Current Topics in Biophysics 2010, 33 (suppl A), 73-76

FREE RADICALS OF THE PINE NEEDLES AS AN INDICATOR OF DAMAGE TO FORESTS CAUSED BY CAR EXHAUST GASES.

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Received June 22, 2010; accepted July 26, 2010; published online November 20, 2010.

Electron Spin Resonance (ESR) spectra have been recorded from needles of pines growing deep in a forest, far from municipal areas, and of pines growing in the vicinity of roads with heavy traffic. An analysis of the spectra has revealed that pine needles may serve as a sensitive indicator of damage to forests caused by chemical pollutants (car exhaust gases). Two effects have been observed: (1) loss of manganese ions and (2) in vitro detection of a weak signal from the ascorbyl radical, indicating loss (utilization) of vitamin C in vivo (defense reaction of needles to oxidative stress). A new method (preliminary paper) is presented which makes it possible to perform such studies using an ESR spectrometer with a relatively low sensitivity, making the method of accumulated spectra obsolete.

INTRODUCTION

The antioxidative system (ascorbic acid) of pine needles may serve as an indicator of damage to forests caused by chemical pollution. Stegman (1989, 1990) have shown that pine needles from forests exposed to gas smog generate electron spin resonance (ESR) signals (doublet) corresponding to the $\dot{A}H^{-}$ ascorbate free radical (Buettner 1995). These signals either reflect antioxidative properties (free radical scavenging) of vitamin C or result from its direct oxidation. ESR signals from pine needles present ex vivo are relatively weak, requiring a sensitive spectrometer and spectral accumulation for detection. A different approach to this problem is now presented. It is known that an ESR spectrum from tissue in vitro (lyophilization and exposure to air) contains a strong signal from the $\dot{A}H$ ascorbyl free radical (singlet) produced from vitamin C (Lohmann 1984, Gonet 1994, Buettner 1995). The author has noted that signals of this type appear in vitro in pine needles subjected to aging (natural drying).

MATERIAL AND METHOD

Needles of pines (Pinaceae) growing deep in a forest, far from municipal areas (30 km in the vicinity of Goleniów, Buk and Brzózki) served as control (group A, 10 samples). Group B (5 samples) was composed of needles from pines growing near a road with heavy traffic (Szczecin – Lubieszyn). Group C (5 samples) included needles collected from pines growing in Szczecin (Ku Słońcu and Gen. Sikorski streets), continuously exposed to car exhaust gases. The study was performed in summer time. Measurements were performed using an XE 2544 ESR spectrometer from Radiopan Firm, under the following measurement conditions: cylindrical resonance chamber RCX660, 9.45 GHz frequency, 3mW power, 100 kHz modulation of amplitude 0.25 mT, scan 200 mT/4 min, time constant 1s, amplification $1x10^4$. DPPH (diphenylpicrylhydrazyl) was used to determine the position of line (g). Spectra were recorded immediately (2 hours) after collection of needles, and 7, 30 and 90 days thereafter. Details of ESR method are in earlier papers (Ikeya 1993, Gonet 1994). Needles were stored in an air-conditioned room (temp. 23 ± 2^0 C, humidity $33 \pm 5\%$).

RESULTS

ESR spectra in group A recorded immediately after collection demonstrate six strong signals corresponding to Mn^{2+} ions (see Ikeya 1993, p.43). On the contrary, ESR spectra in group C (same analytical conditions as for group A) did not contain (fig. 1) or contained very weak (group B) signals from Mn^{2+}. Seven days later, the Mn^{2+} signal amplitude in group A was reduced and a new signal $g = 2.005$, $\Delta B_{pp} = 1$ mT appeared, representing the ascorbyl radical. A weak ascorbyl signal was detected in groups B and C. This trend continued beyond 30 days (fig. 2) and by the 90th day the signals from manganese ions in group A disappeared altogether. Instead, a very strong ascorbyl signal was detected. The amplitude of the ascorbyl (not ascorbate) signal increased in groups B and C, albeit less than in group A (fig. 3). After 90 days these changes were more accentuated. Signal amplitudes in groups A, B and C against time, and variability are shown in Table 1. All differences for X (M^{2+}) and Y ($\overset{\bullet}{A}H$) parameters in the relations A-B, A-C and B-C of groups at each time (immediately, 7, 30, 90 days) are statistically significant ($p<0,01$).

