Contribuţii Botanice, XLI, (1), **2006**
Grădina Botanică "Alexandru Borza"
Cluj-Napoca

CHLORORESPIRATION AND THE KINETICS OF PLASTOQUINONE POOL REOXIDATION IN THE PRESENCE OF MITOCHONDRIAL RESPIRATION INHIBITORS IN THE ALGA *Mougeotia* sp. STRAIN AICB 560

Victor BERCEA[1], Cătălina VASILESCU[1], Bogdan DRUGĂ[1], Nicolae DRAGOŞ[2]
[1] Institutul de Cercetări Biologice, str. Republicii, nr. 48, **RO-400015 Cluj-Napoca**
[2] Universitatea "Babeş-Bolyai", Facultatea de Biologie şi Geologie, str. Clinicilor, nr. 5-7, **RO-400006 Cluj-Napoca**
e-mail: bercea_victor@yahoo.com

Abstract: The involvement of chlororespiration electrons in plastoquinone reoxidation under the action of photosynthetic and mitochondrial electron transporter chain inhibitors was studied in the green algae. Under the action of DCMU, 32.7% of plastoquinol pool was reoxidized. The plastoquinol reoxidation has diminished 25% in the presence of sodium azide. It was established that 72.7% of plastoquinol pool was reoxidized in the presence of salicylhydroxamic acid and n-propyl gallate, while 38.2% in the presence of thenoyltrifluoroacetone. In the presence of dithiothreitol the amount of plastoquinol reoxidized at dark was of 40%. In the condition of plastoquinol reoxidation at dark following light reduction takes place the change in initial fluorescence. The minimum and maximum fluorescence are maintained high, along with the diminishing of the efficiency and the quantum production. Propyl gallate makes an exception, which increased the initial fluorescence, and the salicylhydroxamic acid has inhibited it respectively. The increase of F_0 in light-dark transition results from the accumulation of Q^-_A as a consequence of plastoquinone reduction at dark. The clear reoxidation of Q^-_A has diminished due to the reduced state of plastoquinone at dark.

Keywords: DCMU, fluorescence kinetic, mitochondrial respiration inhibitors, plastoquinone pool, reoxidation

Introduction

The photosynthesis reactions at light encompass the transport of photosynthetic electrons between the two photosystems PSI and PSII, involving different carriers at the level of chloroplasts thylakoids. The plastoquinone (PQ), involved in this transport, is reduced by NAD(P)H dehydrogenase and is reoxidized on the account of the molecular oxygen, reaction mediated by a chloroplastic oxidase [1, 2, 3, 4]. The electron transfer from respiratory chain to oxygen *via* NAD(P)H is elecrogenic, being thus responsible for the permanent membrane potential.

Small quantities of chloroplastic homologues of the mitochondrial NADH dehydrogenase were found in thylakoids [14]. The stimulation of oxygen absorption was observed after lighting at *Chlamydomonas reinhardtii* mutants lacking the PSII reaction centers. This absorption was attributed to the presence of plastoquinol: oxygen oxidoreductase sensitive to n-propyl gallate [7], an inhibitor of alternative mitochondrial oxidases [12]. The chloroplastic complex NAD(P)H-dehydrogenase represents a part of an essential metabolic path in certain conditions of stress [9, 17].

The activity of **Ndh** complex can be monitored by the transitory increase of chlorophyll fluorescence after light exposure [6, 13, 16].

Research supported by CERES Program nr. 4-10/2004

Studying the chlororespiration in the green algae by monitoring the chlorophyll fluorescence, in this paper was aimed the emphasis of plastoquinone reoxidation in the conditions of photosynthetic and mitochondrial transporter chain inhibitors.

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) [8]. 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 DCMU before using, fact that permitted 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 per active reaction center of 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 amount of Q_A 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 $1\text{-}Q_A$.

