Journal of Radiation Researches, vol.7, №2, 2020, Baku
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pp. 34-38

UOT: 504.75

DETECTION OF COPPER EPR SIGNALS IN THE PHOTOSYNTHETIC APPARATUS OF THE PLANTS: *IN VITRO* INVESTIGATIONS

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Abstract: The article is devoted to the study of the detection in the subchloroplast particles of PS 2 a copper signal close in form to the EPR signal of plastocyanin observed in the same temperature region and corresponding to the weakly saturated component of the signal in the chloroplasts. This fact and the recently detected reactivation of the chloroplasts by copper ions and the influence of chelators on the reaction of PS 2 plus detection of the copper fraction firmly held by the fragments of PS 2 suggest that the copper-containing complexes form an integral component of PS 2.

Key words: EPR spectra, chloroplast, photosystem 2, plastocyanin.

1. Introduction

To detect copper in live tissue optical methods are mainly used [1]. The EPR spectra proper of Cu-containing natural complexes are observed only for a small fraction of copper since much of it is in the reduced diamagnetic state. In [2] the authors proposed the use for the detection of copper of the EPR spectra at 77K of tissue treated with xanthogenates. In this paper, this method is tested to elucidate the localization of Cu in chloroplasts. It did not prove quite acceptable for plant specimens so that copper complexes with fructose are proposed for the same purposes.

2. Materials and methods.

The test object was provided by *Vicia faba* chloroplasts and their fragments enriched with photosystem 2 (PS 2) isolated as described earlier [3]. The model complexes were obtained from $CuCl_2$ in different organic solvents and from ethyl xanthogenate as in [2]. The fructose complexes were obtained by the method described in [4] replacing nitrate and nitric acid with copper chloride and HCl.Treatment of the biological material was as follows: to 2 ml of the chloroplast suspension or fragments of PS 2 containing 1 mg chlorophyll per ml was added 2 ml of solution of ethyl xanthogenate in dimethylformamide (100 mg/ml). Complexing occurs very slowly and is terminated in a few days. To determine Cu using fructose complexes to 2 ml of the suspension was added 1 ml of 0.1 M HCl, held for 15 min at room temperature, 1 ml of 0.5 M solution of fructose added, and the pH adjusted to 7.5 with unimolar NaOH. The EPR spectra were recorded at 77K with an EPR spectrometer in conditions excluding saturatíon with the power of the ultra high frequency (UHF) field. Chlorophyll was determined after Arnon [5].

3. Results and discussion

It was not possible by selecting the medium to ensure the complete similarity of the EPR spectra of the model xanthogenate complexes and those extracted from the plant material (fig.1).

