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CHLORORESPIRATION AND THE EFFECTS OF MITOCHONDRIAL OXIDASES INHIBITORS ON PLASTOQUINONE REDOX STATE IN THE ALGA *Mougeotia* sp. STRAIN AICB 560

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Abstract: Plastoquinone redox state, in the presence of mitochondrial respiration inhibitors, correlated with the activity of NAD(P)H-quinone oxidoreductase and with the evolution of photosynthetic O_2 have been studied. In the presence of DCMU, the kinetics reflects plastoquinone reduction at dark and the fractions of Q^- have increased. Mitochondrial inhibitors (NaN_3, SHAM, PG, rotenone) have inhibited plastoquinone oxidation at dark resulting the diminishing of available plastoquinone function of their specificity action, and the fraction of Q^- has increased. The dithiothreitol inhibited plastoquinone oxidation at dark. The partial plastoquinone reduction is accompanied by changes of initial fluorescence. The plastoquinone reduction due to plastidial **Ndh** complex was intensified in correlation with light intensity. Under the action of different intensities light the concentration of photosynthetic oxygen decreased in the majority of experimental variants. The sustained release of photosynthetic oxygen was registered under the action of propyl gallate, TTF, and rotenone in which the amount of oxygen increased.

Keywords: electron acceptors, fluorescence kinetic, NAD(P)H-quinone oxidoreductase, photosynthetic oxygen evolution, plastoquinone pool

Introduction

Chlororespiration has been defined as the interaction of respiratory electron transporters situated at mitochondrial level with the transporter chain of photosynthetic electrons located in the chloroplast thylakoids. The chlororespiration model of [3, 4] defines the changes in the plastoquinone redox state at dark and in the transthylakoidal potential detected in the absence of chloroplastic ATP-synthase. Plastoquinone reduction (PQ) takes place *via* NAD(P)H dehydrogenase, and the reoxidation involves the molecular oxygen, reaction mediated by a chloroplastic oxidase.

As a response to stress factors, the chlororespiration and the expression of **Ndh** complex are being intensified [5]. The photooxidative stress increases the nonphotochemical reduction of intersystemic electron transporters [6, 14]. The chlororespiration may play a photoprotective role in photosynthesis [11] by: dissipation of PS II over excitation; energy dissipation by the electron cyclic flow around PS I [12]; membrane protein resynthesis after photoinhibition [13]; activation of enzymes which breaks down the free radicals [6].

In this paper it was made an evaluation of plastoquinone redox state in the presence of mitochondrial respiration inhibitors correlated with the activity of NAD(P)H-quinone oxidoreductase and with the photosynthetic O_2 evolution.

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Materials and Methods

The green alga *Mougeotia* sp. Agardh (AICB 560) comes from the Culture Collection of Algae at I.C.B. Cluj-Napoca (AICB) [10]. The strain AICB 560 has been grown on Bold nutritive medium (BBM), in conditions of continuous stirring by air bubbling, continuous lighting of 400 $\mu mol.m^{-2}.s^{-1}s^{-1}$, at a temperature of 20°C.

Measurements of the amount of electron acceptors to PS II by fluorescence. The algal suspension was incubated at dark one hour with 3-(3,4-dichloropheny)-1,1'-dimethylurea (DCMU) before using for the complete oxidation of plastoquinone. For electron acceptors measurement was used the Bennoun's procedure [3] which sustain that the area delimitated by the fluorescence rise and its asymptote is proportional with the amount of electron acceptors available at PSII. In the presence of DCMU is possible only one charge separation per PSII reaction center and this is reflected by a fast fluorescence rise.

Excepting the control, all experimental variants were incubated with 3.27 μM DCMU before adding the specific inhibitors: sodium azide (1mM NaN_3), *n*-propyl gallate (1mM PG) salicylhydroxamic acid (1mM SHAM), thenoyltrifluoroacetone (10 μM TTF), 20 μM rotenone and dithiothreitol (20 mM DTT). The Q_A quantity of oxidized primary acceptors of PSII centers was estimated in the presence of DCMU by measuring the fluorescence rise area, and the reduced acceptor Q^-_A was calculated as $1-Q_A$ [2].