DISCUSSION

The results clearly reveal that needles of pines growing away from sources of chemical pollution (group A) ions. It is believed that Mn^{2+} ions mediate in the transport of electrons from water to photosynthetic system II. Therefore, this aspect of photosynthesis seems undisturbed in these trees. Needles of pines exposed to car smog (groups B and C) may demonstrate abnormalities of photosynthesis due to the loss of manganese ions following their reactions with exhaust gases (O_3, SO_2, NO_2). One may infer, therefore, that the content of manganese ions is an indicator of impaired photosynthesis in pine forests. This finding was reproducible in all cases, with the exception of some pine needles in group B in which trace amounts of Mn^2 were detected. Time changes in ESR spectra from pine needles (2h – 90 days) were uniform in that the ESR signal from manganese ions disappeared and a new

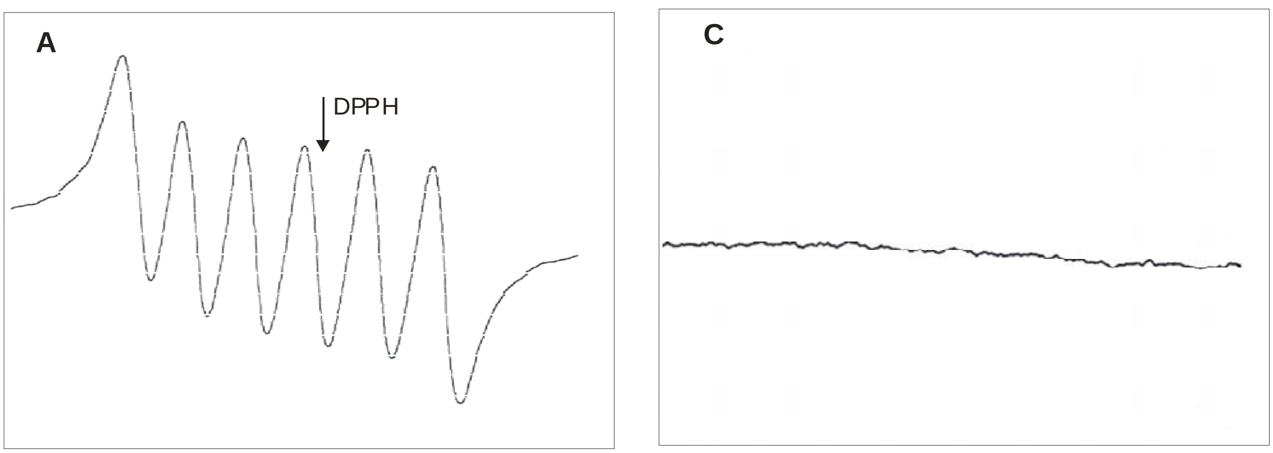

Fig. 1. ESR spectrum from pine needles immediately after collection, growing away from municipal agglomerations (group A), or exposed to car exhaust gases (group C).

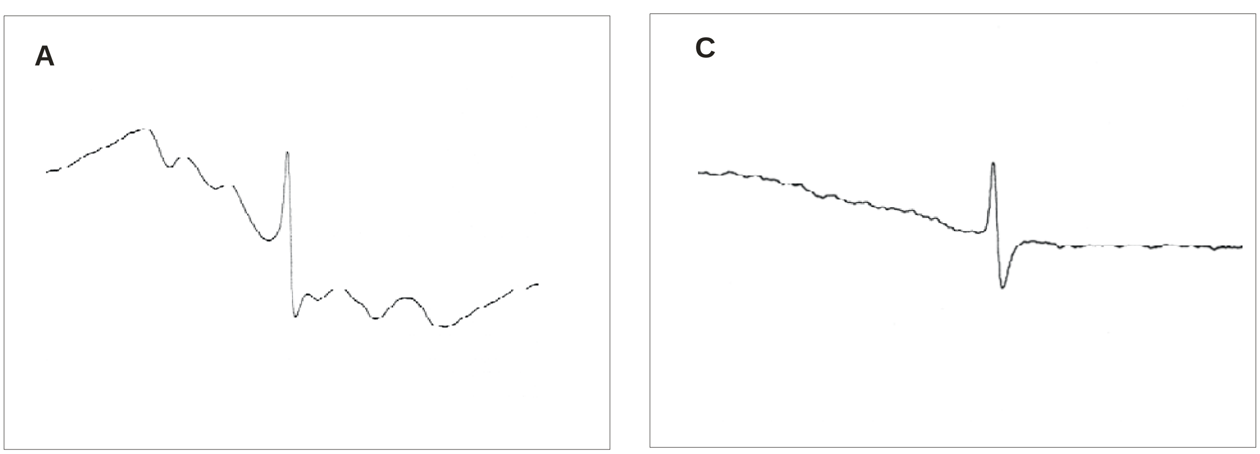

Fig. 2. ESR spectrum from pine needles 30 days after collection, growing away from municipal agglomerations (group A), or exposed to car exhaust gases (group C).

