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REDUCTION OF THE REACTION CENTRES OF PHOTOSYSTEM I OF PHOTOSYNTHESIS: EPR STUDY

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Abstract. The paper considers the behavior of the photo induced EPR signal I in blue- green algae and chloroplasts of higher plants in different regimes of illumination and preliminary treatment. Shown that the main contribution to the kinetics of reduction of the centers $P700^+$ are made by the reduced components of the electron transport chain between the two photosystems. If the conditions of illumination or the presence of inhibitors of non-cyclic electron transport, exclude the accumulation of such products then the kinetics of reduction determined by the reaction with the reduced compounds in the incubation medium formed in the light in the preceding period. These exogenous reductants may be remove from the incubation medium of the chloroplasts by repeated washing out and from the algae by prolonged incubation in the dark. The relation between the cyclic and non-cyclic transport in the presence and absence of exogenous mediators of cyclic fluxis discussed.

Keywords: *chloroplasts, photosystem, EPR signal, inhibitor, transport.*

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1. Introduction

It has been repeatedly noted that the rate of delay of the photo-induced EPR I signal in leaves, chloroplasts of higher plants and algae and also its steady intensity under light fundamentally depend on the preceding treatment of the preparation, in particular, the preliminary illumination of the samples (spectral composition, intensity, duration of illumination) (Goldfield & Blumenfeld 1981; Khalilov & Nasibova, 2008; Rosa, 2007; Nasibova *et al.*, 2007).

The factors determining the steady concentration of the paramagnetic centers and the rate of their decay are the relations between the intensity of light (the number of quanta absorption per unit time), the rate of the back reaction between the primary acceptor and the photo-induced electron donor $P700^*$ (this process apparently plays a role only at low temperatures). The cyclic electron transfer with the participation of a specially introduced mediator of the type phenezine methosulphate (PMS) or an endogenous mediator (Atanasova *et al.*, 2003; Zivcak *et al.*, 2014), the non- cyclic flux of the electrons from the photosystem II and also the reductive reaction through the reductants earlier accumulated in the system. The latter factor is rarely taken into consideration in discussing the kinetics of changes in the intensity of the EPR signal I and the corresponding optical absorption at 700 mμ.

The present paper outlines the results of investigation of the kinetics of rise and delay of the EPR signal of the blue-green algae *Anabaena variables* and a suspension of chloroplasts of *Vicia faba.* It is showing that many aspects of behavior of the EPR signal are due to the accumulation of reductants in the process of the vital activity of organisms in the light in the period preceding the experiment and the reduction of the components of the electron transport chain directly during the experiment also. The influence of the cyclic flux on the behavior of the EPR signals I, as a rule, is masked by other processes even when the transport of electrons between the two photosystems is interrupted by the interaction of 3-(3,4- dichloropheny1)-1,1′- dimethylurea (DCMU).

2. Materials and methods

The chloroplasts were isolated as described earlier (Khalilov *et al*., 1993) and the conditions of cultivation of the algae are described in (Illerhaus *et al*., 2000; Nasibova *et al*., 2016). The techniques of the kinetic measurements of the EPR signal of the photosynthesizing organisms are presented in (Goldfield & Halilov, 1979). Pulsed illumination of the preparation in the resonator of the EPR radio spectrometer was carried out as in (Terao *et al*., 1996; Zuk-Golaszewska *et al.,* 2003).

Experiments with algae. It has long been noted that cultures of some photosynthesizing microorganisms are characterized by a high negative value of the redox potential of the medium (Baig *et al*., 2005; Anderson *et al.,* 1997). In our experiments the EPR I signal of the algae sediment by centrifugation and adjusted with the supernatant to a chlorophyll content of about 1 mg/ ml was observed in the light only after prolonged (about 8 hour at 30̊) keeping of the cells in the dark. In algae taken directly from the phytotron the EPR signal in the light did not appear. Figure 1 shows how the amplitude of the photo-induced signal changes under light of 710 and 634 mμ against a background of 710mμ.

