Across the Tree of Life: Radiation Resistance Gauged by High-Field EPR

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Introduction

This report provides evidence that small high-symmetry antioxidant complexes of manganous ions with metabolites (H-Mn^{2+}) are responsible for cellular resistance to gamma ionizing irradiation (IR), and that H-Mn^{2+} protects the proteome from IR-induced reactive oxygen species. The cellular content of H-Mn^{2+}, as measured by EPR of live, non-irradiated Mn-replete cells, is now the strongest known gauge of biological IR resistance between and within organisms representing all three domains of life. To establish that antioxidant H-Mn^{2+} complexes, not the antioxidant enzyme, Mn superoxide dismutase (MnSOD), governs IR survival, required characterization of the spin Hamiltonian of MnSOD and quantitation of the enzyme in cells. X-band and even Q-band measurements are unable to acquire this information satisfactorily. In this study HFHF EPR provided this key information

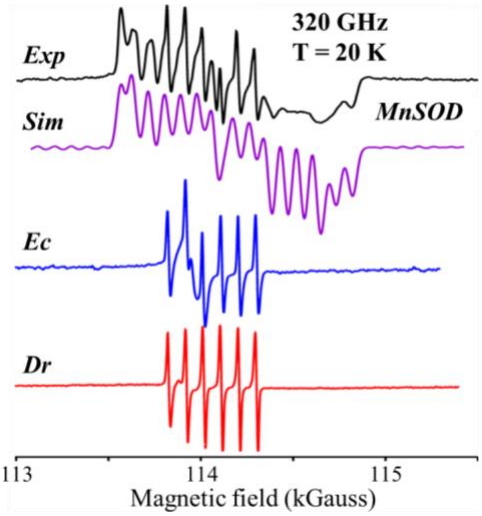

Fig.1 HFEPR spectra at 320 GHz and 20 K. The MnSOD spectrum was simulated (magenta) with parameters given in the text.

Experimental

The HFEPR spectra of frozen aqueous solutions of MnSOD (Mn-superoxide dismutase) as well as cultures of bacteria *Deinococcus Radiodurans* (*Dr*) and *Escherichia coli* (*Ec*) (**Fig.1**) were recorded on the transmission instrument of the EMR facility at temperatures 3 – 20 K and frequencies up to 416 GHz. The maximum magnetic field reached was 14.9 T.

Results and Discussion

The sextet (S = 5/2) spectra of the d^5 Mn(II) ions were interpreted in terms of the spin Hamiltonian

$$\hat{H} = \mu_B \boldsymbol{B}\{\boldsymbol{g}\}\hat{\boldsymbol{S}} + D\left\{\hat{S}_z^2 - \frac{1}{3}S(S+1)\right\} + E\left(\hat{S}_x^2 - \hat{S}_y^2\right) + \hat{\boldsymbol{S}}\boldsymbol{A}\hat{\boldsymbol{I}} \quad [1]$$

Relatively large zero-field splitting parameters, D = –0.350 cm^{-1}, E = –0.026 cm^{-1} were found for MnSOD, while the EPR spectra of Dr ad Ec (**Fig.1**) are characteristic of the low zero-field splitting and high symmetry H-Mn^{2+}. EPR spectra intensity measurements revealed that the concentration of MnSOD in the bacterial cultures was negligible, compared to the high-symmetry Mn^{2+} species.

Conclusions

This study shows that the amount of H-Mn^{2+} in non-irradiated living cells is readily gauged by absorption-display electron paramagnetic resonance (EPR) spectroscopy at Q band, and is highly diagnostic of DNA repair efficiency and survival after gamma radiation exposure. Importantly, the high resolving power of high-field EPR was essential for proving that the enzyme manganese superoxide dismutase (MnSOD) is present in negligible amounts in the bacterium *Deinococcus Radiodurans*, which is capable of surviving radiation doses 20-fold greater than *Escherichia coli*, thereby disproving previous assertions that MnSOD is critical in the IR survival of Dr. The results of this work were published in [1].

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References

[1] Sharma, A., *et al*., Proc. Natl. Acad. Sci. USA, **114**, E9253-E9260 (2017).