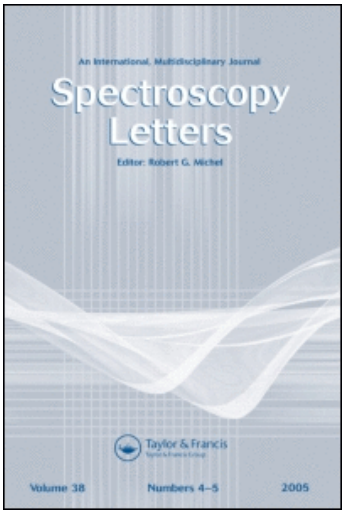

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ELECTRON SPIN RESONANCE INVESTIGATION OF FREE IRON IN TOBACCO LEAVES

Key words: ESR, free iron, plant leaves, tobacco leaves

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ABSTRACT

A loosely bound form of non-haem iron, i.e. free iron capable of forming dinitrosyl complexes with a pair of protein RS^- groups, characterized by an ESR signal with $g_{av}=2.03$ has been found in tobacco leaves by the ESR-method.

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INTRODUCTION

It has been shown that in animal tissues and microorganisms, loosely bound form of non-haem iron, so called free iron is also present in addition to well studied form of haem and non-haem iron existing in hemoglobine, cytochrome, iron sulphur containing proteins and other proteins (1,2). This iron does not form stable bonds with important biopolymers and can be easily removed from tissues by standard methods of dialysis (1,2). Free iron is one of the most important intracellular component and its presence in cell circumstances is necessary for the ferments functioning, sythesis of DNA and division of cells (3-6). In previous studies (2,7) it was reported that free iron can form paramagnetic complex with two NO molecules and a pair of RS^- groups of proteins.This complex is characterized by an ESR signal with axial symmetrical tensor of the g-factor ($g_{\perp}$=2.037, $g_{//}$=2.012) and g_{av} = 2.03. Therefore in accordance with the average value of the g-factor of the ESR signal, the complex is usually refered to as 2.03 complex (2,7). In all previous experiments, the free iron content was studied only in animal tissues. To our knowledge no study on the investigation of the presence of free iron in plants has been published in the literature. Therefore it is interesting to investigate the presence of the free-iron in plant objects such as tobacco leaves. On the other hand it has been reported that smokers' lungs have very significantly elevated levels of iron (8). Thus, in order to make clear the actual source of this iron, it is found to be interesting to investigate the presence of free iron, particularly, in tobacco leaves.

EXPERIMENTAL

Green leaves of tobacco of Samsun sort were used. In the leaves and homogenized leaves, the 2.03 complexes were obtained by nitrogen oxide treatment for 20 to 30 minutes under a pressure of 2.7-4.0 10^2 Pascal. The tobacco leaf homogenate was obtained by crushing the leaves in liqiud nitrogen. In order to separate the supernatant fraction (the cell cytosol), the homogenate was centrifuged for 20 minutes at 20 000 g acceleration . The ESR spectra of the leaves and their preparations were recorded by using "RE-1306" ESR-radiospectrometer and "Radiopan" ESR-radio spectrometer at 77K and 290K.

RESULTS AND DISCUSSION

ESR spectra of nitrogen oxide treated tobacco leaves recorded at 77 and 290K, together with ESR spectrum of untreated leaves recorded at 290K are given in Figure 1. The 2.03 signal ($g_{\perp}$=2.037, $g_{//}$=2.012), characterized 2.03 complex is observed in the ESR spectrum of NO treated sample, which is not present in that of untreated sample. Free radical signal, which obscures the $g_{//}$ component of this 2.03 signal is also observed in the ESR spectrum of the NO treated sample. The obscurity of the $g_{//}$ component was confirmed by subtraction of the ESR spectrum of untreated sample from that of the NO treated sample recorded at the same temperature (290K) on the "Radiopan" radiospectrometer, by using computer facilities. The 2.03 signal is clearly seen in the difference spectrum (Fig. 1 D) and it is also found to be identical to that observed in the ESR spectrum of the NO treated animal tissues (7).

The comparison of the ESR spectrum of the NO treated tobacco leaves recorded at 77K with that of at 290K (See Fig. 1 A and B) indicates that the anisotropy of the g factor of the 2.03 signal is conserved. This result shows that 2.03 complex in the tobacco leaves is formed with a pair of RS^- groups of a protein (or proteins) but not thiol containing relatively small molecules. Since if free iron in tobacco leaves complexed with thiol containing relatively small molecules in comparison to proteins, high mobility would lead to an average of the g factor's anisotropy and hence to narrowing of the ESR signal at room temperature (9).