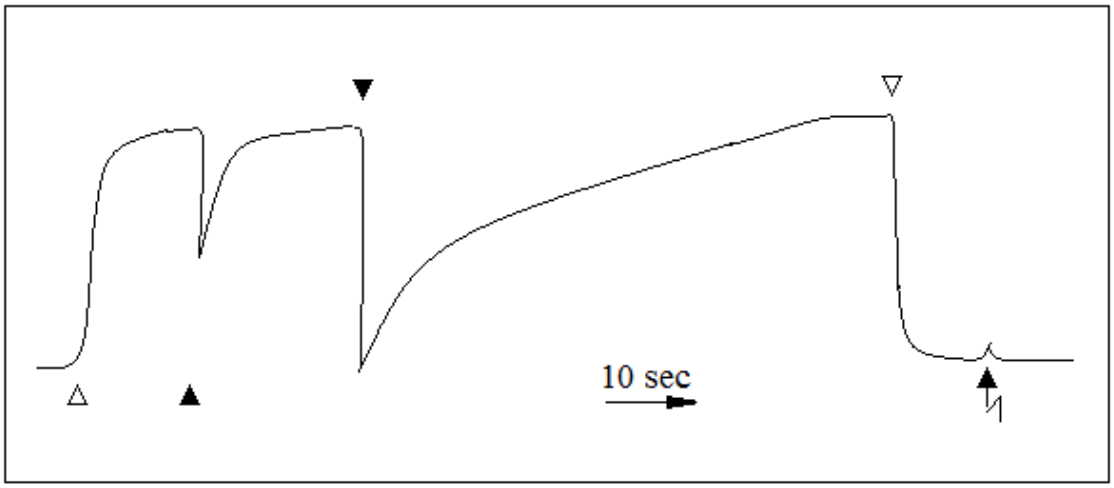

Fig. 1. Change in amplitude of photoinduced EPR signal (maximum of low field side, rel. units) in suspension of *Anabaena*, Δ ▽ ▲▼ switching on and off light 710 mμ (1650 erg/sm^2 sec) and intense white light (~7000 lx) respectively. Broken arrow - flash of white light

The figure shows that light of 634 mμ exciting both photosystems induces the decay of the paramagnetic centers $P700^+$ accelerates the reduction of $P700^+$ in the dark.

The nature of the non-monotonic transitional effects (as exemplified by the leaves and the chloroplasts of higher plants) was discussed earlier (Beardall *et al*., 2011). The inhibitor of non-cyclic transport of electrons DCMU reverses the qualitative differences in the action of light of 634 and 710 mμ although the decay of the signal after switching off the light was also observed in the presence of DCMU (Fig. 2).

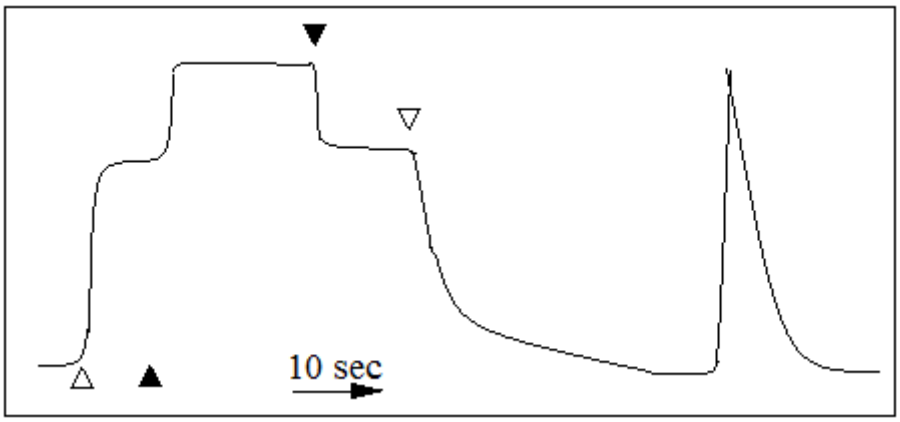

Fig. 2. Same as Fig.1 in the presence of 10^{-5} m DCMU

On illumination of algae with a flash of white light (lasting 20 μsec) in the presence of DCMU we observe the appearance of a photo-induced signal disappearing in the dark. Without DCMU the signal in response to the flash did not appear. The differences in the behavior of the EPR I signal of the cells kept in the dark and cultured in the light immediately before the start of the experiment apparently indicates the accumulation in the light of reduced products expended in the dark. The behavior of the cells kept in the dark not subjected to dark adaptation also differs in the presence of DCMU. In this case the non-cycle flux does not make a contribution to the reduction of $P\ 700^{+}$ nevertheless, algae not subjected to dark adaptation did not give a signal in weak far- red light (710 mμ, 1650 erg/sm^2 sec) or gave in white light a signal rapidly decaying in the dark. At the same time, the algae kept in the dark gave on illumination an intense EPR signal and thus even in presence of an inhibitor of transport the kinetics of reduction, at least in part, is due to the reactions with the external reductants expended in the dark.

