High-Field EPR Investigation of Bacterial Mn(II)-binding Proteins

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Introduction

Staphylococcus aureus and *Streptococcus pneumoniae* are two bacterial pathogens that cause human disease and require Mn(II) during infection.[1-4] These bacteria scavenge Mn(II) from the host using the solute-binding proteins (SBPs) MntC (*S. aureus*) and PsaA (*S. pneumoniae*).[5,6] While crystal structures have been reported for Mn(II)-MntC and Mn(II)-PsaA, the electronic structure of these Mn(II) sites is not well defined.[7,8] Our low-temperature X-band EPR data for Mn(II)-SBPs revealed broad spectral signals, indicating large zero-field splitting (ZFS) and systems that are not within the "high-field" limit.[9] We therefore studied these sites with multi-frequency high-field EPR spectroscopy.

Experimental

All samples (0.75:1 Mn(II):SBP ratio) were prepared in 75 mM HEPES, 100 mM NaCl, pH 7.5 buffer in 1-mL LDPE vials. The samples were incubated for at least 15 min prior to being frozen in liquid nitrogen. The samples were analyzed using the transmission spectrometer and its 15/17 T SC magnet in the Electron Magnetic Resonance (EMR) facility of the NHMFL using multiple frequencies. Frequencies of 64, 124, 235, and 388 GHz were utilized at 30, 5 and 3 K. Data was collected using 5 G or 25 G modulation amplitude.

The Mn(II) EPR spectrum (S = 5/2) can be interpreted with the phenomenological spin Hamiltonian given below.[9]

$$\widehat{\boldsymbol{H}} = \mu_B \boldsymbol{B}\{\boldsymbol{g}\}\widehat{\boldsymbol{S}} + a_{iso}\widehat{\boldsymbol{S}}\cdot\widehat{\boldsymbol{I}} + D\left\{\widehat{\boldsymbol{S}}_z^2 - \frac{1}{3}S(S+1)\right\} + E\left(\widehat{\boldsymbol{S}}_x^2 - \widehat{\boldsymbol{S}}_y^2\right)$$

Results and Discussion

The six line pattern present in the center of the Mn(II)-SBP spectra appearing at ~13.85 T at 388 GHz (**Fig.1**) is attributed to the coupling of the unpaired electron spins with the I = 5/2 ^{55}Mn nucleus observed in the transition between the m_s = ±1/2 spin sublevels. The features flanking the central six line pattern are attributed to transitions between the Kramers doublets with m_s = ±1/2 and ±3/2 as well as those between ±3/2 and ±5/2. The ZFS (D) was determined to be +2.72 GHz and +2.87 GHz for Mn(II)-MntC and Mn(II)-PsaA with an E/D ratio of 0.18 and 0.12 respectively. These values indicate that Mn(II) is bound to the SBPs in a tetrahedral or trigonal prismatic coordination environment. The hyperfine coupling constant for the ^{55}Mn ion was determined to be 241 MHz and 236 MHz for MntC and PsaA, respectively. The relatively low hyperfine constant value indicates a more covalent interaction with the protein compared to water where the ^{55}Mn hyperfine is ~265 MHz.

Conclusions

We determined the ZFS and ^{55}Mn hyperfine constant values of Mn(II)-MntC and Mn(II)-PsaA. The magnitudes of the ZFS indicate that the Mn(II) coordination environment is either tetrahedral or trigonal prismatic. The low hyperfine constant value is indicative of a relatively covalent interaction between the Mn(II) ion and protein. This covalency contributes to the high affinity binding of Mn(II) to the SBPs.

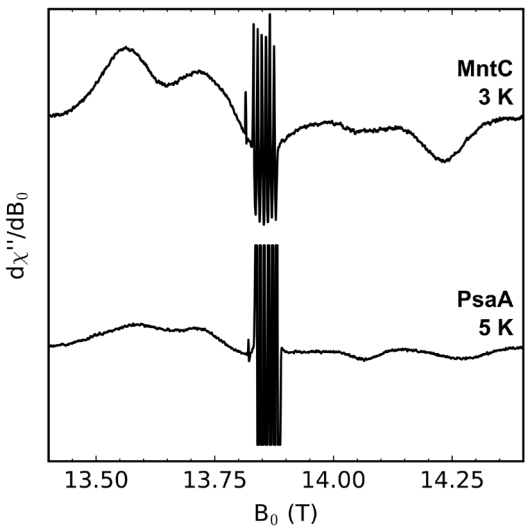

Fig.1 388 GHz, low temperature EPR spectra of Mn(II) bound to MntC and PsaA. Settings: 25 G modulation at 50 kHz, sweep rate 2.0 mT/s.

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