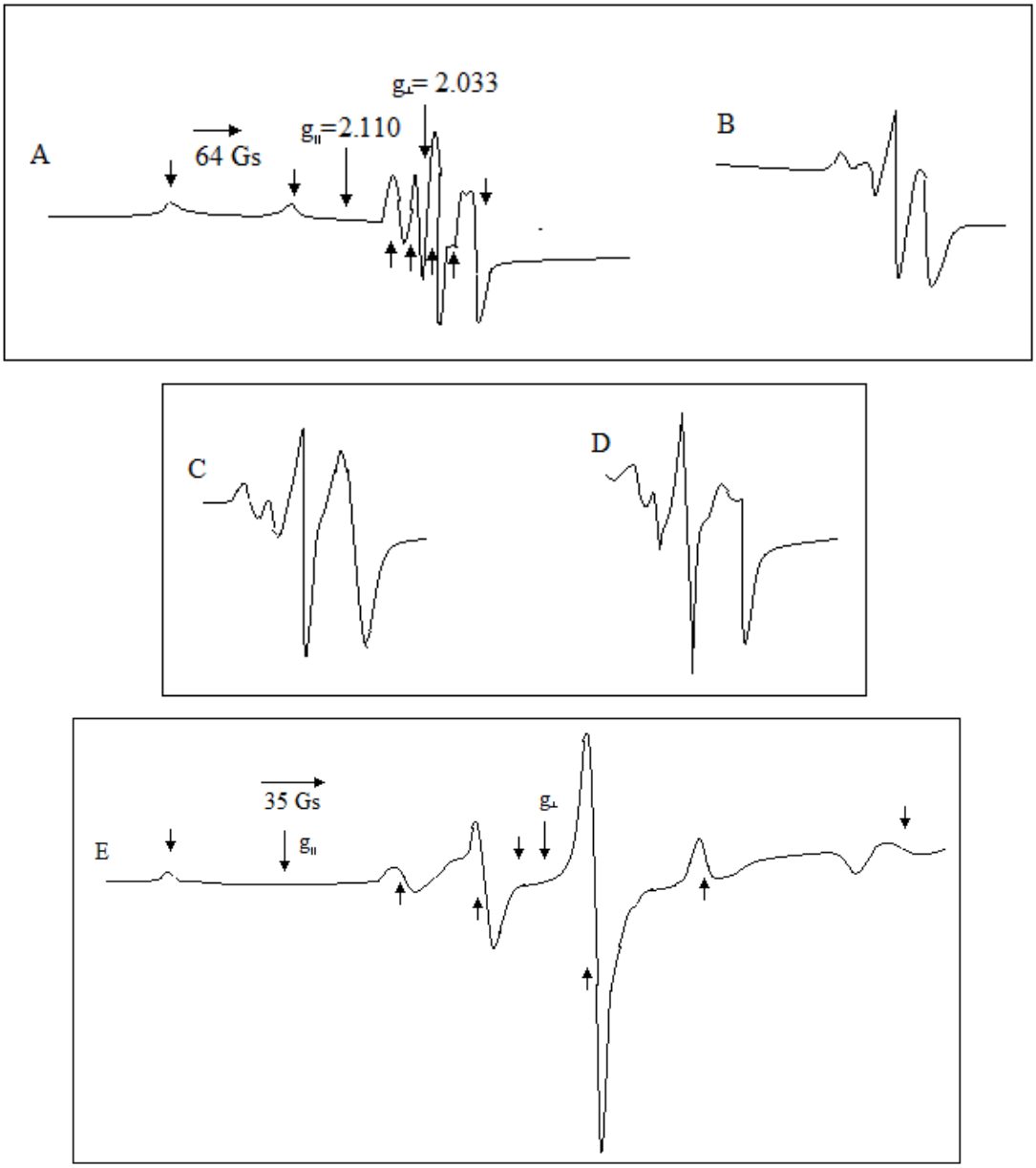

Fig.1. EPR spectra of ethyl xanthogenate complexes of Cu^{2+} in different media and chloroplasts: A – 10^{-3} M $CuCl_2$ in dimethylformamide + 200 mg/ml ethyl xanthogenate; B – $CuCl_2$ in acetone + ethyl xanthogenate; C – $CuCl_2$ in dimethylformamide + ethyl xanthogenate in a mixture of chloroform and toluene (1:1:1); D – $CuCl_2$ in dimethylformamide + ethyl xanthogenate in acetone; E – chloroplasts treated with ethyl xanthogenate in dimethylformamide.

This is probably determined by the inclusion in the ligand sheath of copper of a component extracted from the chloroplasts as in [2]. The absence of similarity of the EPR signals makes difficult quantitative comparison since further operations of calibration and integration of the spectra are necessary. We, therefore, looked for other complexes.

In [4] the authors recorded the formation of stable complexes containing 2 moles of fructose per mole of Cu^{2+} with a characteristic EPR spectrum (fig. 2A) with the parameters of the signal g_{II}=2.256, $g_{\perp}$=2.052, A=188 Gs, B=25 Gs.

Treatment of the chloroplasts and the fragments with the solution of fructose gave EPR spectra (fig. 2B) which in the form are quite similar to the spectra of the model complexes. Thus,

with their aid, it is convenient to make the quantitative determination of Cu in biological material by directly comparing the amplitude of the signals of the test sample and solution of $CuCl_2$ of known concentration. This showed that the fragments of PS II contain per 100 moles of chlorophyll, 0.8 mole Cu while the initial chloroplasts contain 2.0 mole Cu. For spinach preparations containing a higher admixture of P700, the literature gives the following values: 1.8 mole Cu in the chloroplasts and 1.2 mole Cu in the particles of PS 2 [6].

The only characterized Cu-containing protein in the lamellae of the chloroplasts is plastocyanin the functions of which are related to PS 1 [7]. It is known that it is readily washed out of the thylacoid membranes so that its presence in the fragments of PS 2 obtained with the use of detergents is unlikely. From the available evidence [8] the EPR signal proper of Cu-containing chloroplast complexes is inhomogeneous and the components forming it is characterized by a different temperature dependence of ultra high frequency saturation in the temperature interval 13-77K.

