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A FREE RADICAL UBIQUITOUSLY ASSOCIATED WITH SENESCENCE IN PLANTS: EVIDENCE FOR A QUINONE

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The interpretation of EPR and ENDOR measurements on an organic free radical which appears to be a universal concomitant of senescence in plants is discussed. On the basis of EPR spectra obtained at 95 GHz it is speculated that the radical is derived from a quinone.

KEY WORDS: Senescence, quinone.

INTRODUCTION

In several recent studies in Sheffield biochemical assays have established a correlation between the onset of senescence and loss of viability in plants with the development of an EPR signal characteristic of an organic free radical. The radical has been studied in a range of photosynthetic tissues including mosses[1], various gasses[2], and autumn leaves of *Aesculus hippocastanum*[3], and in the non-photosynthetic tissues of germinating maize (*Zea mays* L.)[4], and seeds of oak (*Quercus robur L.*)[5]. We have also followed qualitatively the development of the EPR signal in many other photosynthetic materials including, for example, the autumn leaves of beech (*Fagus sylvatica*), lime (*Tilia x europaea*), tomato (*Lypersicon esculentum*), and *Geranium spp.*, and noted its occurence in samples of herbs dried for culinary use, for example marjoram, oregano, parsley, and thyme, and in commercially dried tea leaves[6]. There have also been other reports of the appearance of the radical in desiccated and damaged plant material, for example, in seeds of soya (*Glycine max*)[7], in various seeds and pollens[8], in diseased potato tubers[9], and in leaves of radish (*Raphanus sativus*), bluegrass (*Poa pratensis*), and ryegrass (*Lolium perenne*)[10]. It is clear that damage to plant tissue is characterised by the formation of the free radical so its identification is a matter of some interest and importance. In this note we comment on problems in making this identification and report some new observations which are helpful.

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METHODS

Electron paramagnetic resonance (EPR) and electron nuclear double resonance (ENDOR) spectra run in Sheffield were obtained using a Bruker ER-200D EPR (X-band) spectrometer with a 100W ENDOR unit and Aspect 2000 minicomputer. The low temperatures at which ENDOR measurements were made were achieved and maintained using a Bruker ER 4111VT variable-temperature unit. Following examination in Sheffield plant material from the same batch was sent to Berlin where it was examined using a spectrometer operating at 95 GHz (W-band). This instrument is built round a Fabry-Perot type resonator with a low-noise solid state Gunn oscillator or klystron as the microwave source[11].

The moss, *Dicranella palustris* (Dicks.) Crundw. ex E.F. Warb was collected from Rydal Fell, Cumbria, and subsequently allowed to dry out under open laboratory conditions. Other plant material was collected from the Sheffield area, or obtained commercially. Quinones were purchased from Aldrich and purified by sublimation.

RESULTS AND DISCUSSION

The X-band EPR spectrum of the radical is uninformative: it is a structureless line, slightly broadened on the high field side, with a breadth between points of maximum slope of 1 mT, and the lineshape is independent of temperature down to 100 K. A typical example, obtained from a dry beech leaf, is shown in Figure 1a. Values of the g-factor reported in the literature vary over the range 2.0045–2.0055. This range of values, which corresponds to a field range of 0.175 mT for a species having $g = 2.0050$ at an observing field of 0.35 T, may seem surprisingly large. However the signal is often observed together with that of a naturally-occurring manganese (Mn^{2+}) species, centred on $g = 2.00$, whose relative intensity varies with the plant species and its history. Further, both of these spectra may be superimposed on a broad underlying feature which may be assigned to an Fe^{3+} species. These underlying features from transition metal ions may cause variations between samples of the field corresponding to the peak of the radical absorption. A further point is that the radical spectrum overlaps that of 1,1-diphenyl-2-picrylhydrazyl (DPPH) which has been commonly used as a g-marker. This is illustrated by the spectrum of Figure 1b, which is of a sample of tea including a DPPH marker. We measure the separation of the peaks in this spectrum as 0.308 ± 0.014 mT and infer a g-value of 2.0053–2.0054 for the radical. In the Sheffield work[1-6] the identities of the radicals formed in different materials have been confirmed as a matter of routine by running mixed samples of two different materials, one of them often moss[1], which separately give spectra of comparable intensity, and checking that there was no change in lineshape for the mixed sample.

