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OXYGEN-INDUCED FREE RADICAL IN WHEAT ROOTS

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ESR experiments have shown that free radicals are formed in wheat plant roots as a result of exposure to O_2. Although the radical(s) has not been positively identified, the nature of the spectra allows simple oxygen-derived radicals, such as O_2^-, $HO_2^{\cdot}$ and $OH^{\cdot}$, to be excluded as possibilites. Adsorption of Cu(II), but not Zn(II) or Cd(II) by the root results in a rapid decay of the radical signal to reach a level of 10% of its original intensity after a few minutes.

KEY WORDS: ESR, O_2-induced free radical, wheat roots

INTRODUCTION

In previous work on the uptake of copper(II) and oxovanadium(IV) ions by wheat roots[1-3], electron spin resonance (ESR) spectra demonstrated the presence of free radicals whose concentrations varied during the course of the experiments. This free radical is the subject of the present investigation which has been performed with the objective of identifying the origin of its signal. As the plant roots used in the earlier papers were exposed to high levels of transition metals and, in addition, were confined in a moist atmosphere in a narrow bore (3 mm internal diameter) quartz tube, the present experiments are concerned with identifying the possible effects of metal ion exposure and oxygen depletion and enrichment on the free radical concentration. A spin trap has also been used in an attempt to identify radical species that may have been formed, but which had half-lives too short for them to be seen in the direct ESR experiments.

MATERIAL AND METHODS

Sterilized wheat (Triticum aestivum L.c.v. Capelle) seeds were germinated under sterile conditions in half-strength Hoagland's nutrient solution[4] for 6 days and, after washing with distilled water, the roots of intact plants were threaded into open-ended quartz tubes of 3 mm internal diameter.

In the experiments in which the plants were exposed to transition metal ions, the tubes were placed for 30 minutes in solutions containing Cu(II) (100 μM or 100 mM), Zn(II) (100 mM) or Cd(II) (100 mM) as the sulphate salts, after which they were immersed several times in distilled water, to remove any excess metal. The tubes were then sealed at the lower end with a small amount of water in the bottom of the tube

to maintain humidity around the roots and then inserted into the ESR spectrometer cavity, taking care to ensure that the water was below the bottom of the cavity.

For oxygen enrichment and depletion experiments the open-ended tubes containing the wheat roots were placed in the spectrometer cavity, the lower end of the tube being connected to a system (shown diagramatically in Figure 1), which allowed a flow of humidified oxygen or nitrogen gas to be passed over the root surface. The effects of the gasses on the ESR signal were monitored by holding the spectrometer magnetic field constant at the maximum turning point of the 1st derivative of the radical signal so that a plot of signal intensity against time was recorded on the spectrometer. Separate measurements of complete spectra were performed to show that the position of the signal was unaltered during these experiments.

In the spin-trap experiment the tubes containing the wheat roots were immersed in a 0.032 mM solution of α-(4-pyridyl 1-oxide)-N-tert-butylnitrone (4-POBN) for one hour prior to exposure to oxygen or nitrogen gas as described above.

All ESR spectra were recorded at ambient temperature on a Varian E104A X-band spectrometer.

RESULTS

A typical ESR spectrum from a wheat root in an environment of moist air is shown in Figure 2. It consists of two clearly identifiable features, one a sextet with $g = 2.0022$ and $A = 9.47$ mT from the solvated Mn(II) ion and the other a single, comparatively narrow, peak (1st derivative peak to peak width 0.74 mT) with $g = 2.0045$. It is this latter free radical component that is the subject of the present communication.