Chlorophyll fluorescence analysis

The chlorophyll fluorescence was measured with PAM-210 fluorometer as described by Schreiber *et al.* [15]. Fluorescence parameters and quenching analysis were assayed 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 ($F_V/F_M = F_M\text{-}F_0/F_M$) 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 samples were exposed to saturable light intensities in the presence of DCMU to induce the complete oxidation of plastoquinone (PQ) and then incubated at dark for 30 s to permit the partial oxidation of plastoquinol ($PQ\text{-}H_2$). Without the PSII activity, inhibited by DCMU, and in the presence of PSI activity, the $PQ\text{-}H_2$ pool becomes rapidly oxidized.

The reoxidation of plastoquinol pool at dark following the light reduction is presented in Fig. 1. The kinetics of chlorophyll fluorescence induction was determined on *Mougeotia* sp. filaments, as follows: M_1 – dark adaptated cells, and M_2 – cells preilluminated for 700 ms and incubated 30 ms at dark before the second illumination for fluorescence recording. The M_1 fluorescence rise reflects the complete reduction of plastoquinone pool induced by light. After 30s of dark adaptation for permitting plastoquinol oxidation, was applied the second saturation pulse which reduced the remained plastoquinone, as it is revealed by the M_2 fluorescence rise.

The area above the fluorescence rise expresses the amount of available plastoquinone pool. By comparing the two areas it is found that 43% of plastoquinol pool was reoxidized in the dark period. This reoxidation of plastoquinol was made through the oxygen consumption in the chlororespiration process [3].

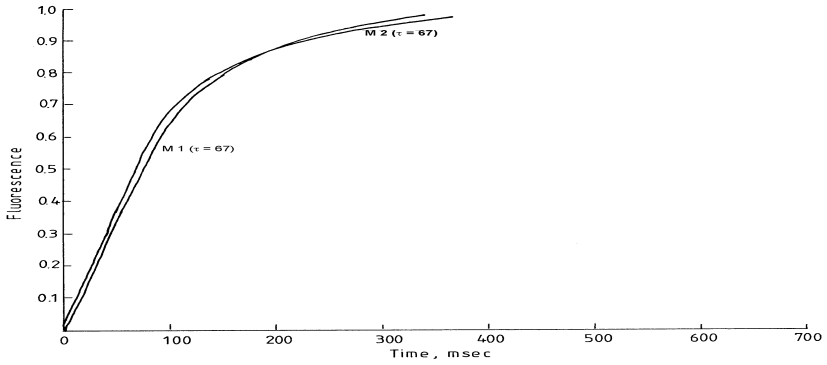

Fig. 1: The plastoquinol reoxidation at dark following the light reduction.
M_1 = dark adaptated cells; M_2 = cells preilluminated for 700 ms and then incubated 30 seconds at dark; τ = half time

It was previously established that the inhibitors of mitochondrial respiration, in general, induce the plastoquinone reduction at dark [5]. It was considered that these inhibitors determine the decrease of ATP concentration which conducts to the intensifying of glycolytic metabolism in chloroplasts resulting in the increase of reductants amount capable of plastoquinone reduction [3]. To block the mitochondrial respiration were used inhibitors that action at the level of electron transport, inhibiting the cytochrome c-oxidase sensitive to sodium azide, and respectively inhibiting the quinol:oxygen oxidoreductase sensitive to n-propyl gallate (PG) and salicylhydroxamic acid (SHAM).

In cells treated with DCMU (photosynthetic inhibitor), only 32.7% of plastoquinol pool was reoxidized in the 30 s dark period, comparing to the control (Fig.2). The amount of the reoxidized plastoquinol was diminished in the presence of DCMU. In the presence of sodium azide (NaN_3) the amount of plastoquinol reoxidized at dark decreased at 24.9% comparing to the control.

