptake in Mehler’s reaction, as in control, as in uncoupled thylakoid membranes, are given in Fig. 3. Gramicidin A was used as uncoupler in the concentration of 0.5 µM. The uncoupling was accompanied by the acceleration of light-dependent oxygen uptake (pH 7.8), more than by 77%. Addition of 25 µM Zn^{2+} and Cu^{2+} caused additional stimulation (mean value more than 2 times higher than the control one), whereas in coupled chloroplasts such effect was not found.

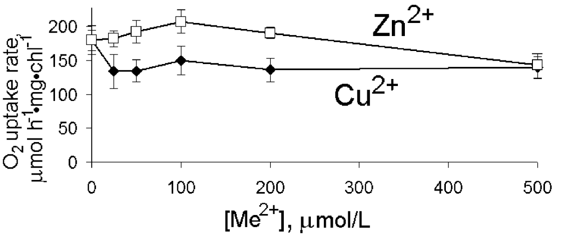


Fig. 3 The effect of Cu^{2+} and Zn^{2+}on light-dependent oxygen uptake rate in Mehler's reaction.

With the increase of concentration under conditions of uncoupling an increased inhibiting effect of these ions was observed. Lowering of the rate of light-dependent oxygen uptake at the increase of the concentration of these ions from 25 µM to 500 µM had quasi-exponential decay nature; at that, the action of ions of zinc was more expressed. At the concentration of 500 µM Zn^{2+} was suppressing this reaction by about 60% from the maximum value, and cuprum—only by 40%. Hence, Zn^{2+} affect the potential maximum rate of electron transport, not limited by ΔpH regulating influence, stronger than Cu^{2+}. These heavy metals represent effective inhibitors of electron transport under uncoupling conditions.

And in coupled chloroplasts, the activity of the abovementioned ions was quite different. Zn^{2+} in the concentration range of 50–200 µM manifested the tendency towards a weak stimulating influence. And Cu^{2+} effected inconsiderable inhibiting influence, starting from concentration of 25 µM. At the concentration of Cu^{2+} and Zn^{2+} of 500 µM , the rate of light-dependent oxygen uptake was decreased by 24% and 21%, correspondingly. At concentrations causing the saturating inhibition of light-dependent proton uptake—50 µM Cu^{2+} and 200 µM Zn^{2+}—light-dependent oxygen uptake in Mehler's reaction was suppressed only by 24% or was not reduced, correspondingly.

Thus, in the presence of 50 µM Cu^{2+} and 200 µM Zn^{2+} alternative electron transfer pathway at the level of PS II is induced which is accompanied by much lower proton uptake than the complete linear electron transfer.

The control value of [PSII-Q_B-non-reducing]$_{rel}$ in our investigations on isolated chloroplasts varied within rather wide limits – from 20% to 45%. Cu^{2+}, Cd^{2+} and Hg^{2+} caused an insignificant growth of the quantity [PSII-Q_B-non-reducing]$_{rel}$, whereas in the presence of Zn^{2+} and Pb^{2+} this value considerably increased (more than by 25%, Fig. 4).

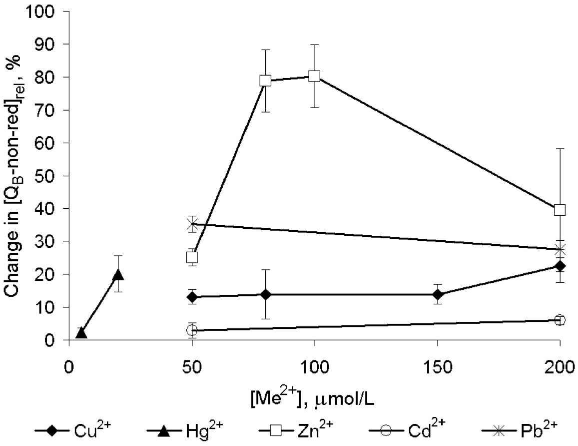


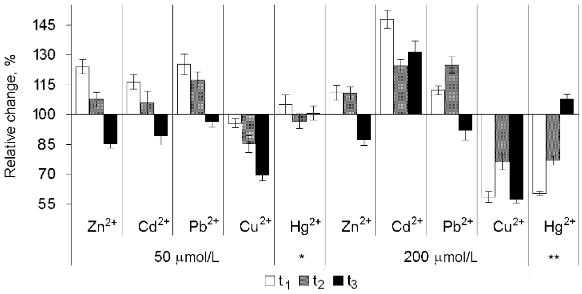
Fig. 4 Changes of the relative content of Q_B-non-reducing PS II centers after addition of ions of heavy metals.