After exposure of roots to Zn(II) and Cd(II) ions, no change in free radical peak intensity was observed over a period of 4 hours, but with Cu(II) there was a marked decrease in free radical concentration with time (Figure 3). Similar effects were

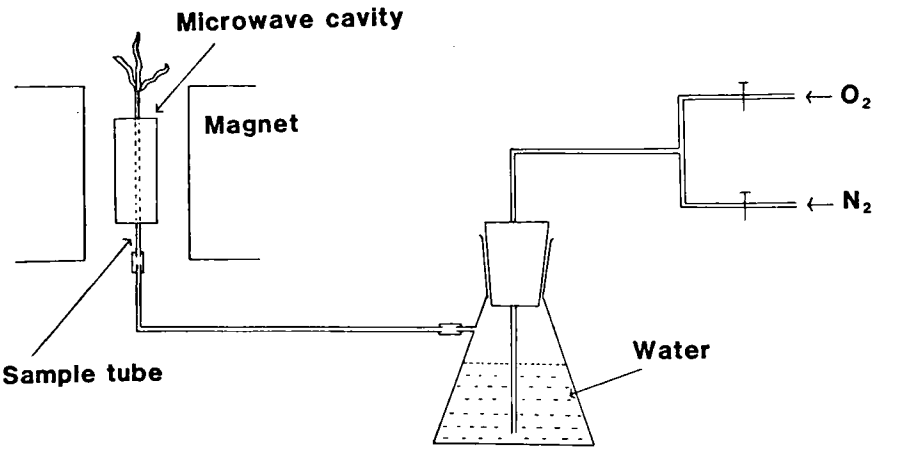

FIGURE 1 Diagramatic representation of the experimental procedure used for passing humidified oxygen or nitrogen gas over root surfaces in the ESR spectrometer cavity.

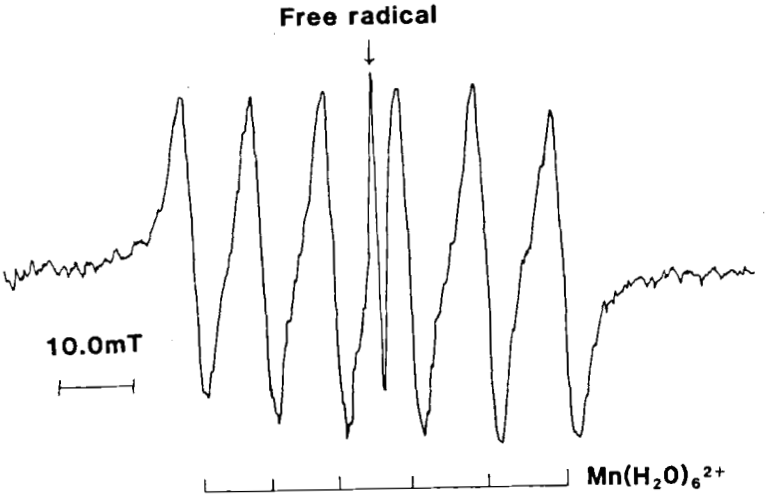

FIGURE 2 Typical ESR spectrum of a root of a wheat plant.