We have endeavoured to obtain information about small hyperfine couplings by making ENDOR measurements on various samples down to 100 K. Results for moss have already been reported[1] but another characteristic spectrum is displayed in Figure 1c; it was obtained from the beech leaf whose EPR spectrum is shown in Figure 1a. It is a typical matrix proton ENDOR signal, the sharp dip at the free proton frequency being dependent on microwave power. We have run long accumulations over the radiofrequency range which would embrace any proton hyperfine interactions lying within the EPR linewidth but the ENDOR spectrum is completely flat except for this matrix proton signal. We have also searched the spectral region

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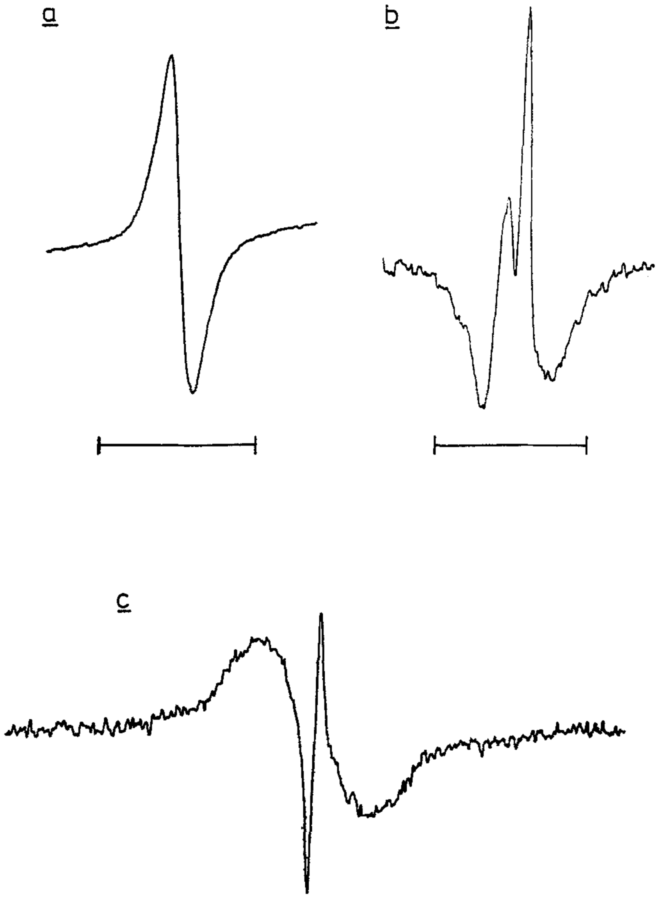

FIGURE 1 X-band EPR and ENDOR spectra. (a) First derivative EPR of dry beech leaf. (b) Second derivative EPR of tea + DPPH. In these EPR spectra, both taken at room temperature, the field scale marker indicates 2.5 mT. (c) First derivative proton ENDOR at 110 K of the beech leaf whose EPR spectrum is shown at (a): frequency sweep, 6 MHz; free proton frequency, 14.56 MHz; microwave power attenuation, 20 dB; radiofrequency power attenuation, 0 dB; 30 scans accumulated.

corresponding to small nitrogen hyperfine couplings, again with no success. These negative observations do not necessarily prove that there are no hyperfine interactions for they may simply reflect the physical nature of the sample. It may be that ENDOR measurements at lower temperatures would be more fruitful but at present there are, to the best of our knowledge, no hyperfine data which would assist in identifying the radical.

A principal object of this note is to report that more encouraging results have been obtained from samples of desiccated moss examined using an EPR spectrometer operating at 95 GHz (W-band)[11]. At this frequency one obtains Zeeman magnetoselection of spectral features with different g-values which is ten times higher than that afforded by measurements at X-band (9.5 GHz). The spectrum of the radical is compounded with one from Mn^{2+} and this shows up very strongly at the high

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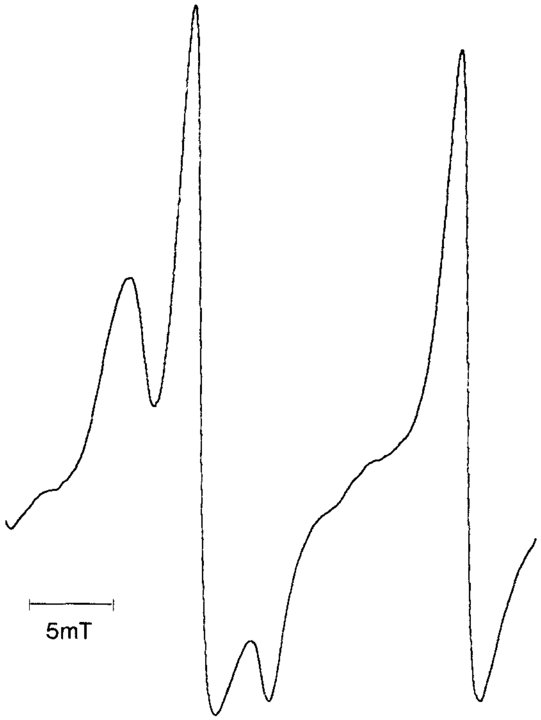

FIGURE 2 First derivative EPR spectrum of moss taken at 95.77 GHz (W-band), 240 K. The strong lines are the two central components, $|M_I| = 1/2$, of the six-line spectrum of Mn^{2+}. The features indicated with arrows are assigned to the *g*-tensor extrema of the radical, the corresponding *g*-values being 2.0054 and 2.0023. The field scale marker indicates 5 mT.