Cd^{2+} caused the increase of [PSII-Q_B-non-reducing]$_{rel}$ value less than by 10%, their action in concentrations 50 and 200 µM did not differ. Cu^{2+} in concentration already of 50 µM caused the increase of the [PSII-Q_B-non-reducing]$_{rel}$ value approximately by 13%. At growing of concentration of ions of cuprum, the tendency towards a greater change of [PSII-Q_B-non-reducing]$_{rel}$ value was observed, which was clearly manifested in its proved growth by nearly 23% at the concentration of 200 µM.

Adding of only 20 µM Hg^{2+} caused a similar increase of [PSII-Q_B-non-reducing]$_{rel}$, as 200 µM of Cu^{2+}. Pb^{2+} more effectively, than Cu^{2+} and Cd^{2+}, increased [PSII-Q_B-non-reducing]$_{rel}$. At concentrations of 50 and 200 µM, they caused the increase of this value approximately by 35 and 28% against the control one, correspondingly. At the concentration of already 50 µM Zn^{2+} increased [PSII-Q_B-non-reducing]$_{rel}$ to 125% against the control value, at the concentration range of 80–100 µM—to 180%, and at the concentration of 200 µM this value was again decreased to 140% against the control one.

Results of the analyses of the decay of variable chlorophyll fluorescence are given in Fig. 5 in the form of relative changes of the time constants of each of three decay components, caused by the addition of ions of heavy metals, compared to the control one.

Zn^{2+} in the concentration of 50 µM slowed the first component more than by 20%, slowed the second one by 8% and accelerated the first one nearly by 15%. At that, the amplitude of the third component significantly increased—more than by 20%. At the concentration of 200 µM action of Zn^{2+} practically did not change. The inhibition of the first component even diminished and amounted to 8%. At the same time, the amplitude changes of the first and the third components strengthened and constituted −5% and +50%, correspondingly.



*- concentration 5 µM

** - concentration 20 µM

Fig. 5 Relative changes of the time constants of three fluorescence decay components (t_1, t_2 and t_3), caused by the addition of ions of heavy metals.

Cd^{2+} in the concentration of 50 µM caused the increase of decay time constant of the first component by 16% – and the decrease of decay time constant of the third component by 11%. At that, the amplitude of the third component increased by 15%. At the concentration of 200 µM Cd^{2+} caused the same amplitude changes of all three fluorescence decay components, just as Zn^{2+} at the similar concentration. At the same time, the increase of time constant of all three components was observed, the first—by 48%, the second—by 24% and the third—by 31%.

Pb^{2+} by their action also reminded the aforesaid heavy metals, and differed by a more expressed influence on the second fluorescence decay component. Thus, at the concentration of 50 µM they were increasing the time constant of the first component by 25%, the second—by 17%, diminishing the amplitude of the first component by 13% and increasing the third one by 80%. At the concentration of 200 µM Pb^{2+} were slowing the second component even by a greater measure (by 24%) as compared to the first one (by 12%). At that, the decrease of the second component amplitude by 27% was observed, and the increase of the third one by 123%. This way, Cd^{2+} and Pb^{2+} in the concentration of 200 µM effectively inhibited electron transfer at the acceptor side of PS II.

The action of Cu^{2+} and Hg^{2+} sharply differs from the action of the aforesaid heavy metals. Thus, Cu^{2+} in the concentration of 50 µM accelerated all the three decay components, at that, the third one—in the greatest measure (by 30%), the second one—by 15% and the first one—by 4%. At the concentration of 200 µM these ions accelerated the first and the third components by 42%, and the second – by 24%. The amplitude of the first, fastest components, which is the indicator of the electron transfer effectiveness, grew by 20% at the concentration of ions of cuprum 50 µM, and by 52%—at the concentration of 200 µM.

Hg^{2+} minutely influenced the rate parameters of fluorescence decay in the concentration of 5 µM, at that, they caused noticeable acceleration of the first (by 40%) and the second (by 23%) decay components at the concentration of 20 µM. The amplitude of the first component grew by 10% at the concentration of 5 µM, and by 44%—at the concentration of 20 µM. The second component, on the contrary, decreased by 16% at the concentration of 5 µM, and by 62%—at the concentration of 20 µM. Amplitude and rate changes of the third component under the influence of ions of mercury were insignificant.

By their influence, heavy metals used can be divided in two groups: (1) Cu^{2+} and Hg^{2+}, accelerating the fast and medium phases of fluorescence decay, increasing the amplitude of the fast phase at the cost of the medium and slow ones; (2) Zn^{2+}, Pb^{2+} and Cd^{2+}, which increase the amplitude of the slow phase and reduce the medium and fast ones.

These results suggest that alternative redox reactions in PS II are induced when Q_B reduction is suppressed by Cu^{2+} and Zn^{2+}. This effect may be caused by overreduction of the photosynthetic electron-transport chain, and may be one of the possible mechanisms that play an important role in photoprotection.

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