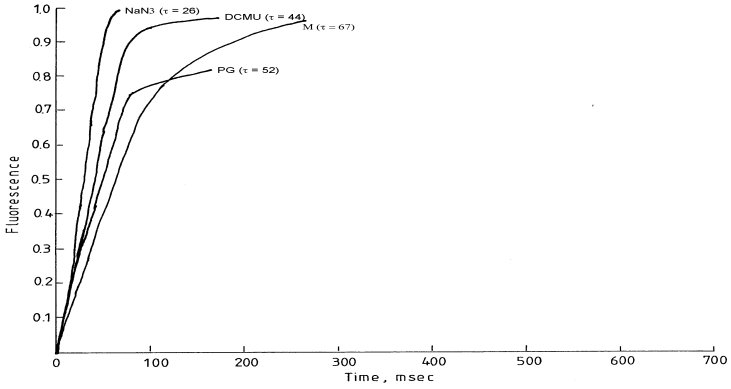

Fig. 2: The plastoquinol reoxidation at dark following the light reduction in the presence of inhibitors. M = dark adaptated cells. Cells treated with 3,27 μM DCMU and then preilluminated for 700 ms, with an incubation at dark for 30 seconds in the presence of 1 mM NaN_3, 1 mM PG.

By comparing the areas above the fluorescence rises, limited by the asymptote, was established that 72.7% of plastoquinol pool was reoxidized in the presence of salicylhydroxamic acid (SHAM) and n-propyl gallate, while 38.2% in the presence of thenoyltrifluoroacetone, comparing with the control (Fig.3). In the presence of dithiothreitol (DDT) the amount of plastoquinol reoxidized at dark was of 40% (Fig. 4).

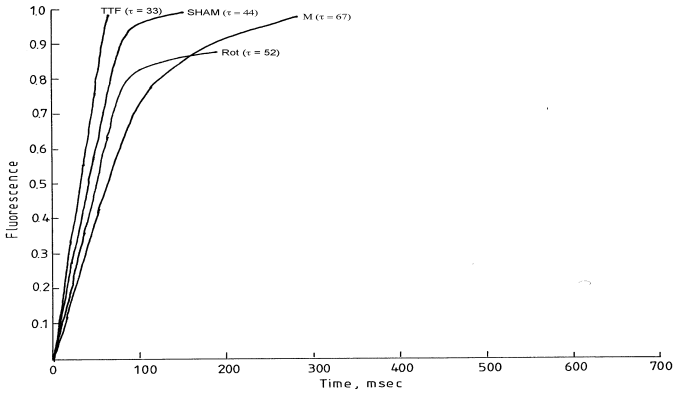

Fig. 3: The plastoquinol reoxidation at dark following the light reduction in the presence of inhibitors. M = dark adaptated cells. Cells treated with 3,27 μM DCMU and then preilluminated for 700 ms, with an incubation at dark for 30 seconds in the presence of 1 mM SHAM, 20 μM rotenone, 10 μM TTF

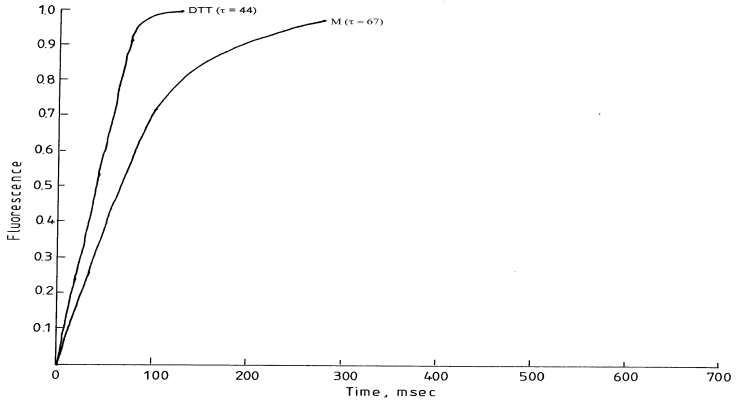

Fig. 4: The plastoquinol reoxidation at dark following the light reduction in the presence of inhibitors. M = dark adaptated cells. Cells treated with 3,27 μM DCMU and then preilluminated for 700 ms, with an incubation at dark for 30 seconds in the presence of 20mM DTT

Electrons mobility (entrances and exits) at the place where plastoquinone is situated in the thylakoidal membrane is reflected in the extent and the slope of fluorescence rise. Thus, it can be remarked the rapid registration of fluorescence rise under the effect of sodium azide and of TTF which increase the rise slope as a result of the decreasing of electron numbers arrived to platoquinone (Fig. 5). The effects of n-propyl gallate and rotenone are similar, as well as the effects of SHAM and DTT.