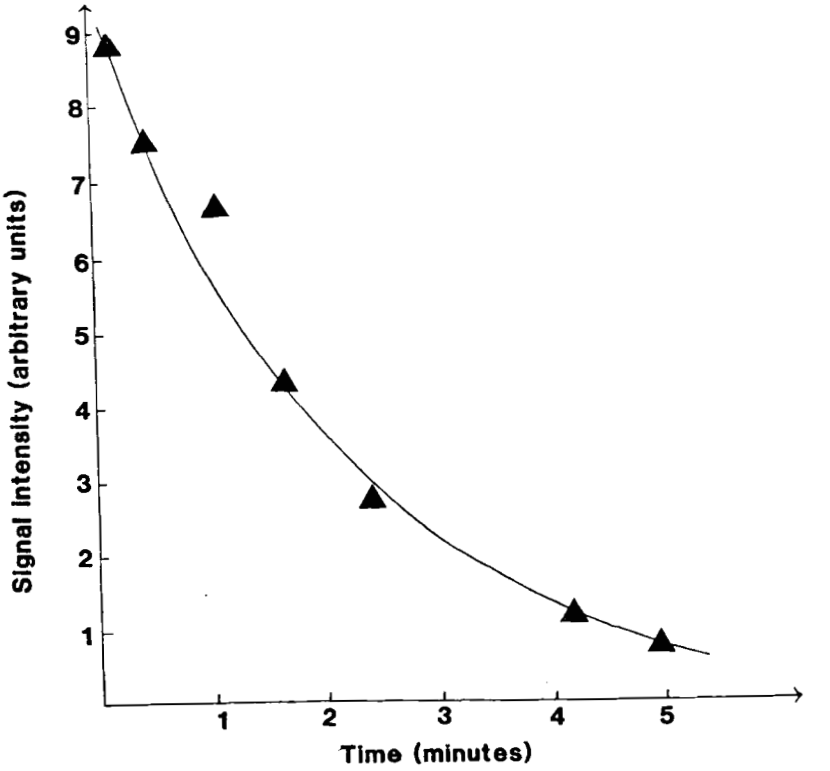

FIGURE 3 Variation in ESR signal intensity from a root of a wheat plant with time commencing approximately 10 minutes after removal from a 100 μM $Cu(II)SO_4$ solution, in which it has been placed for 30 minutes.

observed when solutions of high (100 mM) or low (100 μM) Cu(II) concentrations were used.

Changing the nature of the gas surrounding the root surface resulted in dramatic changes in free radical signal intensity and typical results are illustrated in Fig. 4. In this experiment a steady flow of nitrogen gas was passed over the root surface for 15 mins., during which time there was little change in signal intensity. After 15 mins. the gas was changed from nitrogen to oxygen and within seconds a rapid increase in free radical concentration commenced. The results in Figure 4 show that there was a 12–15 fold increase in signal intensity before it stabilized. This factor varied from one experiment to another, but in every case exposure to oxygen gas resulted in a marked increase in free radical production. After the system had stabilized the oxygen was exchanged by nitrogen and within seconds a rapid decrease in signal intensity commenced.

The times for increase or decrease to half maximum signal varied somewhat from one experiment to another, but always they were of the order of a few minutes. This time represents a maximum for the half-life of the free radical in the wheat root, but the true half-life of the radical could be considerably less, because no account could be taken of the rate of diffusion of oxygen through the tissue, or of the possibility that the radical was being formed from a precursor, not detectable by ESR, but with a much greater half-life. Also, although variations in free radical signal strength could be observed on repeated nitrogen/oxygen cycling, the effect became less pronounced with increasing numbers of cycles. This was seen as progressively lower maxima and higher minima in signal intensity in curves like that in Figure 4.

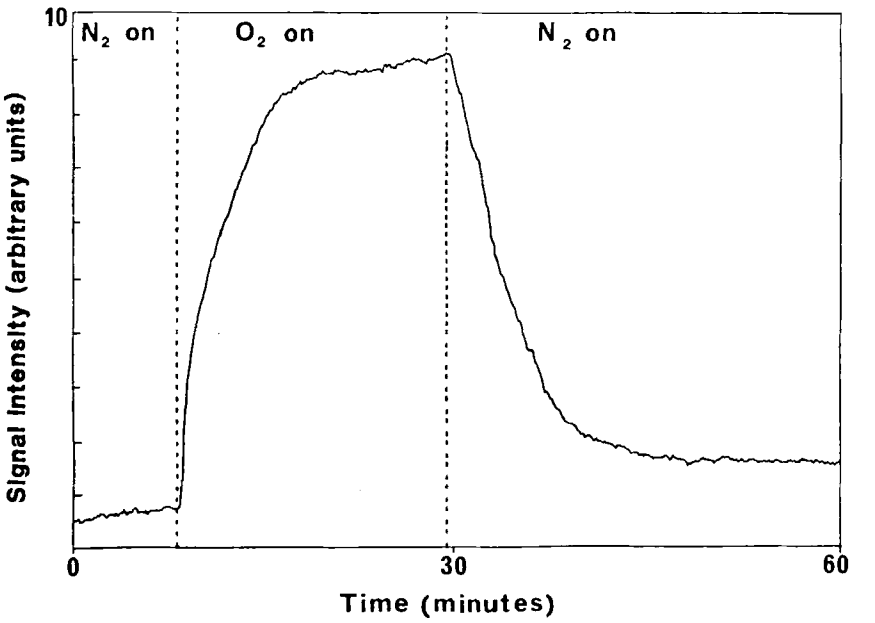

FIGURE 4 Variation in ESR signal intensity from a root of a wheat plant with time, showing the effects of variation in the composition of the gaseous environment of the root.

