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Influence of UV and Gamma Radiation on Paramagnetic Properties in Fragments of Photosystem 2



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ABSTRACT

In the present work, investigation was done to determine the influence of radioecological factors on paramagnetic centers in subchloroplasts particles of photosystem 2 (PS2). It was revealed that these factors do not render serious influence on signal EPR 2. Differently, tyrosine radicals which are source of this signal are not exposed to strong changes. With addition of a strong acceptor of kremniy molibdat (KM) in fragments PS 2 is induced signal EPR 1 on light and thus the amplitude of signal EPR 2 decreases. Simultaneously, it was also observed to increase signal EPR of Mn^{2+} . Radiation samples with UV was done by DRT-230 lamp and by the use of UFS-2 filters. The intensity of radiation was 20 Vt/m^2 . For source of γ -radiation used ^{57}Co isotope. The power of radiation was 670 Qr/h. The spectrum of EPR 2 signal in dark PS 2 fragments is shown in figure 1. As shown in the figure, this signal had a fine structure and consists of 6 components. The parameters of signal were in room temperature $g=2,0049$, $\Delta H_{\max}=20 \text{ Gs}$. When the properties of paramagnetic centers in PS 2 studied in our experiments we observed dependence of signal from power of level of extremely high-frequency area. It was determined on the basis of our experiments, ionized radiation damage electronic transport with influence to the integrity of feofitin molecule which placed in the acceptor part of PS 2 [3,8,10]. But in that time different component which consists of PS 2 macrocomplex, also reaction center P680, light-collecting complex, cytochrome b-559, protein D1 and etc. doesn't change. In fact, effect of ionized radiation to the paramagnetic centers does not lead to noticeable changes.

INTRODUCTION

In the present work, investigation was done to determine the influence of radioecological factors on paramagnetic centers in subchloroplasts particles of photosystem 2 (PS2). It was revealed that these factors do not render serious influence on signal EPR 2. Differently, tyrosine radicals which are source of this signal are not exposed to strong changes. With addition of a strong acceptor of kremniy molibdat (KM) in fragments PS 2 is induced signal EPR 1 on light and thus the amplitude of signal EPR 2 decreases. Simultaneously, it was also observed to increase signal EPR of Mn^{2+} . In recent years, some work on the influence of photosynthesis with ultraviolet and ionized rays has been published [2, 5, 7]. The study mainly focuses on the primary processes of photosynthesis. Isolated chloroplasts and intact sheet are determined that ionized and ultra-violet radiation, particularly affects negatively to the structure-functional condition of PS 2. Due to the fact, macrocomplex PS 2 which is located in the thylakoid membranes in the process of evolution which are later created structure directly involved in the formation of molecular oxygen [6]. In many publications, it was shown that macrocomplexes PS 2 are very sensitive to many stress factors [1, 5, 7]. It was observed that the experiments carried out with the EPR method, at room temperature in *in-vivo* and *in-vitro* research observed two types of EPR signal [8]. EPR 2 signal, which is called darkness signal belong to tyrosine radical which is located in PS 2. But EPR 1 signal belongs to the reaction center of PS 1 and generated in light [5, 9]. It should be noted that for observing EPR 2 signal PS 2 subchloroplast particles is the most convenient object. In this present work, for clarifying the mechanism of action radioecological factors in PS 2 fragments which have high biochemical activity, we studied paramagnetic centers which belong to PS 2 macrocomplex with EPR method.

MATERIALS AND METHODS

Separation of the PS 2 chloroplast fragments was conducted properly [4]. Chloroplasts slurried in conditions with 0.07 M phosphate buffer (pH 7.0), 0.5 M sugar and 0.035 M NaCl. Then, a solution of 1% digitonin was added to system and used in ultrasonic UZDP-1 (22kQs, 400 Vt, 1 min.) dispersant. Sodium chloride was added to the slurry and it was the incubated with vigorous mixing in ice for 40 minutes. 4000 g homogenate was centrifuged for 15 min., sediment was taken, Triton X-100 were quickly added to supernatant with concentration 0.1-0.15% and again 20000 g homogenate was centrifuged for 45 min. The sediment, DT-20 fragments or particles gathering and slurried again in medium which compound 0.015 M Tris-HCl (pH 8.0), 0.035 M NaCl and 0.002 M $MgCl_2$.