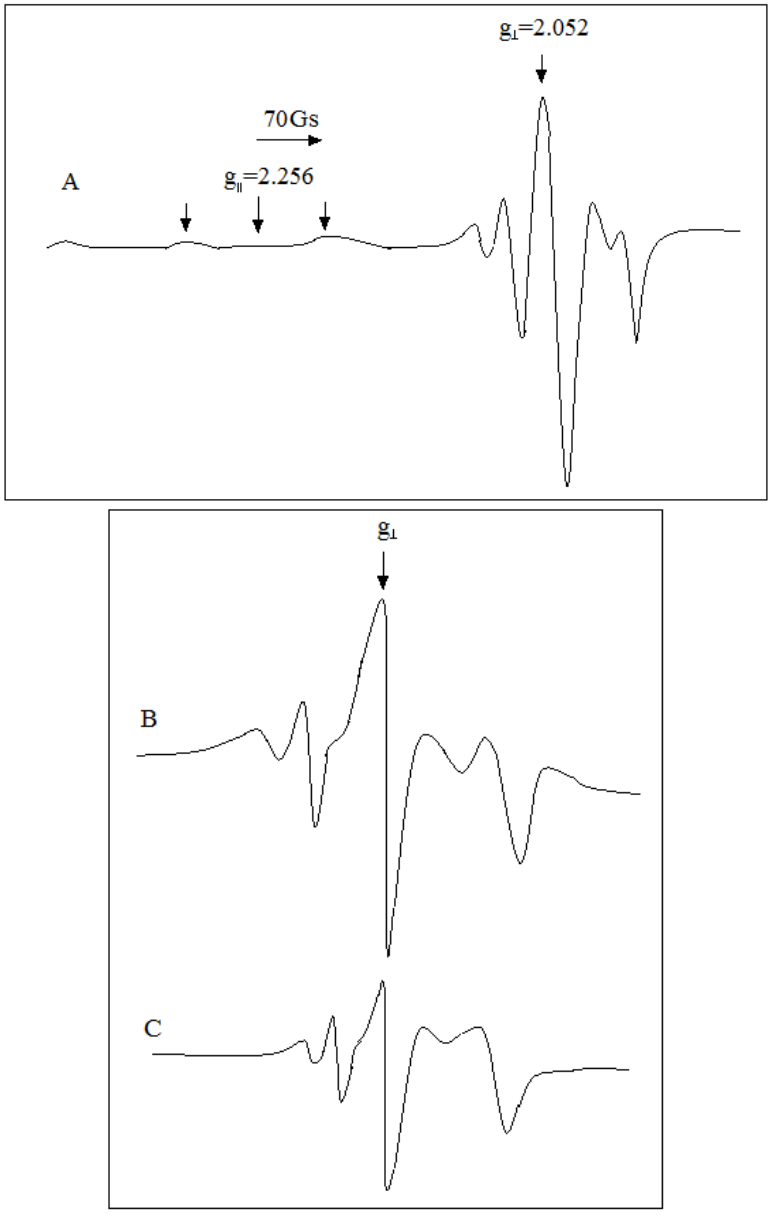

Fig.2. EPR spectra of fructose complexes: A- model complex with $CuCl_2$; B – chloroplasts treated with fructose; C – fragments of PS II treated with fructose.

In the subchloroplast particles of PS 2, we found a copper signal close in form to the EPR signal of plastocyanin observed in the same temperature region and corresponding to the weakly saturated component of the signal in the chloroplasts.

This fact and the recently detected reactivation of the chloroplasts by copper ions [9] and the influence of chelators on the reaction of PS 2 [10,11] plus detection of the copper fraction firmly held by the fragments of PS 2 suggest that the copper-containing complexes form an integral component of PS 2 and take part in its electron transport reactions.

Acknowledgement

The author expresses her acknowledgment to Prof. R.I. Khalilov for help in conducting individual experiments.

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ОБНАРУЖЕНИЕ СИГНАЛОВ ЭПР МЕДИ В ФОТОСИНТЕТИЧЕСКОМ АППАРАТЕ РАСТЕНИЙ: *IN VITRO* ИССЛЕДОВАНИЯ

А.Н. Насибова

Резюме: Статья посвящена изучению обнаружения в субхлоропластных частицах ФС 2 сигнала меди, близкого по форме к сигналу ЭПР пластоцианина, наблюдаемому в той же области температур и соответствующему слабо насыщенной составляющей сигнала в хлоропластах. Этот факт, а также обнаруженная реактивация хлоропластов ионами меди и влияние хелаторов на реакцию ФС 2, обнаружение фракции меди, прочно удерживаемой фрагментами ФС 2, позволяют предположить, что медьсодержащие комплексы образуют неотъемлемый компонент ФС 2.

Ключевые слова: спектры ЭПР, хлоропласт, фотосистема 2, пластоцианин.

BİTKİLƏRİN FOTOSİNTETİK APARATINDA MİSİN EPR SİQNALININ AŞKAR EDİLMƏSİ: *İN VİTRO* TƏDQİQATLARI

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Xülasə: Məqalə, FS2 subxloroplast hissəciklərində, eyni temperatur aralığında müşahidə edilən və xloroplastlarda zəif doymuş siqnal komponentinə uyğun olan, plastosianinin EPR siqnalına oxşar bir mis siqnalının aşkar edilməsinə həsr edilmişdir. Bu fakt mis ionları ilə xloroplastların aşkar edilmiş yenidən aktivləşdirilməsi və xelatatorların FS 2 reaksiyasına təsirini, FS 2 fraqmentləri ilə saxlanılan mis komponentlərinin aşkar edilməsini, mis tərkibli komplekslərin FS 2-nin ayrılmaz tərkib hissəsini təşkil etdiyini göstərir.

Açar sözlər: EPR spektrləri, xloroplast, fotosistem 2, plastosianin.