No qualitative differences in the free radical signal were observed for those roots that had been treated with 4-POBN, and certainly no signals that could be attributed to free radical adducts were seen in these experiments. A spectrum of a root cooled to 77 K in liquid nitrogen was also recorded and found to be qualitativley identical to that obtained at ambient temperature. Although there was an apparent decrease in intensity, this may have been the result of experimental conditions, and no new features characteristic of anisotropic free radicals were observed.

DISCUSSION

The presence of inorganic, oxygen-based radicals such as superoxide (O_2^-), its protonated equivalent, hydroperoxide, (HO_2) and hydroxyl (OH) are well established in biological systems. Of these radicals the short-lived and highly reactive hydroxyl species is considered to account for most of the damage seen in such systems[5] and is also implicated in lipid peroxidation[6]. Production of this radical probably occurs through the redox-catalysed reaction[7] (Scheme 1).

The hydroxyl radical has not been observed directly in biological systems by ESR spectroscopy, but its presence has been demonstrated by the use of spin traps[8]. In particular, 4-POBN has been shown to be highly suitable in the detection of this radical in solution between pH 6 and 7[9]. The superoxide radical is not seen at ambient temperature, but at 77 K it gives an anisotropic ESR spectrum with axial symmetry. The $g_\parallel$-values are typically 0.07 to 0.11 higher than free spin ($g = 2.0023$), the exact magnitude depending on the environment surrounding the radical[10]. The hydroperoxy radical has spectral characteristics similar to the superoxide radical but with a lower value of g (e.g. $g_\parallel = 2.049$ in a γ-irradiated hydrogen peroxide-urea compound[11]). In general, peroxy radicals typically show an increase in $g_\parallel$ from free spin of 0.03 to 0.04[10].

Although it is related to O_2 metabolism the free radical observed in the present experiments is not the superoxide ion (O_2^-), the hydroperoxy radical (HO_2) or the hydroxyl radical (OH). The existence of any of these radicals as a precursor cannot, however, be discounted, even though no adducts were observed in the experiment with 4-POBN. The observation of such adducts in intact biological systems is frought with difficulties requiring (a) the spin trap to be stable in the absence of free radicals, (b) the spin trap to approach the sites of formation of free radicals in amounts sufficient to give reasonable concentrations of adducts and (c) the adducts to be comparatively unreactive so that they have half-lives long enough to permit observation. The negative result obtained here, therefore, provides no information on the free radical pathways within the plant root.

By a process of elimination it seems possible that the free radical observed in the wheat roots is an organic radical formed as a by-product during O_2 metabolism. The present experiments do not provide sufficient information for its identification and a number of possibilties exist. The most obvious of these are organic oxide or peroxide radicals, which would have much greater stability than their simpler precursors and have been shown to produce simple spectra observable at ambient temperature. The

Scheme 1

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH$$
$$Fe^{3+} + O_2^- \rightarrow Fe^{2+} + O_2$$

comparative stability of the radical indicates a degree of protection for the free radical site, possibly as a result of its incorporation in high molecular weight species, e.g. lipids, or alternatively the radical may not be in solution, but immobilized in the cell structure as a product of tissue oxidation. Spectral characteristics are in fact quite similar to those of melanins, which are complex organic polymers which have incorporated a number of aromatic moieties, including dihydroxy phenols.

The result with the root that had been exposed to copper is interesting in that it suggests that copper, but not zinc or cadmium, can either inhibit the rate of oxygen metabolism or, more probably, increase the efficiency with which the free radical or its precursors are inactivated in the plant.

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