EPR spectrometers were noted at room temperature in <Varian E-4> (USA) with range 3-cm. Some of experiments were conducted in RE 1306 spectrometer. To mark EPR signals of samples which were studied, we put them in resonator. Magnetic area was fixed in peak point of lower area derivative of EPR 2 signal for kinetic measurements. We mark changes of EPR 2 signal with help of registering device or computer. For detecting the fast changes of EPR 2 signal used amplifier devices.

Radiation samples with UV was done by DRT-230 lamp and by the use of UFS-2 filters. The intensity of radiation was 20 Vt/m^2 . For source of γ -radiation used ^{57}Co isotope. The power of radiation was 670 Qr/h.

RESULTS AND DISCUSSION

The spectrum of EPR 2 signal in dark PS 2 fragments is shown in figure 1. As shown in the figure, this signal had a fine structure and consists of 6 components. The parameters of signal were in room temperature $g=2,0049$, $\Delta H_{\text{max}}=20 \text{ Gs}$. When the properties of paramagnetic centers in PS 2 studied in our experiments we observed dependence of signal from power of level of extremely high-frequency area. During study of radio spectrometric parameters of this signal, firstly we looked at dependence of high-frequency electromagnetic waves which came from klystron. It was revealed that now there is already strong as 20 mVt signal saturation. When ΔH reaches to $\Delta H=2000 \text{ Gs}$ the study of fragments with EPR shows that, at room temperature there is exists only EPR 2 signal. A Light in different spectral composition (707 nm, 650 nm) doesn't affect to EPR 2 signal. Only white light with high-intensity increase amplitude of signal. In PS2 fragments in room temperature, we use strongly oxidator kremniy molibdat for observation reaction center P680. In our experiments, adding KM (10^{-4} M) in small quantities decrease amplitude of EPR 2 signal. In parallel, we observed formation of signal with 6 components of Mn^{2+} in range of $\Delta H=2000 \text{ Gs}$. Depending on the duration of exposure, growth of signal with 6 components and reduction of EPR 2 signal was observed. It may be because KM changes the oxidation and reduction potential of tyrosine molecule in donor part, by implementing the system, the process of destruction of the water-splitting enzyme cause Mn^{2+} ions getting out. Depending on exposition time of KM and PS 2 fragments there are increased yield of Mn^{2+} ions. Addition of methyl viologen (MV) which is a mediator for molecular oxygen doesn't create noticeable changes in parameters. Amplitude and structure of signal 2 doesn't affect from radiation of PS 2 fragments during 30 min. with UV lights (UFS -2 filters)(fig. 1). Even increasing of UV radiation to 50 min. amplitude and

structure doesn't change noticeably. As can be seen in control and radiated samples EPR 2 signals are absolutely same. Similarity of dependency curves in magnetic field with high frequency shows that UV radiation doesn't affect to the tyrosine radicals which source of EPR 2 signal even in high dose.