Inorder to investigate the localization of the 2.03 complex in tobacco leaves, a homogenezation of the NO treated leaves was performed. Then the homogenate was centrifuged at 20 000g. As a result, approximately 75% of the total amount of 2.03 complex was found in the supernatant fraction i.e in the cell sap (Fig. 2 A and B). The same distribution was also observed in the experiments where the 2.03 complex had been obtained by nitrogen oxide treatment of pre-separeted homogenate fractions. In the ESR spectrum of the sample obtained after 3-4 hours long dialysis of the supernatant fraction from 0.01 M sodium citrate solution, the 2.03 signal was not observed any more (Fig 2 D). But, when a bivalent iron salt was introduced into the dialysed supernatant, with a subsequent nitrogen oxide treatment of this preparetion again the 2.03 signal was observed in the ESR spectrum of the sample (Fig 2 C). Analogous result was observed with the whole tobacco leaves in dialysis experiments. We also recorded the ESR spectrum of the

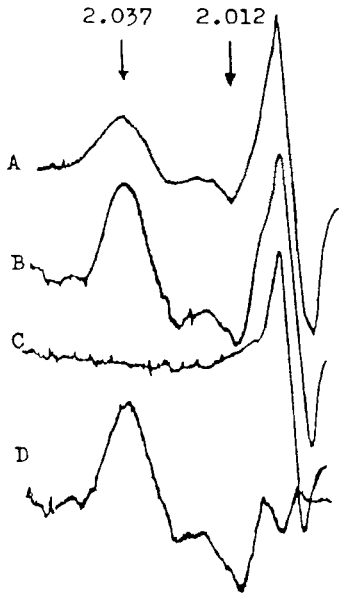

Fig.1. A and B- ESR spectra of nitrogen oxide-treated tobacco leaves recorded at 77 K (A) and 290 K (B) . C- ESR-spectrum of untreated tobacco leaves at 290K. D- Difference spectrum obtained by subtraction of spectrum C from B. The spectra were recorded with radiospectrometer ESR-"Radiopan". Modulation amplitude H_{mod} =0.05 mT, SHF-power P=0.5 mW.

leaves that were left for an hour in a 0.1 M sodium citrate solution and after then treated with NO. The comparison of this spectrum with the ESR spectrum of the leaves pre-treated by NO and after treated by sodium citrate, indicated that the amount of the 2.03 complex in the former was approximately five times less than that of the latter. But if a bivalent iron salt (0.01 M) was introduced into the sodium citrate solution, the amount of the 2.03 complex increased sharply to a level twice or three times as high as that of the reference leaves (kept in physiological solution). These results can be interpreted as follows; when the solution contains bivalent iron, the iron enters the leaves which leads to an increase in the amount of the 2.03 complex in it as compared to the reference level.

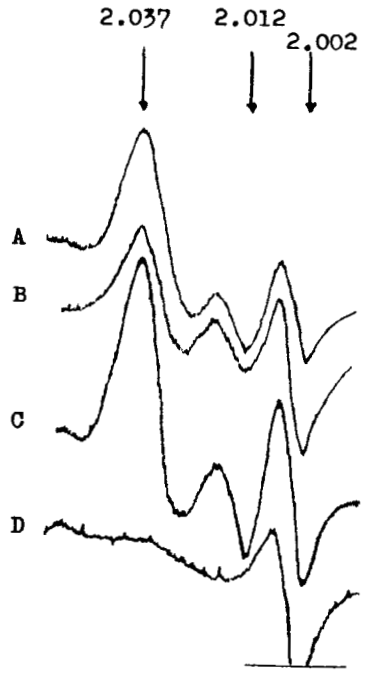

Fig.2. ESR-spectra of nitrogen oxide treated homogenized tobacco leaves and their preparations. A- the leaf homogenate B- supernatant fraction of the leaf homogenate C- preparation D after the introduction of Fe^{2+} salt into it and its subsequent treatment with NO. D- preparation B after 3-4 hours dialysis. The spectra were recorded at 77K by radiospectrometer ESR-"Radiopan". H_{mod}=0.05 mT, P=0.5 mW.

The obtained results were identical with those observed in dialysis experiments with non-cellular preparations of animal tissues (1,2) On the basis of ESR spectroscopic results, it can be concluded that there is a loosely bound form of non-haem iron (free iron) in tobacco leaves and its preparations. The concentration of free iron in tobacco leaves is found to be ca. 4-6 μg/g of moist tissue.

We propose that free iron in tobacco leaves plays analogous role to that in the animal tissues, namely, it involves in the sythesis of metalloferments which is

necessary for the functions of some proteins and cell divison. On the other hand the presence of loosely bound form of non-haem iron in tobacco leaves is significant with respect of the toxicology of smoking. Since it has been reported that the lungs and especially the alveolar macrophages of cigarette smokers contain high concentrations of iron (8,10,11). Our results together with those in the literature (8,10,12) lead us to suggest the following hyphotesis; cigarette smoke, which contains NO (13) induces the formation of nitroxil complexes of non-haem iron in organs and tissues of human body, as a result, iron accummulates in the lungs of smokers.

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