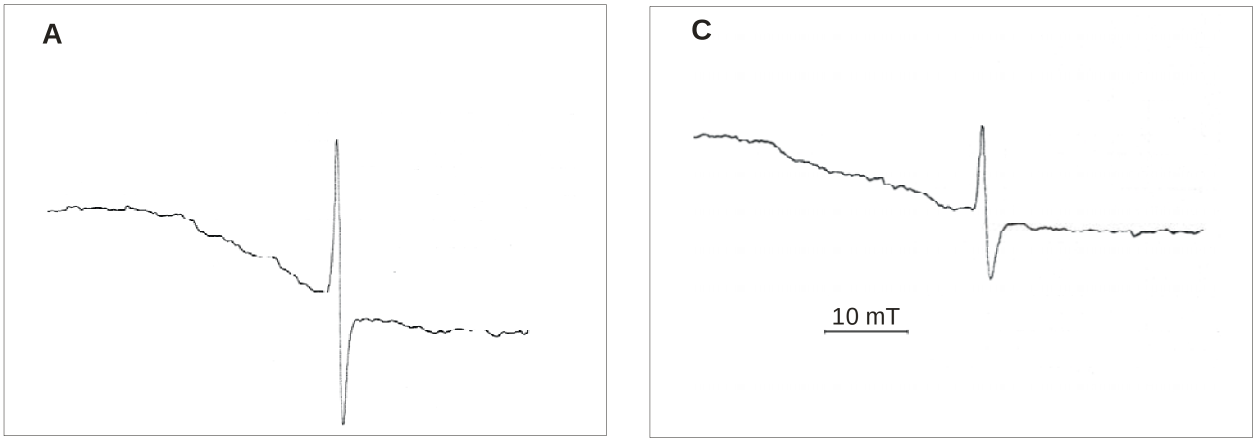

Fig. 3. ESR spectrum from pine needles 90 days after collection, growing away from municipal agglomerations (group A), or exposed to car exhaust gases (group C).

Table 1. Amplitudes of ESR signals (relative units) as a function of time for manganese ions (X) and ascorbyl radical (Y) (s - standard deviation), n – number of samples.

days / Groups	Immediately	7 days	30 days	90 days
A n=10	X ± s	72 ± 9	54 ± 6	12 ± 2
	Y ± s	0	5 ± 2	41 ± 5
B n=5	X ± s	5 ± 1	0	0
	Y ± s	0	9 ± 2	30 ± 4
C n=5	X ± s	0	0	0
	Y ± s	0	4 ± 0,5	17 ± 2

signal (singlet, g = 2.005, ΔB_{pp} = 1 mT), representing the ascorbyl free radical, appeared. The ascorbyl signal was detected in all groups (A, B and C); the signal was weakest in needles exposed to car smog (group C).

What is the possible mechanism of the ascorbyl signal? It has been shown in numerous studies (Cimbolaityte 1982, Lohmann 1984, Neubacher 1984, Gonet 1994) that the ESR signal in lyophilized tissues (blood, liver, lungs) measured with exposure to air in vitro originates from the ascorbyl free radical $(\dot{A}H)$ as an in vivo product of vitamin C. It is produced during the interaction of AH^{-} ascorbic acid (ionized form) with membrane matrices, after lyophilization and exposure to ambient air. Presumably, the storage of pine needles is, in some respects, similar to slow lyophilization, and by analogy, the ascorbyl free radical $(\dot{A}H)$ is generated appropriately to in vivo vitamin C levels. Pine needles exposed to smog have consumed their vitamin C during scavenging of free radicals generated by chemical pollutants, and a consequence, ESR signals are weaker as compared to these of "healthy" pines (group A). Considering the fact that the free radical signal always has the same shape, the relative height of the signal (peak vs peak) may be taken as a measure of the number of unpaired electrons and amount of forest contamination. The weaker this signal is, the greater is the loss of vitamin C and the more contaminated is the environment. In line with the present results, Stegman (1989, 1990) have observed an in vivo increase of the ascorbate radical signal (doublet) in pine needles exposed to smog as a reflection of intense scavenging of free radicals by vitamin C and its subsequent loss.

CONCLUSION

ESR spectra from pine needles may serve as an indicator of damage to forests by chemical pollutants (car exhaust gases). Two effects are observed in this case: loss of manganese ions and appearance in vitro of a weak ascorbyl free radical signal reflecting the loss (consumption) of vitamin C in vivo (defense reaction of pine needles to oxidative stress). This approach allows for the use of an ESR spectrometer with a relatively low sensitivity, without the need for spectra accumulation.

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