Determination of NAD(P)H-quinone oxidoreductase activity. The algal suspension was concentrated by filtration, was grind to disrupt the cell walls and was suspended in 50 mM phosphate buffer pH=6.6. The chlorophyll was eliminated with benzene, and the mixture was diluted with 50 mM Tris-HCl buffer pH=7.8, containing 10 mM $MgCl_2$, 10 mM NaCl, 0.1 M sorbitol, 0.5 mM phenylmethylsulfonyl fluoride (PMSF), 5% glycerol, 1% Triton X-100, and then was used for enzyme activity determination. The reaction medium contains 3 ml of cellular extract in Tris-HCl buffer, 200μM NAD(P)H, and 120 μM duroquinone. The NAD(P)H-quinone oxidoreductase oxidation at dark was spectrophotometrically registered by the absorption diminishing at 340 nm. Enzyme activity was reported in μmol/min/mg chlorophyll *a*, using an extinction coefficient of 6.23 mM^{-1} cm^{-1}.

Hill reaction. The measurement of photosynthetic oxygen evolution was made by measuring the reduction rate of electron artificial acceptor 2,6-dichlorophenolindophenol (DCPIP) in the presence of specific inhibitors. The filaments of *Mougeotia* were filtrated and roughly stirred in a Blender for cell disruption and for chloroplasts releasing. This material was suspended in phosphate buffer containing: 50 mM buffer phosphate pH = 7.0, 1mM L-ascorbic acid, 1 mM EDTA, 0.1M sorbitol and 1% PMSF. Absorption modification per hour was calculated on the basis of regression coefficient, and the released O_2 was correlated with chlorophyll concentration.

Chlorophyll fluorescence analysis. The chlorophyll fluorescence was measured with PAM-210 fluorometer as described by Schreiber *et al.* [22]. Fluorescence parameter and quenching analysis were done by saturation pulse method. The quantum yield of photochemical energy conversion was determined by the equation Yield = $\Delta F/F'_M$, and the ratio F_V/F_M ($F_V/F_M = F_M-F_0/F_M$) is expressing the photochemical quantum yield of the closed PSII reaction centers. The fluorescence parameters of algal suspensions grown in normal conditions were used as control sample.

Results and Discussion

The effects of specific inhibitors on the oxidation and reduction at dark of plastoquinone

The chlorophyll fluorescence kinetics *in vivo*, measured by the illumination of *Mougeotia* sp. cells adapted to dark and treated with DCMU, is presented in Fig. 1. In the presence of DCMU which breaks down the link between Q_A, Q_B and plastoquinone pool, the kinetics reflects

the plastoquinone reduction at dark under chlororespiration pressure. The plastoquinone amount have decreased at 23,6% comparing to the control. The ratio between areas showed the existence of two molecules of electron acceptors per active PSII reaction center. The fraction of acceptors in the reduced state (Q^-_A) has increased at 89% in the presence of DCMU (Fig. 1). The lifetime of Q^-_A in the DCMU presence has increased [20].

To highlight the catalytic reaction involved in the *in vivo* plastoquinone oxidation, it was tested the effect of mitochondrial oxidases inhibitors in the DCMU presence (Fig. 1). Thus, the salicylhydroxamic acid (SHAM), considered as an inhibitor of mitochondrial alternative oxidases, has inhibited the plastoquinone oxidation at dark. The available plastoquinone has diminished at 78.2%, and the molecular ratio has revealed the existence of 1.3 molecules of electron acceptors per active reaction center with a proportion of Q^-_A of 62.4%. SHAM led to the increase of plastoquinone reduction due to the inhibition of mitochondrial respiration [8].

Propyl gallate (PG), inhibitor of mitochondrial alternative oxidases, has inhibited the plastoquinone oxidation at dark, decreasing the available electron acceptors at 18.2%, and respectively to 2.7 acceptor molecules per PSII active reaction center. The Q^-_A fraction has risen to 91.2% (Fig. 1). The complete reduction of plastoquinone highlights that the redox state of plastoquinone at dark is controlled as following: the reduction is controlled by stromatic reducers which redox state depends on metabolic and mitochondrial activity, and the oxidation is subjected to propyl gallate-sensitive oxidase [8].