Experiments with the chloroplasts. Chloroplasts in the presence of methylviologen (and without an exogenous acceptor) after single sedimentation by centrifugation did not give in the light an EPR signal (710 mμ, 2000 erg/sm^2 sec). After washing out with the isolation medium and repeat sedimentation we observed (in the presence of methylviologen) in the light the appearance of an EPR signal. Suspension in the supernatant of the first centrifugation of the chloroplasts repeatedly washed out with the isolation medium had the result that the behavior of the EPR signal became the same as in the non-washed chloroplasts. After the third centrifugation, the chloroplasts in the light at 710 mμ gave a maximum EPR signal, which did not increase with rise in the intensity of light or in white light (Fig.3). The redox potentials of the supernatants of the first, second and third centrifugations were 260, 340 and 430 mV respectively. Thus, the supernatant acts as an effective reductant preventing rise in the concentration of the oxidized centers of $P700^{+}$. This reductant is evidently of a non-protein nature and is highly stable; boiling of the supernatant in air for 5 minute did not remove its reductive

action in relation to $P700^+$. We would note that in the repeatedly washed chloroplasts after illumination with light of 710 mμ, the rate of the dark reduction of $P700^+$was extremely low ($T_{1/2}$=20- 30 sec), less by one order of magnitude than after the light of 634 mμ (when the reductive plastoquinone pool between the two photosystems is charged)or after light of 710 mμ and singly sedimented chloroplasts.

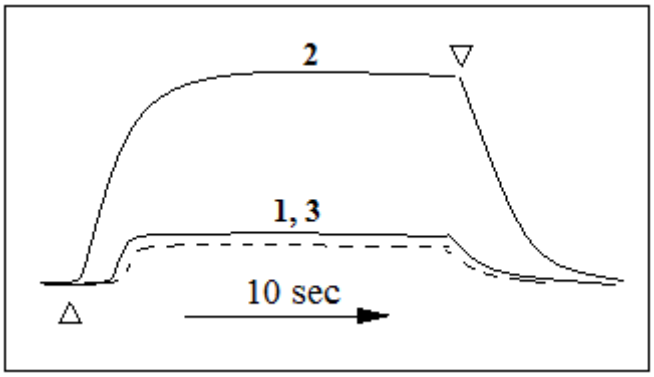

Fig.3. Changes in a amplitude of EPR signal in chloroplasts: 1- control; 2- after threefold washing; 3- washed chloroplasts resuspended in supernatant of first centrifugation, boiling for 5 min. Everywhere end concentration of methylviologen 10^{-4} M. Light 710 mμ, 150 erg/sm^2 sec.

The character of the link between the two photosystems expressed by the form of the transitional region and the relation of the steady signals under light of 710 and 710+ 634 mμ was not disturbed during washing out of the external reductants.

3. Results and discussion

The results outlined of the experiments with algae and the chloroplasts thus show that by varying the regime of illumination and the composition of the medium it is possible to carry out reduction of the reaction centers of photosystem I in one of two ways: in the presence of a link between the two photosystems (in the washed chloroplasts) the reduction of $P700^+$ proceeds through electron transfer from the photosystem II or from the reduced pool filled as a result of absorption of near - red light. In the absence of such a link (chloroplasts with DCMU or on illumination with far-red light) the rate of reduction of $P700^+$ is determined by the reaction with the external reductants accumulated earlier. Without preliminary treatment standardizing the state of the test system the observed kinetics of reduction may contain a different contribution from each of these processes. To explain the behavior of P700 apparently it is not necessary to resort to the cyclic flux of electrons in photosystem I.

In conclusion, we shall consider the conditions in which the cyclic flux of electrons is realized in the presence in the system of the mediator of cyclic transport PMS. In fact, the only manifestation of the cyclic flux in the chloroplasts in the presence of PMS consists in cyclic photophosphorylation. According to the published findings (Nasibova *et al.*, 2017; Barnes *et al.*, 2013) for a sufficient concentration of DCMU or monuron the cyclic photophosphorylation in the chloroplasts are suppressed. Consequently, PMS as such is not capable of ensuring prolonged (of the order of several minutes necessary for the formation of appreciable amounts of ATP) functioning of the cyclic chain.

Figure 4 shows that in the presence of DCMU and PMS the EPR signal I in the light appears in a period of about 20 sec while in the dark the signal disappears comparatively slowly. Thus, simultaneous with the cyclic flux there is "evacuation" of the electrons to the final acceptor (oxygen).