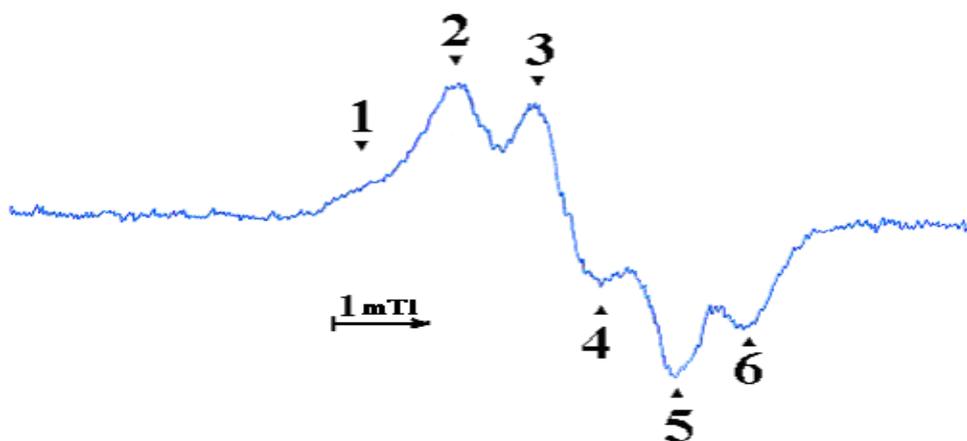


Fig.1. EPR 2 signal of subchloroplast particles in PS 2

($g=2,0049$, $H_{max}=20$ Gs), mod.=3,2; $\tau' =0,3$

Only the Mn^{2+} signals seem to have been formed. After UV radiation giving white light to sample as before irradiation doesn't affect noticeably. In EPR 2 signal UV radiation doesn't change noticeably dependence curves of high-frequency electromagnetic waves. With the addition of a small amount of KM to PS 2 fragments which exposed to UV radiation decrease amplitude in EPR 2 like in control, and increase output of Mn^{2+} signal. It should be noted that, providing white light amplitude of EPR 2 signal was increased. With addition less oxidative ferrocyanide the following changes are taking place. Firstly EPR signal of Mn^{2+} ions. EPR 2 signal grow in the darkness. By giving white light it is observed the grow of a signal. Analysis of kinetic changes shows that differences emerge in kinetics caused by switching off the light. So that, addition of ferrocyanide and half-drop moment decrease. It shows changes in redox potential of ferrocyanide and KM in nature. In PS 2 fragments changing concentration of KM cause disappearing signal 2 in darkness, and generation of new signal (EPR1') in light. This signal is getting more likely from reaction center $P680^+$. $P680$ cation radical has the highest concentration in the presence of KM. The reason for this is in donor part the issue of the speed of transfer of electron to reaction center but in acceptor part $P680^+$ and Q_A decreasing speed of recombination.

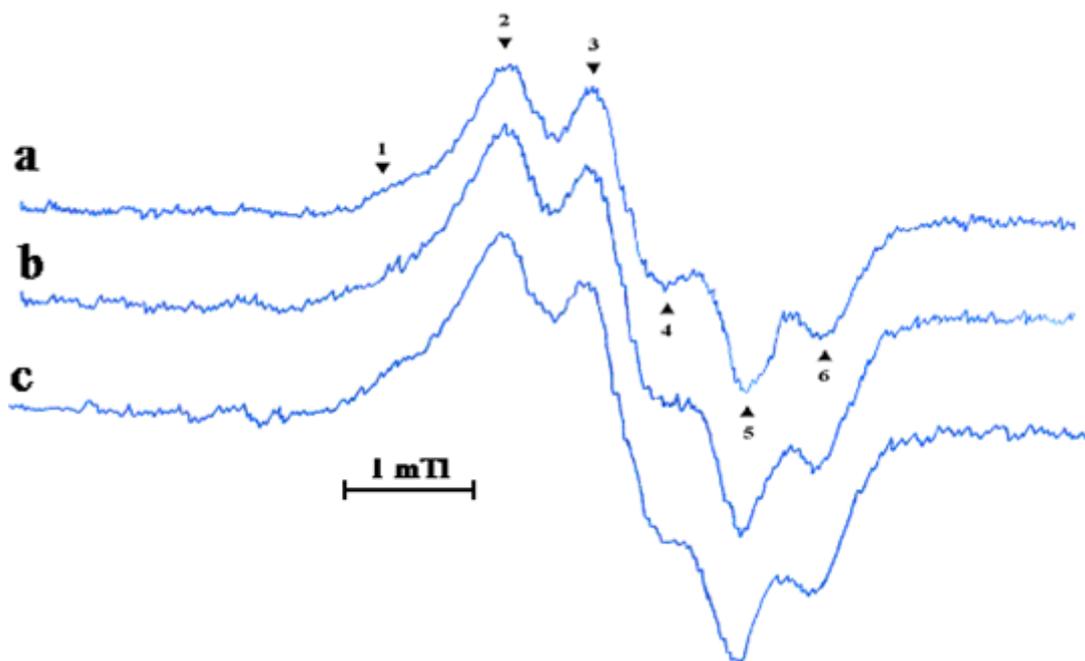


Fig.2. Dependence of EPR 2 signal from the duration of radiation in subchloroplast fragments in PS 2. a-control, b-30 min. UV radiation, c-50 min. UV radiation. Mod.=5 G; force= 3,2*1000; t=1"