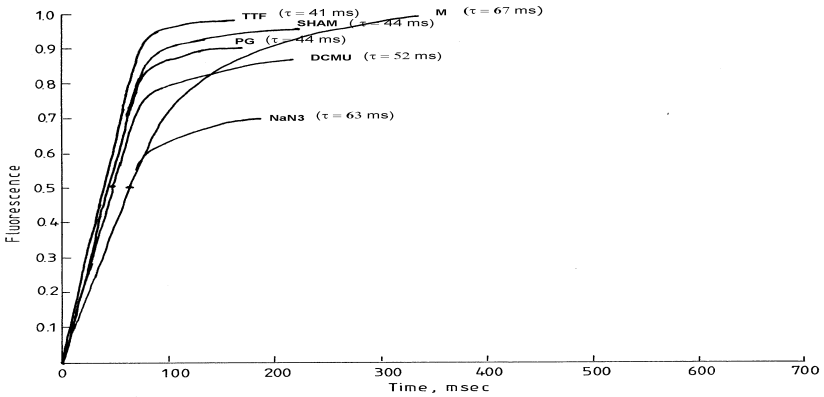

Fig. 1: The chlorophyll fluorescence kinetics in *Mougeotia* sp. Area interval = 700 ms; M=dark-adapted cells (control). Cells treated with 3,27 μM DCMU and then inhibited for 4 minutes at dark with: 1 mM SHAM, 1 mM PG, 1 mM NaN_3, 10 μM TTF, τ = half time

Sodium azide (NaN_3), inhibitor of mitochondrial *c*-cytochrome oxidase, has reduced at dark the plastoquinone pool at 16.4%, respectively to 3 molecules of electron acceptors per active reaction center, and the Q^-_A fraction increased at 92% (Fig. 1). Our results regarding the effects of SHAM, PG and NaN_3 are comparable with those obtained by Bennoun [3].

Thenoyltrifluoroacetone (TTF) is an inhibitor of diaphorase activity of ferredoxin-$NADP^+$ oxidoreductase (FNR) without affecting the NDH-dehydrogenase activity. Guedeney *et al.* [14] showed that the FNR flavoprotein is bound to the majority of **Ndh** polypeptides within thylakoids, and thus it was suggested that the FNR within thylakoids may be functionally equated with the NADH oxidizing domain existing in complex I. Teicher şi Scheller [26] brought sufficient arguments against FNR intervention in **Ndh** activity. In the presence of TTF, at dark, the available electron acceptors have diminished at 45.4% comparing with the control. The ratio of 2.2 between areas revealed the presence of a single plastoquinone molecule per reaction center. The electron acceptor fraction in a reduced state (Q^-_A) was of 78% (Fig. 1).

Rotenone is a classical inhibitor of mitochondrial complex I, acting on Fe-S reduced centers of ubiquinone. It was observed that rotenone inhibited the plastoquinone oxidation at dark, diminishing the available electron acceptors at 63.6% comparing with the control. The 1.6 ratio between fluorescence areas highlights the existence of 0.8 molecules of electron acceptors per reaction center. The amount of electron acceptors in a reduced state (Q^-_A) was of 69.4% (Fig. 2). The inhibition of plastoquinone oxidation at dark by rotenone is explained by the inhibitory action on NAD(P)H oxidation, confirming that this enzyme activity is of complex I type [26].

Dithiothreitol (DTT) was proved to be an *in vivo* and *in vitro* inhibitor of violaxanthine de-epoxidation, contributing to the increase of Q_A reduction by the diminishing of photochemical coefficient (q_p) facilitating thus the photoinhibition [19]. The decrease of available zeaxanthin as a consequence of the inhibitory effect of DTT stimulates the sensitivity to photodamages. Also DTT possess additional effects *in vivo* on carbon metabolism enzymes, especially on thioredoxin regulated reactions, and on ATP synthesis [23]. In the presence of DTT was inhibited the plastoquinone oxidation at dark, decreasing the available electron acceptors at 32.7%, respectively 1.5 molecules of acceptors per reaction center. The amount of acceptors in reduced state (Q^-_A) increased at 84% (Fig. 2). The specific inhibitors may block the plastoquinone pool oxidation at dark, and also the various species of algae may respond differently to inhibitors [1].

