

Simultaneous CW-EPR imaging of isotopic nitroxyl radicals

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Introduction

The distribution of enantiomers can be visualized with electron paramagnetic resonance (EPR) imaging if they are labeled with stable spin-probes such as nitroxyl radicals. To make *in vivo* imaging of chiral drugs possible, we first need to perform synthesis and toxicological studies for spin-labeled chiral drugs, as well as develop a simultaneous imaging method to visualize two enantiomers. A recent study showed that the anticancer drug Lomustin can be successfully labeled with a nitroxyl radical, without affecting its toxicological activity [1]. This study highlights the potential for the labeling of chiral molecules with isotopic nitroxyl radicals for the visualization of different EPR spectra.

Three imaging methods based on magnetic resonance phenomena were reported for the simultaneous imaging of two or more kinds of free radicals: (i) spectral-spatial EPR imaging [2], (ii) Overhauser-enhanced magnetic resonance imaging [3] and (iii) EPR imaging based on a process of iterative deconvolution [4]. However, these methods are time-consuming and not suitable for three-dimensional (3D) imaging of short-lifetime radicals. As part of the development of *in vivo* imaging of chiral molecules, this study describes a method of *simultaneous* EPR imaging, which can visualize the spatial distributions of both ¹⁴N- and ¹⁵N-labeled nitroxyl radicals with no additional acquisition time.

Methods

Separation of spectra The process of spectral separation of ¹⁴N- and ¹⁵N-labeled nitroxyl radicals was carried out by subtracting the spectrum of ¹⁴N-labeled nitroxyl radicals from overlapping spectra. Since the line-shapes of the absorption peaks of three-line EPR spectrum are similar to each other, shifting the line-shape of the center peak could approximate that in the lower field. To shift the measured center peak to an arbitrary position in the magnetic field, we used the shifting theorem of Fourier transform.

Phantom In two-dimensional (2D) EPR imaging, six capillary tubes (inner diameter of 1.9 mm and outer diameter of 2.5 mm) were held with a bobbin (22 mm in diameter and 25 mm long) made of cross-linked polystyrene. Each capillary tube contained 0.1 mL of solutions (35 mm long in tubes) of nitroxyl radicals. Capillary tubes were filled with TEMPOL-d₁₇-¹⁵N and/or TEMPOL-d₁₇ in distilled water.

EPR imaging An in-house built 750-MHz continuous-wave (CW) EPR imager was used in the experiments with phantoms. The details of our 750-MHz CW-EPR imager have been described elsewhere [5, 6]. For 2D phantom imaging, the measurement parameters were as follows: RF power 2.5 mW, field scanning 3.0 mT, magnetic field modulation 0.06 mT, field gradient 40 mT/m, duration of field scanning 1.0 s, time constant of lock-in amplifier 1 ms, number of projections 64, and number of averages 2. The total acquisition time was 2 min 41 sec.

Results and Discussion

EPR images for a mixture of the solutions of 4-hydroxyl-2,2,6,6-tetramethylpiperidine-d₁₇-1-oxyl (TEMPOL-d₁₇-¹⁴N) and 4-hydroxyl-2,2,6,6-tetramethylpiperidine-d₁₇-1-¹⁵N-1-oxyl (TEMPOL-d₁₇-¹⁵N) radicals agreed with the concentrations of each kind of radicals. Figure 1A shows a photograph of six tubes filled with a mixture (0.1 mL) of two kinds of nitroxyl radicals in different concentrations. Figure 1B illustrates the arrangement of the tubes used for the experiments. Figures 1C and 1D show 2D images of TEMPOL-d₁₇ and TEMPOL-d₁₇-¹⁵N radicals, respectively.

Our experimental results demonstrate that simultaneous EPR imaging can be used to visualize ¹⁴N- and ¹⁵N-labeled nitroxyl radicals in a single image scan. Despite the fact that the solutions of both radicals were mixed in the phantom and the subject mouse, the EPR spectra of each radical were well discriminated through the process of spectral separation. Because of our ability to successfully separate the EPR spectra from two different radicals measured simultaneously, we believe this technique has potentially important application in the study of the redox status of intra- and extra-cellular environments, where labelling of membrane-permeable and -impermeable nitroxyl radicals with isotopic nitrogen (¹⁴N and ¹⁵N) may be used to determine the reduction rates of nitroxyl radicals.

Conclusion

We demonstrated simultaneous EPR imaging of ¹⁴N- and ¹⁵N-labeled nitroxyl radicals *in vitro*. This technique has the potential of *in vivo* imaging for spin-labelled enantiomers.

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References

- [1] Zhelev Z, Bakalova R, Aoki I, Matsumoto K *et al.*, Chem Comm 2009;53–55.
- [2] Maltempo MM, Eaton SS, Eaton GR, J Magn Reson 1987;72:449–455.
- [3] Utsumi H, Yamada K, Ichikawa K *et al.*, Proc Natl Acad Sci USA 2006;103:1463–1468.
- [4] Czechowski T, Krzyminiowski R, Jurga J, Chlewicki W, J Magn Reson 2008;190:52–59.
- [5] Akaba-Sato H, Fujii H, Hirata H, Rev Sci Instrum 2008;79:123701.
- [6] Akaba-Sato H, Kuwahara Y, Fujii H, Hirata H, Anal Chem 2009;81:7501–7506.

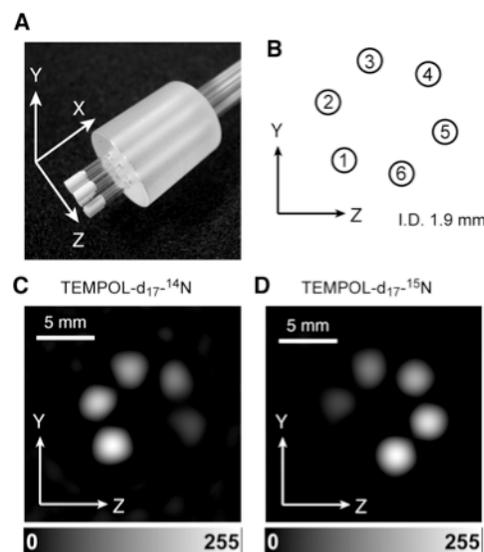


Fig. 1. 2D EPR imaging for nitroxyl radicals in a phantom. A) Photograph of the phantom consisted of six tubes. B) Arrangement of six tubes (inner diameter of 1.9 mm). Each tube contained 0.1 mL of the solutions of nitroxyl radicals (35 mm long in tubes). The tubes from 1 to 5 contained 1.0, 0.8, 0.6, 0.4 and 0.2 mM TEMPOL-d₁₇ solutions, respectively. The tubes from 2 to 6 contained 0.2, 0.4, 0.6, 0.8 and 1.0 mM TEMPOL-d₁₇-¹⁵N solutions, respectively. EPR images of the solutions of C) TEMPOL-d₁₇ and D) TEMPOL-d₁₇-¹⁵N radicals.