KM directly, receiving electrons from Q_A , that's why electrons don't go on opposite way, in other words, recombination does not occur, as a result stationary concentration of P680 increase. As can be seen from Fig. 3 the observed EPR 1' signal like a singlet $g=2,0025$, width is 9 Gs. It should be noted that giving different spectral composition of light ($\lambda=707$ nm, far red light, $\lambda=650$ nm, near red light) doesn't to lead to growth of EPR 1' signal. High intensity white light cause to growing EPR 1 signal. By turning off the light signal is shrinking rapidly. In next experiments, we studied the effect of UV radiation to EPR 1' signal. Small doses of radiation significantly affect to the amplitude of the signal and to the kinetics. As the duration of radiation, in other words increasing the dose of PS 2 fragment amplitude of the signal decreases, sharp changes occur in the kinetics.

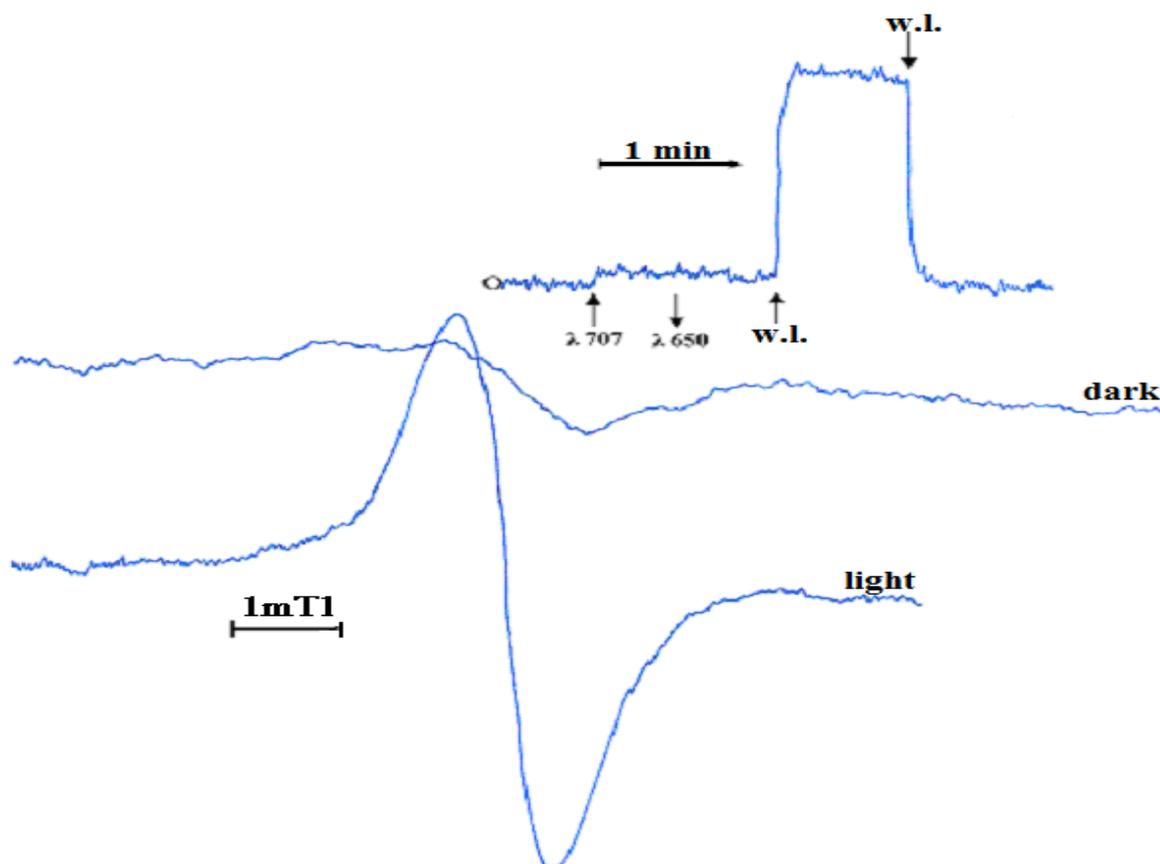


Fig.3. Influence of lights with different intensity to EPR 1' signal.