The change in initial fluorescence in the condition of plastoquinol reoxidation at dark following light reduction is presented in Fig. 6. The approximate equal values of the quantum yield and of photochemical efficiency (F_V/F_M) showed the absence of energized state of thylakoidal membrane. After the light reduction, the minimum and maximum fluorescence are maintained high, along with the diminishing of the efficiency and the quantum yield. Propyl gallate makes an exception, increasing the initial fluorescence, and the salicylhydroxamic acid has inhibited it respectively.

The increase of F_0 in light-dark transition results from the accumulation of Q^-_A as a consequence of plastoquinone reduction at dark. The clear reoxidation of Q^-_A has diminished due to the reduced state of plastoquinone at dark [10].

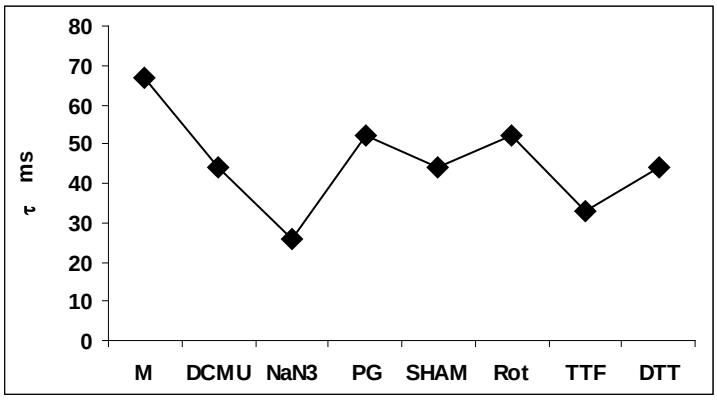

Fig. 5: The evolution of chlorophyll fluorescence half time considering the effects of specific inhibitors

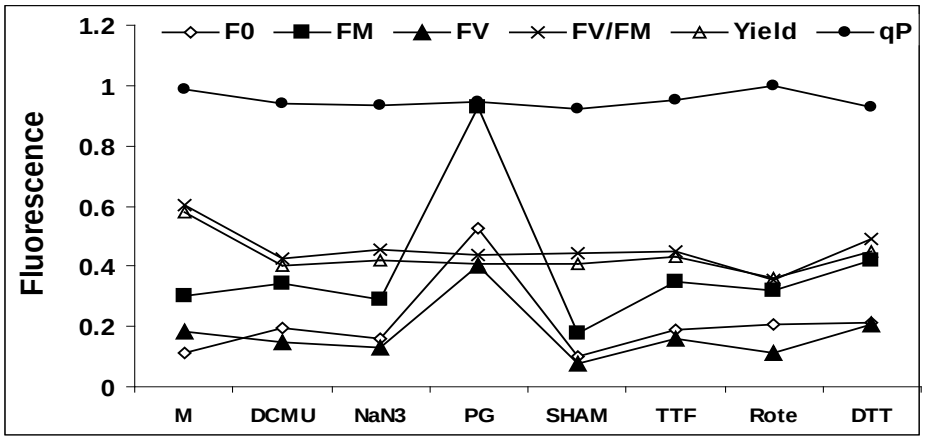

Fig. 6: The evolution of chlorophyll fluorescence under the action of specific inhibitors in the condition of plastoquinone reoxidation at dark following the light reduction

In the presence of DCMU the base fluorescence increase suggests the partial reduction of plastoquinone pool, and the progressive decrease of maximum fluorescence probably reflects the transition between state 1 and 2 at the LHC II antenna level, conducting to the distribution modification of excitation energy between PSII and PSI as a response to plastoquinone pool diminution [11].

