Microelectrode-Detected Electron Spin Resonance (MEDESR) Signals of Free Radicals in Electrolyte Solutions

Hidekatsu Yokoyama, Toshiyuki Satoh, Hiroaki Ohya-Nishiguchi,* Hitoshi Kamada, Nahoko Kasai,† and Tomokazu Matsue†
Institute for Life Support Technology, Yamagata Technopolis Foundation, 683 Kurumanomae, Numagi, Yamagata 990
†Faculty of Engineering, Tohoku University, Aoba, Aramaki, Sendai 980

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The ESR spectra of stable free radical in solution have first been detected through potential change between two electrodes inserted in the solution. The working electrode (ϕ 50 μm) was biased by a constant current source under microwave irradiation and modulated magnetic field. The AC component of the potential on resonance was preamplified and detected by a lock-in amplifier. The sensitivity of this method, 5 X 10^6 spins/G, is 10^3 higher than those of conventional ESR spectrometers.

Since free radicals *in vivo* are considered to play a crucial role in various diseases, measurement of intracellular free radicals is essential for understanding pathogenesis. Electron spin resonance (ESR) technique has been widely used for observation of free radicals *in vitro*, but conventional ESR spectrometer are incompetent to observe the free radicals *in vivo* mainly because of high dielectric losses of biological systems. Recently, to overcome this problem, low frequency microwave, where the dielectric losses is one tenth lower than that at X-band, has been utilized for the *in vivo* ESR techniques to measure lossy samples such as small animals[1-3]. However, the sensitivity of the spectrometers is one tenth lower than those at X-band ESR spectrometers. An alternative approach to the observation of the free radicals *in vivo* is to detect an electric potential change under constant current on resonance through the biological systems. This idea is based on the detection of lattice defects or deep traps in diodes[4] or field-effect transistors[5], the sensitivity of this method being 10^3 to 10^4 spins/G. We have tried to apply this technique to electrolytic solution including typical stable organic free radicals (model systems of biological system) and succeeded to detect them sensitively through the potential change. This is the first report of the microelectrode-detected ESR (MEDESR) signal within our knowledge.

The block diagram of our MEDESR system is shown in Figure 1. The system consists of an electrolytic cell, a homemade bias-current source, a homemade AC preamplifier, an X-band ESR spectrometer (modified TE200, JEOL) and an external lock-in amplifier (model 5210, EG&G). The electrolytic cell was composed of a glass capillary tube (1.1 mm in inner diameter, 15 cm in length), a working microelectrode and its reference electrode. The microelectrode was made as follows. Au wire (50 μm in diameter), which was connected to a copper wire by electric welding, was insulated by a melted lead glass and then enclosed into a glass capillary tube (1 mm in outer diameter) by using epoxy resin. Afterward the tip of the electrode was polished to make a flat surface. An Ag/AgCl electrode was served as the reference electrode, which was connected to the electrolytic cell through salt bridge. An organic free radical, 3-carbamoyl-2,2,5,5-tetramethyl-pyrrolidine-1-oxyl (Carbamoyl PROXYL; g=2.0065, a^N=1.56 mT) was dissolved in saline solution. 40 μl of 25, 50, or 75 mmol/l Carbamoyl PROXYL solution was put into the electrolytic cell, which was set at the center of the ESR cavity. The bias-current source was worked at 0.05 nA. In this condition, the microelectrode was applied at about 0.4 V versus the reference electrode. This potential value was lower than oxidation potential of Carbamoyl PROXYL of 0.68 V. Therefore, Carbamoyl PROXYL itself was not almost oxidized in this experiment. The modulation frequency of 80 Hz was used. The AC component of the potential variation between two electrodes on resonance was amplified by the AC pre-amplifier, followed by the lock-in amplification at 80 Hz for the signal detection. The measurement were made at room temperature with 200 mW incident of X-band microwave power and static magnetic field around 340 mT.

In the present condition all spectra showed drifts of baseline difficult to eliminate technically. When 75 mmol/l Carbamoyl PROXYL was measured, a single broad signal was observed at

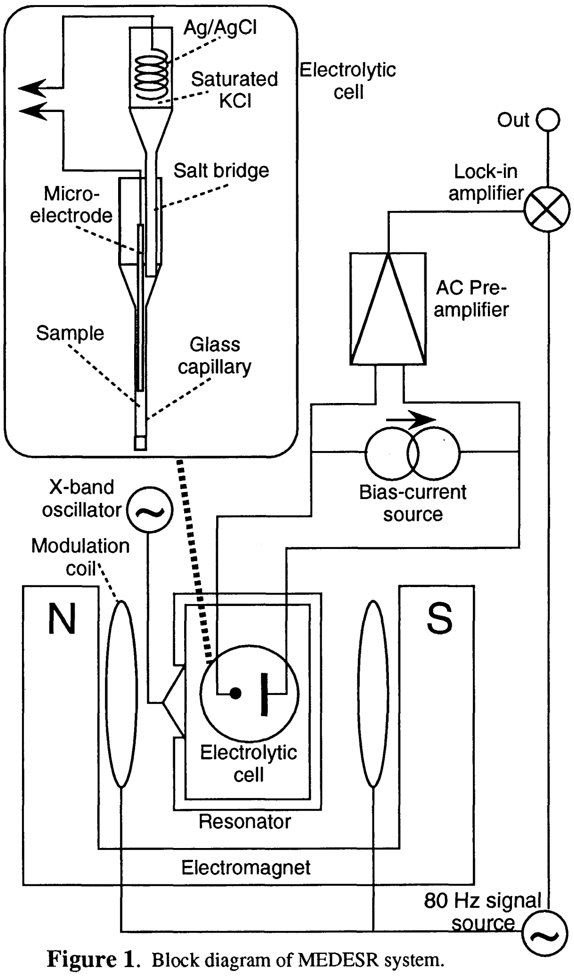

Figure 1. Block diagram of MEDESR system.

