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An Example of *In Vivo* Analysis by L-Band ESR Technique Using a Loop-Gap Resonator

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An absorption behavior of TEMPOL radical in celery has been investigated as an example of *in vivo* analysis by L-band ESR technique using a loop-gap resonator. TEMPOL radical absorbed was found to be diluted in a stalk of celery and disappeare finally at leaves according to the first order reaction.

In our previous paper,[1] it has been reported that L-band ESR spectrometer was constructed by using a loop-gap resonator with an electric shield and that it will be possible to analyze large quantities of wet biological samples at *in vivo* state. As our loop-gap resonator has a diameter of 29 mm and a length of 28 mm, various living things will be put in its resonator. In this paper, the measurement of fresh celery is presented as the first example of *in vivo* analysis by our L-band ESR technique using a loop-gap resonator.

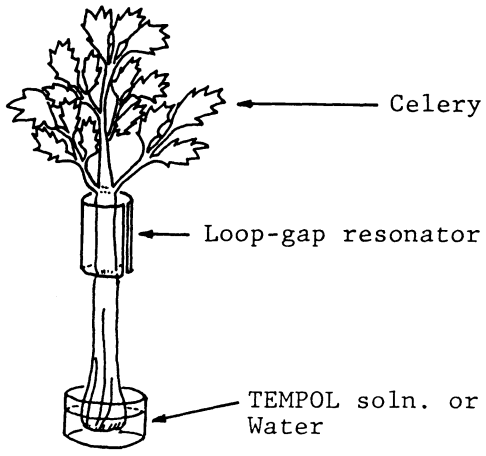

Fig. 1. General view of experimental system.

The absorption of nitroxide radical in celery was examined at room

temperature. A stalk of celery, whose diameter is about 20 mm, was inserted into a loop-gap resonator, and then a cut end of the stalk was soaked in an aqueous solution of 0.1 mol dm^{-3} 4-hydroxy-2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPOL). The TEMPOL solution was placed under the resonator as is illustrated in Fig. 1. For a short time, a typical hyperfine structure of three line due to a nitrogen nucleus in TEMPOL molecule was observed on the ESR spectrum as shown in Fig. 2. The signal intensity increased gradually with time. The intensity of the hyperfine structure at lower field is plotted in Fig. 3. The stalk of celery was soaked after 80 minutes in water in exchange for radical solution. After a few minutes, the signal of TEMPOL began to decrease and finally disappeared.

In Fig. 4, ESR spectrum of 0.1 mol dm^{-3} TEMPOL solution itself is given. The broad line shape of 0.1 mol dm^{-3} TEMPOL is considered to be due to a dipole-dipole interaction because of high concentration of TEMPOL. These results mean that TEMPOL is either diluted simply with tissue water or decomposed by chemical reaction in tracheal tissue, since celery sucks up TEMPOL solution through tracheal tissue in stalk by capillary action.

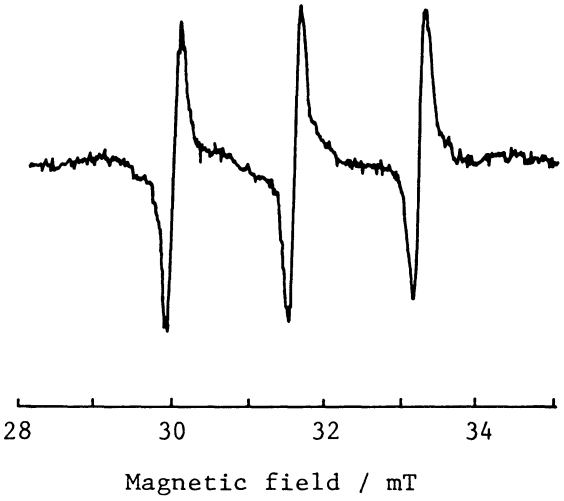

Fig. 2. L-band ESR spectrum of TEMPOL absorbed in celery stalk.
Freq. : 930.26 MHz
Mod. : 0.1 mT
Amp. : 2 x 100
Power : 8 mW

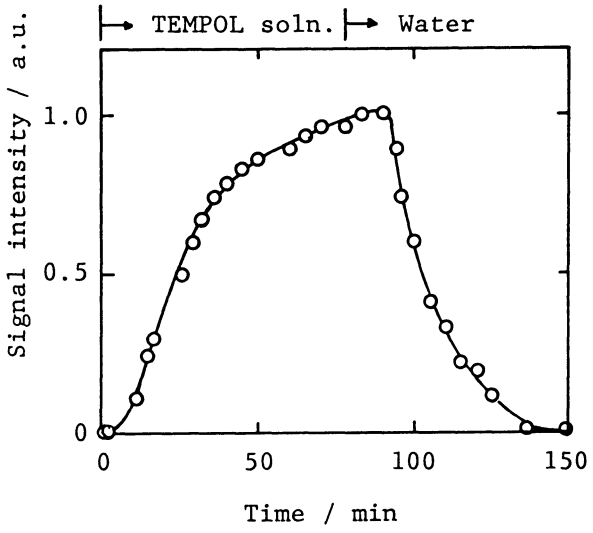

Fig. 3. Absorption behavior of TEMPOL in fresh celery.

