Direct Detection of Hydrogen Peroxide via ^{1}H CEST-MRI

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Introduction

Hydrogen peroxide (H_2O_2) is a well-documented component of living cells. It plays important roles in host defense and oxidative biosynthetic reactions. In additional, there is growing evidence that at low levels, H_2O_2 also functions as a signaling agent. In human plasma, the concentration of H_2O_2 is reported to be 4-5 μM[1] and to elevate to as much as 100 – 600 μM under inflammation[2] or ischemia-reperfusion tissues.[3] Therefore, it is of important and practical demands to develop better imaging techniques to visualize this important metabolite.

Unfortunately, it is difficult to visualize H_2O_2 because of its unfavorable spectroscopic property. To solve this issue, many assistant agents have been invented so far, for example, those agents for fluorescent spectroscopy/imaging[4,5] and for magnetic resonance imaging (MRI).[6,7] In this meeting abstract, however, we will report a new method for MRI measurement, which will take advantage of ^{1}H NMR spectroscopic feature of H_2O_2 per se[8] and a newly developed MRI contrast mechanism of CEST (Chemical Exchange Saturation Transfer).[9]

Results and Discussion

In ^{1}H NMR spectrum of H_2O_2 aqueous solution, two peaks can be detected at 4.7 and 11.1 ppm, which have been assigned to bulk water and H_2O_2, respectively.[8] These two peaks have obvious line broadening due to the proton chemical exchange effect. This feature, in combination with a newly developed MRI contrast mechanism of CEST, has stimulated us to think: (1) whether it is feasible to image H_2O_2 directly via CEST-MRI; and (2) what is the detection lower limit if applicable?

To test our assumption, a series of CEST spectra were recorded at 400 MHz NMR spectrometer at different temperatures ranging from 22.5 to 50 °C. In this initial characterization, a concentrated H_2O_2 aqueous solution was used, which contains *ca.* 10% H_2O_2, namely, $[H_2O_2] \approx 2.94$M *versus* $[H_2O] \approx 50$M, respectively. Very interestingly, two CEST peaks (**Fig 1**) were detected in those spectra, one being assigned to the direct saturation of bulk water (referred to as 0 ppm in CEST spectra) and another due to the chemical exchange saturation transfer of H_2O_2 (sit at 6 ppm downfield). This means that hydrogen peroxide can be detected via CEST-MRI technique!

The experimental CEST data (**Fig. 1**) may be successfully fitted to a 2-pool exchanging model of Bloch theory,[10] which gave the exchanging lifetimes (τ_M) of H_2O_2 as follows: 1200, 961, 763 and 612 μs at 22.5, 30, 40 and 50 °C, respectively. By further fitting to Arrhenius Equations, the thermokinetic parameters of proton exchanging reaction between H_2O_2 and H_2O may be obtained as follows: Ea^{298} = 19.2 kJmol^{-1}, LnA = 14.6, ΔH^{298} = 16.8 kJmol^{-1}, ΔS^{298} = 16.8 Jmol^{-1}K^{-1} and ΔH^{298} = 56.1 kJmol^{-1}, respectively.

With above parameters plus the 2-pool exchanging Bloch theory, we may perform additional theoretical simulations to better understand the CEST-MRI detection lower limit of H_2O_2. **Fig. 2** shows such a prediction. At 500 MHz NMR spectrometer or MR imager and by using an excitation pulse B_1 = 150 Hz for 1 s at ^{1}H resonant frequency of H_2O_2, around 5% CEST decrease may be accessed for a sample containing 10 mM H_2O_2, which implies that hydrogen peroxide may be imaged at mM concentration level.

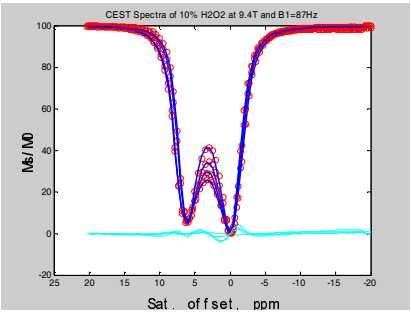

Figure 1. The experimental CEST spectra of 10% H_2O_2 obtained at 400 MHz by applying B_1 = 87 Hz for 1 s at 22.5, 30, 40 and 50 °C, respectively. The blue solid lines are the corresponding fitted curves to a 2-pool exchanging Bloch theory.

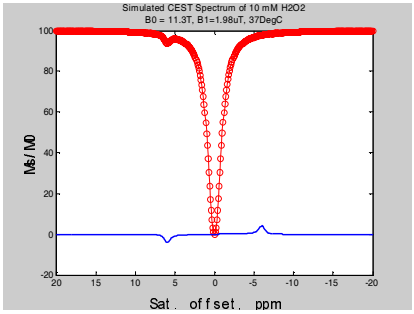

Figure 2. The Simulated CEST spectrum of a sample containing 10 mM H_2O_2 at 500MHz by applying B_1 =150 Hz for 1 s and at 37 °C.

Conclusion

A new method of direct detection of hydrogen peroxide has been introduced, which is based on the CEST-MRI. The experimental data as well as the theoretical simulation revealed that H_2O_2 might be imaged at mM concentration level. The sensitivity will be the most critical issue of this method in comparison with other MRI techniques using exogenous contrast agents. Further in vitro and in vivo demonstrations are on-going.

References

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