Conclusions

By PSII inactivation, which was inhibited by DCMU, and in the presence of PSI activity, the PQ-H_2 pool becomes rapidly oxidized. The reoxidation of plastoquinol pool at dark following the light reduction showed that 43.6% of plastoquinol pool was reoxidized in the dark period. In cells treated with diuron (DCMU), only 32.7% of plastoquinol pool was re-xidized in the 30 s of dark period.

The inhibitors of mitochondrial respiration, in general, induce the plastoquinone reduction at dark. In the presence of sodium azide the amount of plastoquinol reoxidized at dark diminished at 24.9%. It was established that 72.7% of plastoquinol pool was reoxidized in the presence of salicylhydroxamic acid and n-propyl gallate, while 38.2% in the presence of thenoyltrifluoroacetone. In the presence of dithiothreitol the amount of plastoquinol reoxidized at dark was of 40%. Electrons mobility at the place where plastoquinone is located in the thylakoidal membrane is accompanying in the extent and the slope of fluorescence rise. Thus,

the sodium azide and the TTF increased the rise slope, as a result of the decreasing of electrons number arrived to plastoquinone. The effects of n-propyl gallate and rotenone are similar, as well as the effects of SHAM and DTT.

In the condition of plastoquinol reoxidation at dark following the light reduction takes place the change in initial fluorescence. The minimum and maximum fluorescence are maintained high, along with the diminishing of the efficiency and the quantum yield. Propyl gallate makes an exception, increasing the initial fluorescence, and the salicylhydroxamic acid has inhibited it respectively. The increase of F_0 in light-dark transition results from the accumulation of Q^-_A as a consequence of plastoquinone reduction at dark. The clear reoxidation of Q^-_A has diminished due to the reduced state of plastoquinone at dark. The plastoquinone redox state in dark conditions is permanently under chlororespiration control.

REFRENCES

1. Bennoun, P., 1982, Evidence for a respiratory chain in the chloroplast, *Proc.Natl.Acad.Sci.USA*, **79**: 4352-4356.
2. Bennoun, P., 1994, Chlororespiration revisited: mitochondrial-plastid interactions in *Chlamydomonas*., *Biochim.Biophys.Acta*, **1186**: 59-66.
3. Bennoun, P., 2001, Chlororespiration and the process of carotenoid biosynthesis. *Biochim.Biophys.Acta*,**1506**: 133-142.
4. Bennoun, P., 2002, The present model for chlororespiration., *Photosznth.Res.*,**73**: 273-277.
5. Bercea, V., Vasilescu, C., Drugă, B., Dragoş, N., 2006, Clororespirația şi efectele inhibitorilor oxidazelor mitocondriale asupra stării redox a plastochinonei la alga *Mougeotia sp.*, tulpina AICB 560, *Contrib.Bot.* (in press).
6. Burrows, P.A., Sazanov, L.A., Svab, Z., Maliga, P., Nixon, P.J., 1998, Identification of a functional respiratory complex in chloroplasts through analysis of tobacco mutants containing disrupted plastid *ndh* genes. *EMBO J.*, **17**: 868-876.
7. Cournac, L., Redding, K., Ravenel, J., Rumeau, D., Josse, E.M., Kuntz, M., Peltier, G., 2000, Electron flow between photosustem II and oxygen in chloroplasts of photosystem I –deficient algae is mediated by a quinol oxidase involved in chlororespiration, *J.Biol.Chem.*, **275**: 17256-17262.
8. Dragoş, N., Péterfi, L.Șt., Momeu, L., Popescu, C., 1997, An introduction to the algae and the culture collection of algae at the Institute of the Biological Research Cluj-Napoca. Cluj Univ.Press.
9. Endo, T., Shikanai, T., Takabayashi A., Asada, K., Sato, F., 1999, The role of chloroplastic NAD(P)H dehydrogenase in photoprotection, *FEBS Lett.*, **457**: 5-8.
10. Feild, T.S., Nedbal, L., Ort, D.R., 1998, Nonphotochemical reduction of the plastochinone pool in sunflower leaves originates from chlororespiration, *Plat Physiol.*, **116**: 1209-1218.
11. Joët, T., Cournac, L., Peltier, G., Havaux, M., 2002, Cyclic electron flow around photosystem I in C_3 plants. *In vivo* control by the redox state of chloroplasts and involvement of the NADH-dehydrogenase complex, *Plant Pysiol.*, **128**: 760-769.
12. Josse, E.M., Simkin, A.J., Gaffe, J., Laboure, A.M., Kuntz, M., Carol, P., 2000, A plastid terminal oxidase associated with carotenoid desaturation during chromoplast differetiation, *Plant Physiol.*, **123**: 1427-1436.
13. Munshi, M.K., Kobayashi, Y., Shikanai, T., 2006, Chlororespiratory reduction 6 is a novel factor required for accumulation of the chloroplast NAD(P)H dehydrogenase complex in *Arabidopsis*, *Plant Physiol.*, **141**: 737-744.
14. Quiles, M.J., Albacete, M.E., Sabater, B., Cuello, J., 1996, Isolation and partial characterisation of the NADH dehydrogenase complex from barley chloroplast thylakoids, *Plant Cell Physiol.*, **37**: 1134-1142.
15. Schreiber, U., Schliwa, U., Bilger, W., 1986, Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer, *Photosynth.Res.*, **10**: 51-62.
16. Shikanai, T., Endo, T., Hashimoto, T., Yamada, Y., Asada, K., Yokota, A., 1998, Directed disruption of the tobacco *nadh B* gene impairs cyclic electron flow around photosystem I. *Proc.Natl.Acad.Sci.USA*, **95**: 9705-9709.
17. Wang, P., Duan, W., Takabayashi, A., Endo, T., Shikanai, T., Ye, J.Y., Mi, H., 2006, Chloroplastic NAD(P)H dehydrogenase in tobacco leaves functions in alleviation of oxidative damage caused by temperature stress, *Plant Physiol*., **141**: 465-474.