frequency but at low microwave power two features, which we confidently assign to two principal values of the g-tensor of the radical, are evident. The g-values are 2.0054 and 2.0023. They are illustrated in Figure 2, in which the two central lines, $|M_I| = 1/2$, of the six-line Mn^{2+} spectrum are also displayed. We suggest that these two g-values are consistent with the radical being derived from a quinone. The basis of this suggestion is comparison with the values of the principal components of the g-tensors for the radical anions of 1,4-benzoquinone and of 7,8-dimethyl-1,4-naphthoquinone obtained from W-band measurements on frozen solutions.[12] For the former the principal values of the g-tensor are found to be gXX = 2.0065, gYY = 2.0053, and gZZ = 2.0023, where X lies along the C-O bonds and Z is perpendicular to the plane of the radical; for the substituted naphthoquinone the values are gXX = 2.0057, gYY = 2.0050, and gZZ = 2.0022. Our lowest g-tensor component of 2.0023 is clearly assignable as gZZ and we assign the higher value of 2.0054 as gXX: there is nothing evident in the spectrum down to the next Mn^{2+} hyperfine component to low field so we can rule out any g-feature in the range 2.0054 up to at least 2.008. We thus take it that the intermediate g-tensor component is masked by the strong Mn^{2+} line. The X-component is somewhat lower than the values observed in the model compounds but we note that this component would be reduced if the lone pair electrons on oxygen were involved in any interaction of the radical with its environment. We also note that the suggested range of g-values, which corresponds to a spread in field of 0.54 mT at X-band, is perfectly consistent with the linewidth of 0.9 mT observed for the free radical spectrum recorded at that frequency.

In conclusion, we consider it to be established beyond doubt that there is a unique and probably ubiquitous free radical species which reflects damage in senescent plant material and we postulate that this radical derives from a quinone. Whether the precursor is a quinone involved in an electron transport chain, a simple phenolic secondary metabolite, or a more complex polyphenol, is a problem which we expect to address in future work.

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References

1. W. Seel, G. Hendry, N. Atherton, and J. Lee (1991) Radical formation and accumulation *in vivo*, in desiccation tolerant and intolerant mosses. *Free Radical Research Communications*, **15**, 133–141.
2. P. C. Thorpe (1993) *Ph.D. Thesis*, The University of Sheffield.
3. M. N. Merzlyak, G.A.F. Hendry, N.M. Atherton, T.V. Zhigalova, V.K. Pavlov, and O.V. Zhitenva (1993) Pigment destruction, lipid peroxidation and free radical formation in leaves during autumn senescence. *Biokhimiya*, **58**, 240–249.
4. O. Leprince, R. Deltour, P.C. Thorpe, N.M. Atherton, and G.A.F. Hendry (1990) The role of free radicals and radical processing systems in loss of desiccation tolerance in germinating maize (*Zea mays* L.). *New Phytologist*, **116**, 573–580.
5. G.A.F. Hendry, W.E. Finch-Savage, P.C. Thorpe, N.M. Atherton, S.M. Buckland, K.A. Nilsson, and W.E. Seel (1992) Free radical processes and loss of seed viability during desiccation in the recalcitrant species *Quercus robur* L. *New Phytologist*, **122**, 273–279.
6. N.M. Atherton, G. Collins, and G.A.F. Hendry, *unpublished observations*.
7. D.A. Priestley, M.B. McBride, and C. Leopold (1980) Tocopherol and organic free radical levels in soybean seeds during natural and accelerated aging. *Plant Physiology*, **66**, 715–719.
8. D.A. Priestley, B.G. Werner, A.C. Leopold, and M.B. McBride (1985) Organic free radical levels in seeds and pollen: the effects of hydration and aging. *Physiologia Plantarum*, **64**, 88–94.
9. L.S. Monk, D.B. McPhail, B.A. Goodman, and H.V. Davies (1989) An electron spin resonance investigation of internal rust spot, a physiological disorder of the potato tuber. *Free Radical Research Communications*, **5**, 345–350.
10. V.C. Runeckles and M. Vaartnou (1992) Observations on the *in situ* detection of free radicals in leaves using electron paramagnetic resonance spectroscopy. *Canadian Journal of Botany*, **70**, 192–199.
11. O. Burghaus, M. Rohrer, T. Götzinger, M. Plato, and K. Möbius (1992) A novel high-field/high-frequency EPR and ENDOR spectrometer operating at 3 mm wavelength. *Measurement Science and Technology*, **3**, 765–774.
12. O. Burghaus, M. Plato, M. Rohrer, and K. Möbius (1993) 3 mm high field EPR on semiquinone radical anions $Q^{-\cdot}$ related to photosynthesis and on the primary donor $P^{+\cdot}$ and acceptor $Q_A^{-\cdot}$ in reaction centers of *Rhodobacter sphaeroides* R-26. *Journal of Physical Chemistry*, in press.

Accepted by Professor B. Halliwell

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