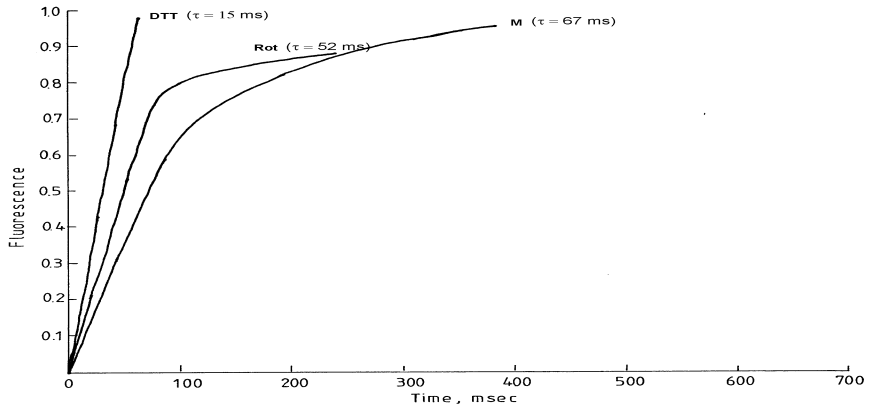

Fig. 2: The chlorophyll fluorescence kinetics in *Mougeotia* sp. The area interval= 700 ms. M=dark-adapted cells (control). Cells treated with 3,27 μM DCMU and then inhibited for 4 minutes at dark with: 20 μM rotenone, 20 mM DTT, τ = half time

The half time in dark conditions (τ) highlights two important phenomena: the recording of fluorescence rise is dependent of plastoquinone amount, and the slope of this rise is in relation with the quantity of electrons arrived at plastoquinone. It may be observed that the sodium azide and the rotenone permit the entrance of an increased electron number through plastoquinone system, reducing the slope of fluorescence rise. PG and SHAM are acting similarly, and DTT is limiting the electron entrance in the transporter chain *via* plastoquinone, conducting to the increase of fluorescence rise slope (Fig. 3).

The partial reduction of plastoquinone pool was accompanied by changes in the initial fluorescence (Fig. 4). Thus, the minimum and maximum fluorescence have increased in the majority of experimental variants, more striking being the case of PG treatment. The photochemical efficiency (F_V/F_M), the quantum yield, and the efficiency of photochemical quanta conversion (q_P) have decreased; in a parallel manner has diminished the variable fluorescence, fact denoting that photoinhibition took place conducting to the closure of PSII reaction centers.

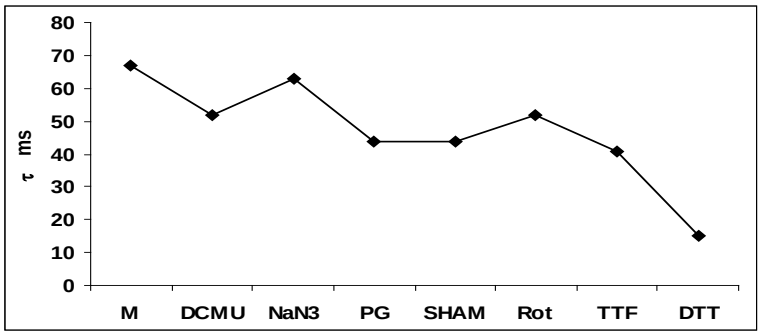

Fig. 3: The evolution of chlorophyll fluorescence half time related to the effects of specific inhibitors

Among the inhibitors, SHAM has reduced the initial fluorescence. PG and rotenone have increased the initial fluorescence, but have diminished the efficiency and the quantum yield. Due to the fact that all experimental variants contain DCMU which blocks the photochemistry of PSII centers, the contribution to the chlorophyll fluorescence should be attributed mainly to PS I activity.

