

216 GHz EPR of KatG

216 GHz Electron Paramagnetic Resonance of Mycobacterium Tuberculosis Catalase-Peroxidase: The Role of the Arg418 Residue

Matt Bawn¹, Jurek Krzystek², Richard Magliozzo^{1*}

¹Department of Chemistry, Brooklyn College of the City University of New York, Brooklyn, New York 11210

²Center for Interdisciplinary Magnetic Resonance, National High Magnetic Field Laboratory, Florida State University, Tallahassee, Florida 32310, USA

*To whom correspondence should be addressed: Richard Magliozzo, ¹Department of Chemistry, Brooklyn College of the City University of New York, Brooklyn, New York 11210. Email:rmaglioz@brooklyn.cuny.edu.

ABSTRACT

The catalase-peroxidase protein from *Mycobacterium tuberculosis* contains a variety of unique structural features including a covalently-linked three amino acid adduct capable of hosting a tyrosine-based radical. Previous work has demonstrated that the Arg418 residue is essential for the catalase but not the peroxidase activity of the protein and crystallography has indicated the residue to be capable of adopting two conformations relative to the adduct-radical. In the present work the WT and Arg418Leu mutant proteins were investigated using high-field electron magnetic resonance spectroscopy. Different sets of g-values were found for each protein indicating different paramagnetic environments. Quantum chemical calculations of model structures were undertaken to elucidate the geometrical environment of the radical. It is proposed that the two sets of g-values correspond to the two conformations of the Arg418 residue. The implications for the catalytic mechanism are discussed.

INTRODUCTION

Mycobacterium tuberculosis catalase-peroxidase (Mtb KatG) is a dual-function heme containing enzyme that also contains a unique covalently-linked three amino acid adduct consisting of residues Trp107, Tyr229 and Met255 (MYW-adduct). The adduct has previously been shown to be necessary for the catalase but not the peroxidase activity of the protein. The adduct is capable of hosting a tyrosine-based radical as part of the catalase reaction, other work¹ has revealed a critical role for the Arg418 residue in this reaction. The residue has been seen to be able to adopt two conformations relative to the MYW-adduct (vicinal and distal) as revealed by x-ray crystallography² and shown in **Figure 1**. Electron Paramagnetic Resonance (EPR) spectroscopy is a technique capable of probing the electronic environment of amino acid radicals and has been used to characterize Tyr radicals in a variety of enzymes. Previous high-field EPR spectroscopy undertaken by the group at D-band (130 GHz) of wild-type (WT) Mtb KatG suggested the presence of two MYW-adduct radical environments characterized by two distinct g_x values³. To determine whether these two environments correspond to the two conformations of the Arg418 residue EPR has again been applied but this time to the Arg418Leu mutant and WT Mtb KatG proteins to produce the distal and vicinal conformations respectively.

EXPERIMENTAL

Sample Preparation: WT and Arg418Leu KatG were grown and purified as described previously¹. Protein was reacted against 8000-fold excess hydrogen peroxide at pH 7.5 and manually freeze-

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quenched (freezing time ~ 2 s) in liquid ethane. Excess liquid ethane was removed and the samples transferred at low-temperature to Teflon EPR cups. Samples were stored in liquid nitrogen at 77K when not in use.

EPR: Field-modulated continuous-wave EPR spectra were measured at 216 GHz. An atomic hydrogen standard was included during measurement for accurate field determination⁴.

RESULTS

HF-EPR: The 216 GHz EPR and simulated spectra measured at 15 and 60 K of WT and Arg418Leu KatG respectively are shown in **Figure 2**. In the case of the WT sample there was a large source of Mn(II) contamination that is commonly detected by HF EPR and becomes more apparent at higher temperatures. Temperatures were chosen in order to enhance the derivative nature of the spectra and facilitate simulation. The simulations clearly show g -values for each protein of $g_x = 2.0064$ $g_y = 2.0038$ $g_z = 2.0024$ for the WT and $g_x = 2.0054$ $g_y = 2.0033$ $g_z = 2.0019$ Arg418Leu. The contributions to both spectra from Mn(II) are also shown in the figure.