In order to examine the reaction of TEMPOL in celery, a new celery was soaked in 0.1 mol dm^{-3} TEMPOL solution. After one hour, the stalk of 3 cm in length was cut out and inserted into a loop-gap resonator. Immediately, the change of signal intensity of TEMPOL was measured. As shown in Fig. 5, the signal intensity of TEMPOL in stalk did not decrease for about one hour. Therefore, it is considered that tracheal tissue in stalk absorbs TEMPOL with dilution of TEMPOL and without decomposition of it by chemical reaction.

On the other hand, leaves of celery treated by the same method as the stalk indicated the decrease of TEMPOL according to the first order reaction as given in Fig. 5. The reaction rate constant is calculated to be 5.3×10^{-4} s^{-1}. Juice of 1 ml squeezed from the stalk or the leaves of fresh nontreated celery was mixed with 1 ml of 1×10^{-5} mol dm^{-3} TEMPOL solution. The decrease of signal intensity was observed only in the case of juice from leaves as shown in Fig. 5. However, the reaction rate constant (4.0×10^{-3} s^{-1}) is larger than that of *in vivo* analysis. The experiment of

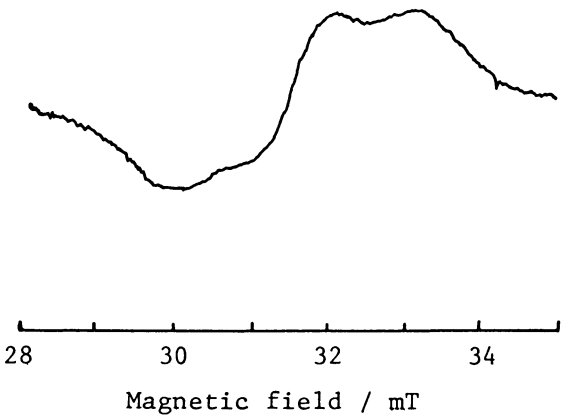

Fig. 4. L-band ESR spectrum of 0.1 mol dm^{-3} TEMPOL (0.3 ml).
Freq. : 928.24 MHz
Mod. : 0.1 mT
Amp. : 1 x 100
Power : 8 mW

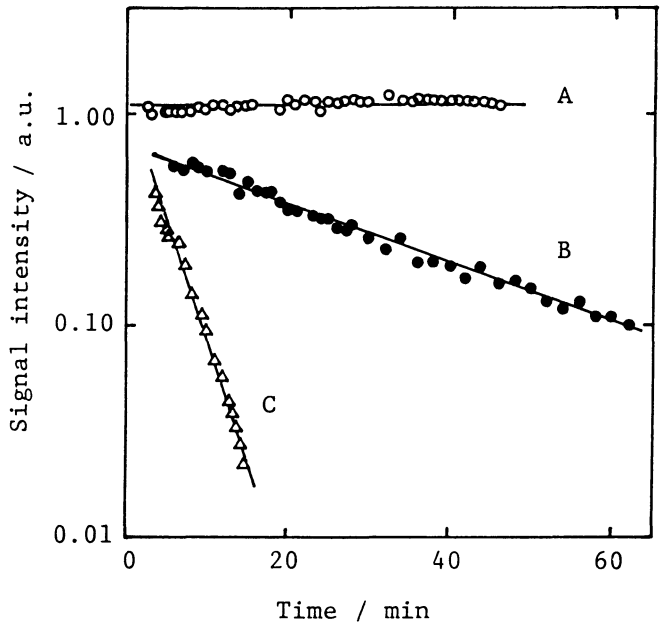

Fig. 5. Change of signal intensity of TEMPOL in celery stalk (A), leaves (B), and juice from leaves (C).

juice was carried out by JEOL FE3X X-band ESR spectrometer using a flat type cell at room temperature.

It is reported that TEMPOL is changed to diamagnetic hydroxylamines by the reaction with ascorbic acid.[2] Therefore, the decrease of TEMPOL in leaves of celery may be due to the reduction by ascorbic acid in leaves. Small value of rate constant for *in vivo* results suggests that there are functions of protection in leaves and the invasion process of TEMPOL radical in cellular tissue containing ascorbic acid may be rate determining step.

At any rate, these results could be obtained only by the *in vivo* analysis, although the detail reaction of TEMPOL in celery must be investigated in future. In our laboratory, the *in vivo* measurements of a whole body of mouse and a head of rat are now being carried out. Furthermore, another loop-gap resonator of 40 mm in diameter with 2 or 4 gaps is made in order to measure samples of larger volume.

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References

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2) W. R. Couet, R. C. Brasch, G. Sosnovsky, and T. N. Tozer, Magn. Reson. Imag., 3, 83 (1985).

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