The increase of plastoquinone reduction is due to chlororespiration intensification. F_M increased as a consequence of Q_A reduction, conducting to the increase of the ratio F_0/F_M which is a measure of Q_A reduction proportion. The plastoquinone redox state regulates the states transition resulting out from the reversible organization of light harversing complexes with PS II reaction centers. In the DCMU presence plastoquinone reduction is made by chlororespiration electron transport reactions, inducing the 2 state of low fluorescence, because LHCs are dissociated of PS II. The illumination is oxidizing the plastoquinone by the intermediate of PS I, and the fluorescence level attains the 1 state when LHCs are reassociated with PS II [18].

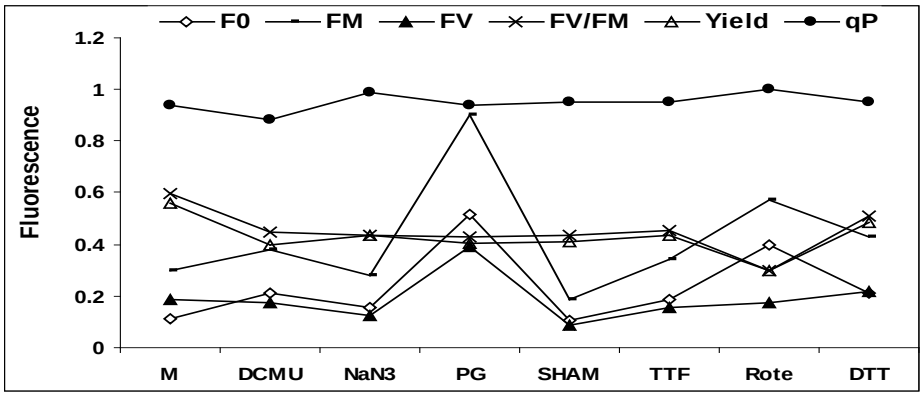

Fig. 4: The evolution of chlorophyll fluorescence under the action of specific inhibitors in the condition of plastoquinone oxidation at dark

The estimation of NAD(P)H-quinone oxidoreductase activity

The chlororespiration involves a sequence of reactions catalyzed by **Ndh** complex which can reduce the plastoquinone *via* NADH and a terminal oxidase, and the reduced plastoquinone is then reoxidized in the same process. An equimolar reaction takes place between NADH and plastoquinone [6].

Ndh in chloroplasts plays a role in the cyclic transport of electrons, the **Ndh** complex bound to thylakoidal membrane mitigating the electron donation from stromatic NAD(P)H to plastoquinone. The **Ndh** contribution to the various metabolic paths is different between species the growth conditions [26].

The plastoquinone reduction activity due to NAD(P)H-quinone oxidoreductase function of **Ndh** complex at the plastidial membrane level has been intensified in correlation with light

intensity (Fig.5). Using duroquinone as substrate, an analog of quinones, was assured the physiological specificity of the enzyme. The function of this NAD(P)H-dehydrogenase in plant cell is the reduction of plastoquinone at plastoquinol through an e^- donation, process which prevents the synthesis of semiquinones and of oxygen free radicals [25]. In the presence of DCMU the activity of this dehydrogenase decreases comparing with control. Low levels of enzyme activity are registered in the presence of sodium azide, propyl gallate and thenoyltrifluoroacetone, and increases appear under the action of salicylhydroxamic acid, rotenone and dithiothreitol.

Rotenone is an inhibitor of mitochondrial type-1 primary oxidase which catalyses electron transfer from NADH to quinone, and is involved in transmembranar proton translocation. The increase of F_0 was inhibited by rotenone, and the reduction rate of plastoquinone at dark decreased, fact suggesting that NAD(P)H-dehydrogenase is the main entrance point for electrons from NAD(P)H to plastoquinone [26]. The electrons are conducted by a quinol-oxidase sensitive to propyl gallate and insensitive to SHAM. The oxidase is immunological similar with a plastidial protein involved in carotenoids biosynthesis [7].

The thenoyltrifluoroacetone – an inhibitory of mitochondrial electron transport chain, is not inhibiting light dependent NADH oxidation, but inhibits the FNR diaphorase activity mediated by duroquinone, as well as NAD(P)H oxidation mediated by duroquinone [26].