DISCUSSION

Previous spectra measured at 130 GHz and 7K of the WT protein at pH 8.5 also revealed a rhombic g -tensor with dual g_x values (2.00550 and 2.00606), $g_y = 2.00344$ and $g_z = 2.00186$. These are in reasonable agreement with the g -values obtained in this study for the WT and Arg418Leu mutant protein. It must also be remembered however, that these previous results were performed on slightly different samples. The main differences to be taken into consideration should be the freezing time (35 ms RFQ for the D-band vs 2 s MFQ in the present study) and the pH (pH 8.5 for D-band and pH 7.5 in

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the present work). Indeed in another heme protein in which an Arg residue has been shown to be able to “swing” into a vicinal and distal position, pH has been indicated to be a factor in the conformation.

The results of the HF-EPR clearly show the presence of two differing conformations of the paramagnetic environment in the WT and ARG418Leu proteins that may be explained by the ARG418 residue being in the vicinal and distal conformations. The presence of a bi-conformational ARG residue has also been seen in the crystal structure of versatile peroxidase¹¹ □ . The Trp164Tyr mutant of the protein however, seemed to stabilize only one of the ARG conformations.

DFT calculations of the g-tensor for the MYW radical in katG with the R418 residue in both conformations indicate that the g_x-value for the vicinal ARG should be lower than for the distal position. This is opposite to what is observed experimentally in the case of the WT and R418L HF-EPR results described in this work.

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FIGURE LEGENDS

Figure 1, MYW-adduct and Arg418 residues from Mtb KatG [PDB:2CCA]. Arg418 is shown in vicinal and distal conformations.

Figure 2, 216 GHz continuous-wave EPR spectra (solid) and simulations (dashed) measured at 15 K and 60 K respectively of WT (A) and Arg418Leu mutant Mtb KatG (B). Simulation parameters for WT were: $g_x = 2.0064$ $g_y = 2.0038$ $g_z = 2.0024$ whilst those for Arg418Leu were: $g_x = 2.0054$ $g_y = 2.0033$ $g_z = 2.0019$. The contributions to the simulation from Mn(II) and KatG species are indicated in blue and red respectively.

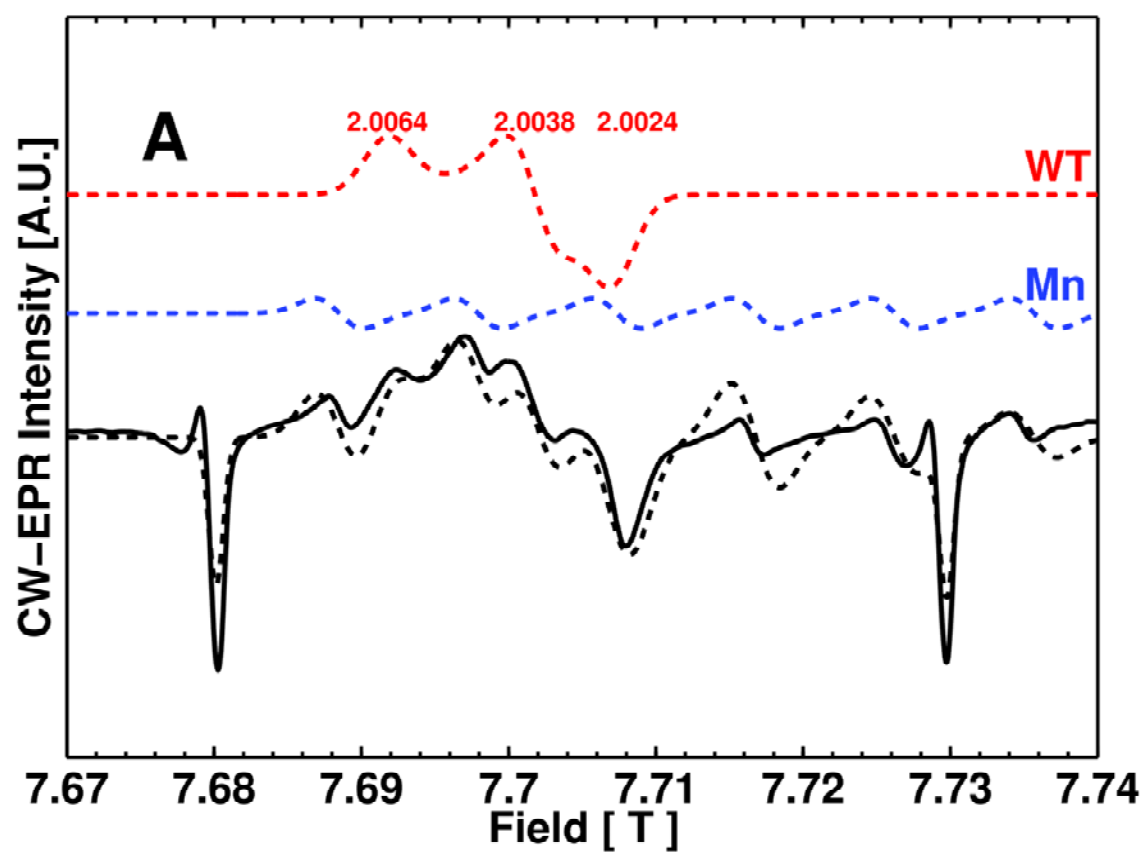
Figure 3, Hydrophobicity of heme binding pocket environment of KatG. [PDB:2CCA] The strength of hydrophobicity is indicated by the degree of redness of the wire-frame representation. Crystallographic water oxygen atoms within 5 nm of the R418 residue are shown as red spheres.