Maybe UV radiation taking effect to the reaction center of PS 2 only in high doses, create destructive changes. Appearing destructive changes decrease amplitude of EPR 1' signal. In the following experiments, we studied dependence amplitude of EPR 2 signal from concentration of KM in nature. The experiment series which we done shows that this dependence is linear. With the increasing concentration EPR 2 signal is shrinking, but EPR 1' signal growth linearly. In our opinion, it is proved that the destruction of tyrosine which is source of EPR 2 signal is a reason of weakening of the speed of electron in reaction center. As a result, the amount of cation radicals increase.

In the next experiments, we took ionized radiation gamma scattering like a stress factor and reviewed the impact of paramagnetic centers in PS 2 subchloroplast particles. In experiments fragments stayed exposed to radiation in different doses of source ^{57}CO isotope. Unlike UV radiation we doesn't observe Mn^{2+} signal in interval $\Delta H=2000$ Gs. In fig. 4 was given series of experiments which belong to ionized radiation effect to the paramagnetic centers in the PS 2 subchloro particles. As shown in figure, observed PS 2 particles which exposed to gamma radiation in 10min. doesn't change 6 components of EPR 2 signal. In 100 Qs interval though

modulation 5 Gs, there are no ingredients of extreme slim structure in multicomponent EPR 2 signal.

Giving intensive white light to the EPR resonator almost doesn't change amplitude of signal. In the maximum of second component caused by fixation of magnetic field doesn't change under the influence of light. By turning off the light again registration of EPR 2 signal remains unchanged previous light and darkness. In large interval ($\Delta H=2000$ Gs) by changing the magnetic field we study appearance paramagnetic centers in PS 2 level in light also in dark environment in experiments by the changing magnetic field in wide-range under the influence of ionized radiation. As can be seen (fig. 4 d, e) in this large interval we doesn't observed signal of ions.