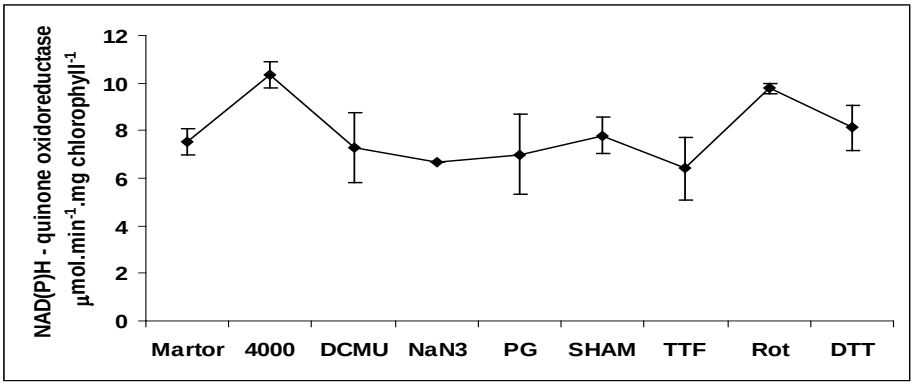

Fig. 5: The NAD(P)-quinone oxidoreductase activity of algal suspensions under light and different inhibitors action

Fluorescence increase after illumination is based on plastoquinone reduction by NAD(P)H or other reducers accumulated as a consequence of illumination, and implies the cyclic transport of electrons around PS I. Type 1 NAD(P)H dehydrogenase (NDH-1) was high in the algae grown in light [9]. The NAD(P)H-dehydrogenase complex codified in plastids is involved in the nonphotochemical reduction of plastoquinone [17].

The photosynthetic oxygen evolution

The isolated chloroplasts preserve the feature of oxygen releasing, phenomenon called the Hill reaction, belonging to photosynthesis photochemical reactions associated with PS II, which intermediates the using of electrons resulted from water photolysis for acceptors reduction. In living organisms, these electrons ultimately reduce $NADP^+$ to NAD(P)H.

In the presence of an artificial electron acceptor as 2,6-dichlorophenolindophenol (DCPIP) the electrons are captured by photosynthetic transport chain when chloroplasts are exposed to light. The blue oxidized form (quinone) of DCPIP reduces (phenolic compound) and becomes colorless. This reaction progress may be recorded by absorbance change at 600 nm of DCPIP solution. The extent of color change is proportional with the number of transfered electrons, respectively with the rate of photosynthetic electron transport.

The evolution of photosynthetic oxygen under the action of different light intensities and in the presence of inhibitors is presented in Fig. 6. Under the light intensity of 1500 μmol.m^{-2}.s^{-1} the concentration of photosynthetic oxygen decreases in the majority of experimental variants. Making abstraction of the fact that a part of inhibition is due to DCMU, with which all experimental variants were treated to block the PS II activity, can be estimated the individual effects of each inhibitory. The respiration inhibition becomes sensitive to DCMU, the cyclic phosphorylation being implicated – responsible for the external formation of ATP [21]. Sodium azide and rotenone inhibits the evolution of photosynthetic oxygen, more negative effects being recorded in the case of salicylhydroxamic acid. The sustained release of photosynthetic oxygen was registered under the action of propyl gallate and TTF, were the amount of oxygen increased.