g=2.006±0.001 (Figure 2a). This might be due to the dipolar broadening of the linewidth at high concentration of paramagnetic species. When the concentration of Carbamoyl PROXYL decreased to 50 mmol/l, a decrease of signal intensity was observed (Figure 2b). At the concentration of 25 mmol/l the signal intensity decreased appreciably (Figure 2c) and triplet hyperfine lines with splitting of about 1.6 mT were observed at the position with same g value. These g and a^N values corresponded to those obtained by conventional ESR in same samples (Figure 2d-f). No signals were obtained under zero microwave power. Another electrode with larger surface area showed a concomitant increase of the intensity (Figure 3). These findings indicate that observed signals were related to the ESR originated from Carbamoyl PROXYL and the intensity depended on the concentrations of the radical dissolved in the solution, incident microwave power, and the surface area of the microelectrode.

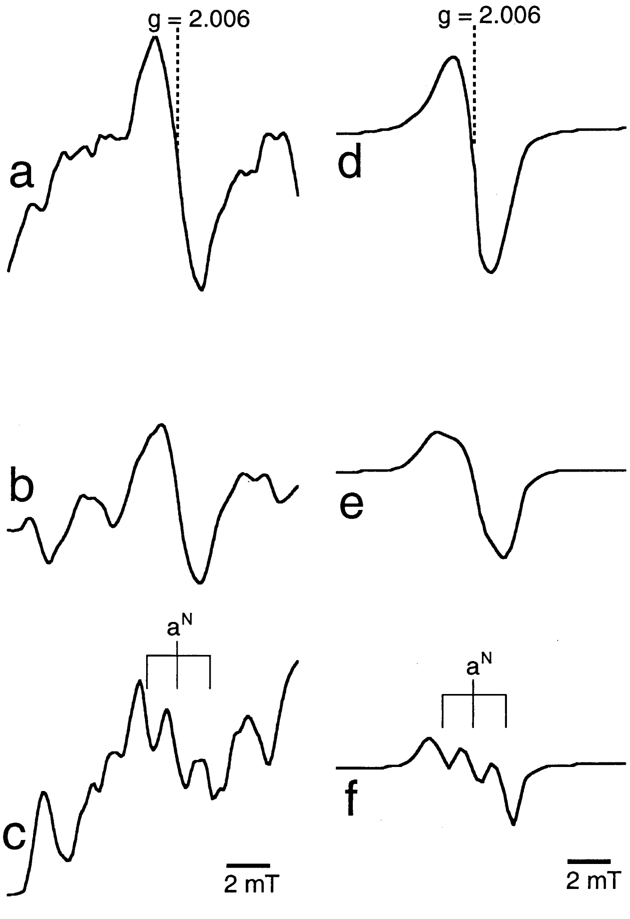

Figure 2. Spectra of MEDESR (a-c) and conventional ESR (d-f) of Carbamoyl PROXYL in saline solution. Concentration of Carbamoyl PROXYL was 75 (a,d), 50 (b,e), or 25 mmol/l (c,f). The diameter of microelectrode was 50 μm.

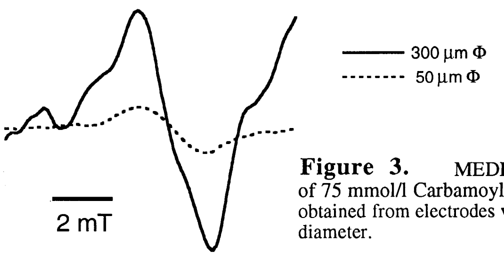

Figure 3. MEDESR spectra of 75 mmol/l Carbamoyl PROXYL obtained from electrodes with different diameter.

Theoretical interpretation of the mechanisms of MEDESR is now under way, but we can say here that MEDESR signals might be derived from an impedance change caused through magnetic interactions of conducting electrons having magnetic moment with paramagnetic species such as Carbamoyl PROXYL in the electrical double layer. The cross section of the paramagnetic species interacting with conducting electrons may change due to the migration of up-spins and down-spins on resonance, which results in an apparent change of the impedance of the electrical double layer. Since the electrolyte is highly conductive and the electrical double layer has high resistance, the impedance between two electrodes almost reflects that of the electrical double layer. If such mechanism is correct, one can estimate the amount of spins detected in our study. As the volume of the electrical double layer of about 3 X 10^{-8} m thick[6] is about 10^{-17} m^3, the amount of spins of Carbamoyl PROXYL in the detected region was estimated to be about 10^{-15} mol. Therefore, the sensitivity of our system was estimated about 5 X 10^6 spins/G. This value is much better than the sensitivity of the conventional ESR at the room temperature, about 7 X 10^9 spins/G[7]. These estimations mean that our system has a possibility detecting free radicals in ultramicro region in biological system much smaller than the volume of moderate-sized cells. The present study is considered to be an essential step for the subsequent development of MEDESR which can detect intracellular free radicals.

References

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