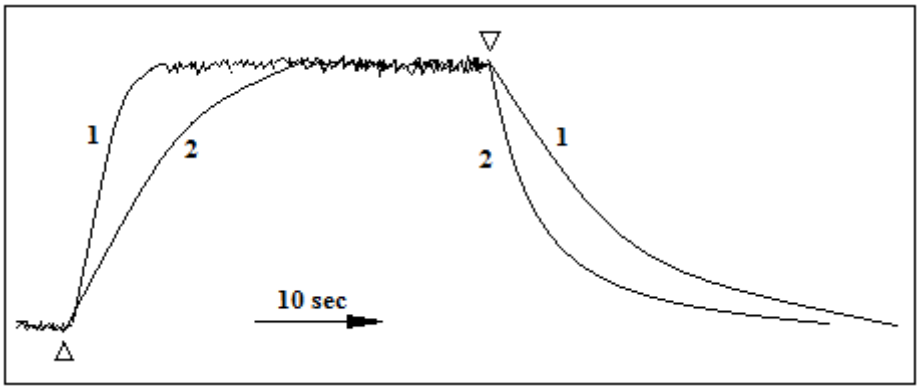

Fig. 4. Chloroplasts washed three times. EPR signal under light of 710 mμ (150 erg/sm^2 sec): 1- in the presence of DCMU (10^{-5} M) and methylviologen (50 mμ); 2- in the presence of DCMU and 10^{-5} M phenazine methosulphate. Chloroplasts first illuminated with light of 710 mμ (1650 erg/sm^2 sec) and kept in dark for 2 min.

As a result all the components of the cyclic chain (including P700) are oxidized and cyclic transport ceases. The time of inactivation of the reaction centers in these conditions determined by the relation between the number of cycles of cyclic transport and the time of electron transfer to the final acceptor. Apparently, the prolonged maintenance of the cyclic photophosphorylation requires compensation of the oxidation of the reaction centers of photosystem I through excitation of photosystem II and the residual non-cyclic electron transfer. At low concentrations of monuron, which however practically completely suppress the Hill reaction we nevertheless observe the spectral dependence of the kinetics of the decay of the EPR signal in the presence of methylviologen (Fig. 5) from which it follows that electron transport between the photosystems are not finally suppressed. This residual transport may ensure the reduction of P 700^+ compensating the electron transfer to the final acceptor. The need forcyclic photophosphorylation of a free source of electrons is also indicated by the findings of (Andrea *et al*., 2002; Khalilov *et al.,* 2015) who showed that in subchloroplasts particles enriched with photosystem I the cyclic photophosphorylation is observed only when a specialexogenous donor is introduced. The inhibitors of photosystem II such as, for example, carbonylcyanide-m-chlorophenylhydrazone possibly suppressed the cyclic photophosphorylation precisely through the inhibition of this compensating flux of electrons from the photosystem I.

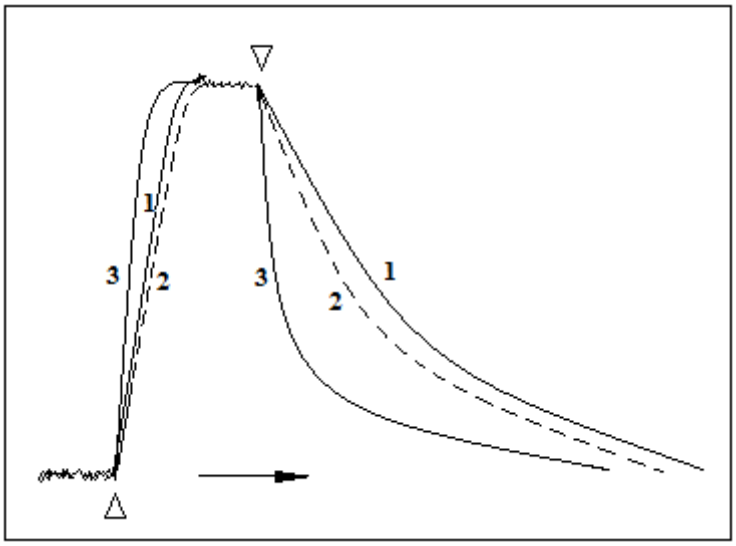

Fig. 5. Change in the amplitude of EPR signal in chloroplasts: 1-light of 710 mμ (150 erg/sm^{2}sec), 5 x 10^{-5} monuron; 2-light of 710 mμ, control; 3-white light (~ 7000 lx), 5x10^{-5} monuron

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