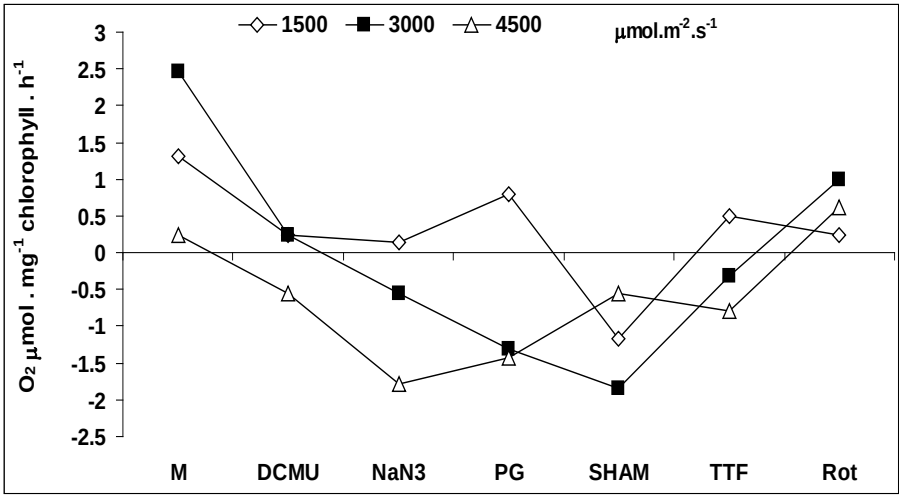

Fig. 6: The evolution of photosynthetic oxygen under the light and specific inhibitors influence

Under the light intensity of 3000 μmol.m^{-2}.s^{-1} the amount of photosynthetic oxygen dramatically decreases, excepting the case of experimental variant treated with rotenone were positive values were obtained. A similar evolution was also registered under the light of 4500 μmol.m^{-2}.s^{-1}. DTT, being a strong reducer, didn't permit the Hill reaction to take place because of the spontaneous reduction of DCPIP. Depending on the physiological state of the algae, the illumination induced a transitory and rapid inhibition of oxygen, due to chlororespiration inhibition [8]. Function of light intensity, our results are in agreement with those of Cournac *et al.* [7]. The oxidase which catalysis the plastoquinol oxidation and oxygen reduction to water is present in thylakoids. Because is sensitive to propyl gallate, this oxidase is thought to be involved in chlororespiration. The sensitivity of photosynthetic flow to mitochondrial respiration inhibitors is indirectly due the metabolic changes produced by mitochondrial inhibitors.

The photosynthesis response to light is fundamental for a good understanding of photochemical efficiency. The obtained negative concentrations of photosynthetic oxygen are due to Kok effect. The linear response of photosynthesis to light is rapidly changing in the vicinity of compensation point, so that the quantum yield decreases when the radiation increases and is due to the progressive inhibition of respiration by light. The Kok effect is an indicator of respiration light induced suppression – the paths of respiratory metabolism continue to operate in the presence of light, but at reduced rate [24]. The degree of photosynthesis inhibition by respiration is maximum at a medium activity of respiration and is implicated the PS I together with the cyclic phosphorilation. The ATP produced in photosynthesis is consumed in cytoplasm during illumination period instead of ATP produced in the cyclic phosphorilation – this means a stoichiometric ratio between ATP and NAD(P)H [21]. DCMU inhibits the O_2 evolution but does not affect the light dependent O_2 exchange, thus the Kok effect still remains. The preferential activity of PS I intensifies the Kok effect, and the cyclic phosphorylation conducted by PS I may

raise the ATP/ADP ratio sufficiently to limit the transport of respiratory electrons. The PS I activity may change the respiratory reducers flow guiding them toward the photosynthetic electron transport [16]. The Kok effect and the state transitions at light are the manifestation of the absorption photodepression of PS I dependent respiratory O_2. The Kok effect inhibits the chlororespiration because the electron flow is deviated from PS I [18].

Conclusions

By treating cells with DCMU, the kinetics reflects the plastoquinone reduction at dark by chlororespiration stromatic reducers. The available plastoquinone pool had decreased at 23,6%, and the fraction of reduced quinone acceptors (Q^-_A) had increased at 89%.

The mithocondrial inhibitors had inhibited the plastoquinone oxidation at dark, diminishing the available plastoquinone function of their specificity, and the fraction of reduced acceptors (Q^-) had increased. The inhibition of plastoquinone oxidation at dark is explained by the inhibitory action on NAD(P)H oxidation, confirming this enzyme activity as being of complex I type.

Dithiothreitol, an *in vivo* and *in vitro* inhibitory of violaxanthine de-epoxilation, inhibited the plastoquinone oxidation at dark, decreasing the available electron acceptors at 32.7%, respectively 1.5 molecules of acceptors, and the amount of reduced acceptors (Q^-_A) increased at 84%.