CLORORESPIRAȚIA ŞI CINETICA REOXIDĂRII STOCULUI DE PLASTOCHINONĂ ÎN PREZENȚA INHIBITORILOR RESPIRAȚIEI MITOCONDRIALE LA ALGA *Mougeotia sp.* TULPINA AICB 560

(**Rezumat**)

Clororespirația s-a studiat la alga verde *Mougeotia sp.* prin cuantificarea fluorescenței clorofilei pentru a evidenția reoxidarea plastochinonei în condițiile acțiunii inhibitorilor lanțului transportor de electroni fotosintetic şi a inhibării respirației mitocondriale. Suspensia de algă s-a incubat la întuneric o oră în prezența inhibitorului fotosintetic DCMU ceea ce a permis oxidarea completă a plastochinonei urmând apoi preiluminarea timp de 700 ms, incubarea la întuneric 30 s şi aplicarea unui puls de saturație luminos atât la martor cât şi la variantele cu inhibitori. Rezultatele au evidențiat că 43,6% din stocul de plastochinol a fost reoxidat în perioada de întuneric. Cantitatea de plastochinol reoxidat a fost diminuată în prezența DCMU. În prezența azidei de sodiu (NaN_3) cantitatea de plastochinol reoxidat la întuneric a scăzut la 24,9% comparativ cu martorul. 72,7% din stocul de plastochinol a fost reoxidat în prezența acidului salicilhidroxamic (SHAM) şi a n-propyl gallate, 38,2 % în prezența tenoiltrifluoroacetonei şi 40% în prezența DTT. Modificarea fluorescenței inițiale în condițiile reoxidării la întuneric a plastochinolului urmând reducerea prin lumină precum şi producția cuantică şi eficiența fotochimică a PS II au evidențiat lipsa stării de energizare a membranei tilacoidale. După reducerea prin lumină, fluorescența minimă şi maximă s-au menținut ridicate, excepție făcând variantele cu propyl gallate care a mărit fluorescența inițială, respectiv acidul salicilhidroxamic a inhibat-o datorită acumulării de Q^-_A ca o consecință a reducerii plastochinonei la întuneric. Prin urmare starea redox a plastochinonei în condiții de întuneric se află în permanență sub controlul clororespirației.