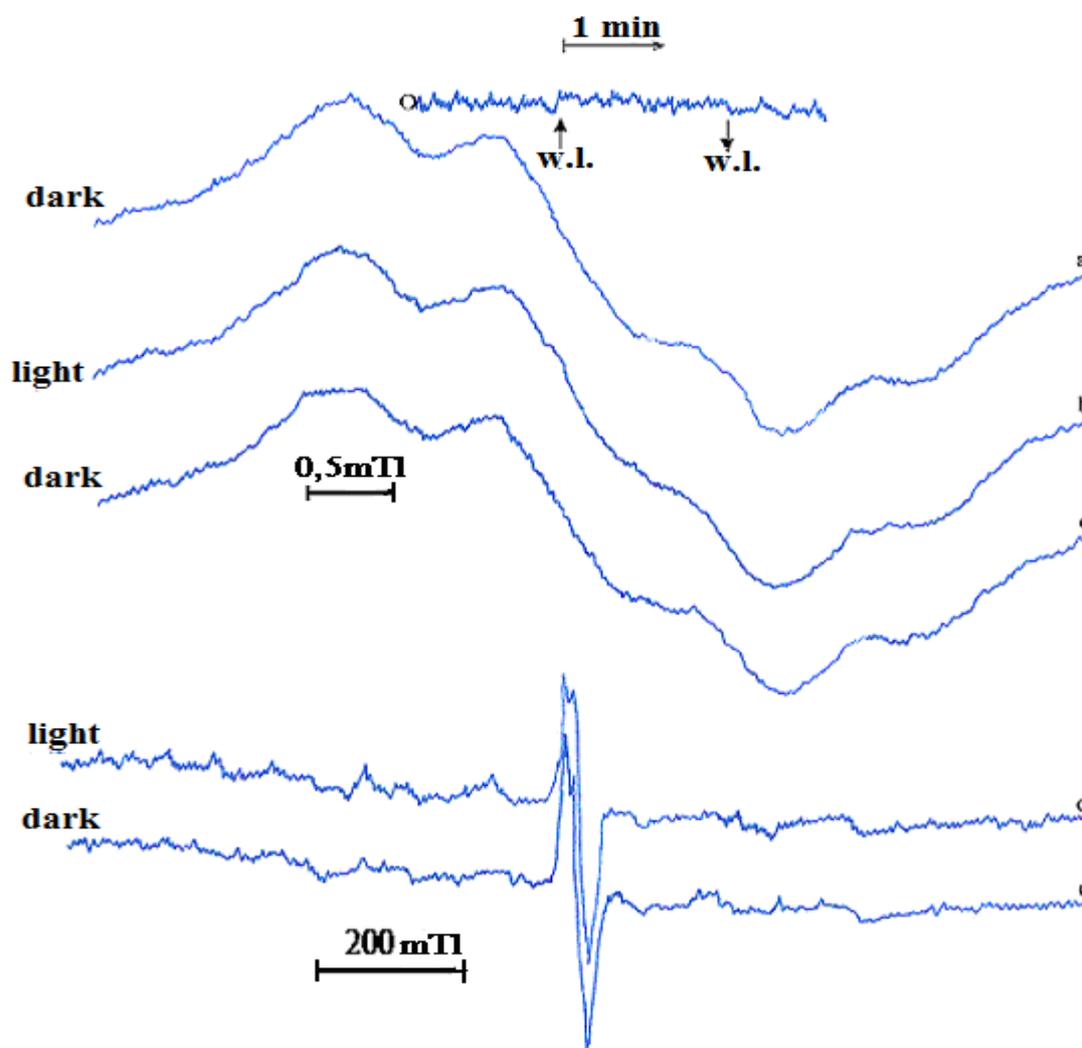


Fig.4. Influence of ionization radiation to paramagnetic centers of PS 2 subchloroplast particles. Mod.= 5 Gs, force = $3,2 \cdot 1000$, $\tau=1$

Here is an idea that can be expressed as follows, observation in room temperature free Mn^{2+} and Fe^{2+} cations don't damage thin structures (fermentative complex which forms water, alternative b-559 electron carriers in PS 2 level and etc.).

In fact, EPR signals observed in the P680(EPR 1'), tyrosine amino acid (EPR 2) at room temperature, there is no seriously change the shape and amplitude in that doses. But unlike them, changes occur in an enzymatic system which divided water have lokus for PS 2 sensitive to stress factors. We observed that like Mn^{2+} ions, which include enzymatic system taking a pass from center take paramagnetic shape. So that, in small doses of these factors of 6 component EPR signal of ions observed at room temperature. With the increase in dose, the amplitude of signal also increase. Of course, in low temperatures (liquid helium 4K, liquid nitrogen 77K) EPR studies that would allow us to observation of behavior under the influence of stress factors in paramagnetic centers of PS 2. This Fe ions belong to that comprise the b-559 cytochrome, feofitin molecule, Q_A and Q_B on Fe and etc. Gamma-radiation which is radiation factor during the effect of different exposition to PS 2 particles we observed resistance of EPR 2 and EPR 1' signal against it. Unlike UV radiation there is no Mn^{2+} ions in free form in the environment under the influence of ionized radiation. Ionized radiation in PS 2 fragments firstly damages electronic transport with influence to the integrity of feofitin molecule in Electron Transport Chain. EPR 1' signal observed with KM in light partly shrinks in large doses of radiation. We know that the data in the literature ionized radiation firstly effect to the energetics of photosynthesis during influence to the photosynthesis process. It was determined on the basis of our experiments, ionized radiation damage electronic transport with influence to the integrity of feofitin molecule which placed in the acceptor part of PS 2 [3,8,10]. But in that time different component which consists of PS 2 macrocomplex, also reaction center P680, light-collecting complex, cytochrome b-559, protein D1 and etc. doesn't change. In fact, effect of ionized radiation to the paramagnetic centers does not lead to noticeable changes.

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