It was observed that the partial reduction of plastoquinone pool was accompanied by changes in the initial fluorescence. The increase of plastoquinone redox state is due to chlororespiration intensification. In the presence of DCMU, when anaerobiotic conditions are created, and the complexes LHCs and PS II are dissociated, the plastoquinone reduction is mitigated by chlororespiration electron transport. In the DCMU presence, the acceptors pool becomes mostly deoxidized in few seconds at dark, due to the cyclic flow of electrons around PS I in which the **Ndh** complex is implicated too.

The plastoquinone reduction activity due to NAD(P)H-quinone oxidoreductase function of **Ndh** complex at the plastidial membrane level was intensified in correlation with light intensity. Low levels of enzyme activity were registered in the presence of sodium azide, propyl gallate and thenoyltrifluoroacetone, and increases appeared under the action of salicylhydroxamic acid, rotenone and dithiothreitol. The increase of F_0 was inhibited by rotenone, and the reduction rate of plastoquinone at dark decreased, fact suggesting that NAD(P)H-dehydrogenase is the main entrance point for electrons passing from NAD(P)H to plastoquinone.

Under the action of light of different intensities the concentration of photosynthetic oxygen decreased in the majority of experimental variants. The sustained release of photosynthetic oxygen was registered under the action of propyl gallate, TTF, and rotenone were the amount of oxygen increased.

The negative concentrations of photosynthetic oxygen are due to Kok effect which shows that in the vicinity of compensation point the quantum yield decreases when the radiation increases and is due to the progressive inhibition of respiration by light. The inhibitors affecting the photosynthesis and respiration do not inhibit the Kok effect.

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CLORORESPIRAŢIA ŞI EFECTELE INHIBITORILOR OXIDAZELOR MITOCONDRIALE ASUPRA STĂRII REDOX A PLASTOCHINONEI LA ALGA *Mougeotia sp.* TULPINA AICB 560

(**Rezumat**)

Clororespiraţia s-a studiat prin evaluarea schimbărilor în starea redox a plastochinonei la întuneric în prezenţa inhibitorilor respiraţiei mitocondriale în corelaţie cu activitatea NAD(P)H-chinonă oxidoreductazei şi evoluţia O_2 fotosintetic. Suspensia de algă verde *Mougeotia sp*. care provine din Colecţia de culturi de alge a I.C.B. Cluj-Napoca (AICB) s-a incubat la întuneric o oră cu DCMU (inhibitor al transportului fotosintetic de electroni) pentru oxidarea completă a plastochinonei apoi s-au aplicat diferiţi inhibitori *in vivo* pentru măsurarea cantităţii de acceptori de electroni ai PS II în stare oxidată şi redusă pe baza cineticii fluorescenţei clorofilei. Astfel, acidul salicilhidroxamic (SHAM), n-propyl gallate (PG) şi azida de sodiu (NaN_3), inhibitori ai oxidazelor mitocondriale alternative, au inhibat oxidarea la întuneric a plastochinonei determinând mărirea stării reductive a aceteia datorită inhibării mitorespiraţiei. Tenoiltrifluoroacetona (TTF), inhibitor al activităţii diaforazice a feredoxin-$NADP^+$ oxidoreductazei (FNR), precum şi rotenone, inhibitor clasic al complexului I mitocondrial, au acţionat negativ asupra oxidării la întuneric a plastochinonei scăzând disponibilul de acceptori de electroni ai PS II. Ditiotreitolul (DTT), inhibitor *in* vivo şi *in* vitro al de-epoxidării violaxantinei, a inhibat oxidarea la întuneric a plastochinonei contribuind la creşterea stării reductive. Activitatea de reducere a plastochinonei datorită complexului **Ndh** de la nivelul membranei plastidale s-a intensificat în corelaţie cu intensitatea luminii şi sub acţiunea acidului salicilhidroxamic, rotenone şi ditiotreitolului. Creşterea fluorescenţei a fost inhibată de rotenone, a scăzut rata reducţiei plastochinonei la întuneric ceea ce a indicat că NADPH-dehidrogenaza este principalul punct de intrare a electronilor de la NAD(P)H la plastochinonă. Evoluţia oxigenului fotosintetic s-a corelat cu schimbările în starea redox a plastochinonei. Ca urmare a rezultatelor, reducerea plastochinonei în prezenţa inhibitorilor se face pe cheltuiala electronilor din clororespiraţie.