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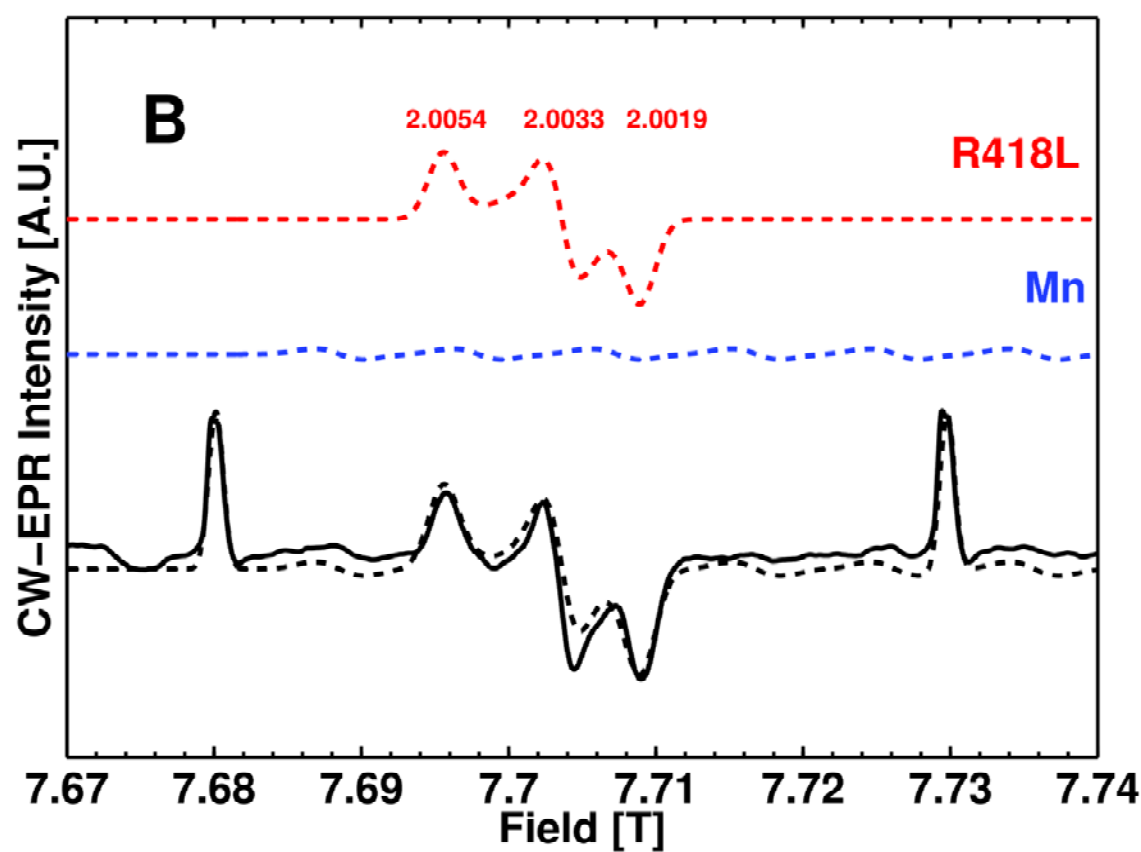
Figure 1



Figure 2



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Figure 3

