The Structure of the Biliverdin Radical Intermediate in Phycocyanobilin:Ferredoxin Oxidoreductase Identified by High-field EPR and DFT

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Introduction

The reduction of biliverdin IXa (BV) to phycocyanobilin by phycocyanobilin:ferredoxin oxidoreductase (PcyA) involves the transfer of four electrons and four protons to the substrate. Signals from two substrate radical intermediates have been observed by EPR [1], but the details of the catalytic cycle are not currently known. EPR at high and very high fields allows the measurement of the g tensor of the substrate radical intermediate with very high resolution. By correlating the measured g tensor to theoretical predictions for a variety of structures using density functional theory (DFT), the protonation state of the substrate radical intermediate can be identified. This contributes to the understanding of the reaction mechanism.

Experimental

Continuous-wave EPR spectra of 400 µM solutions of various mutants of PcyA, treated with dithionite (DT) or reduced ferredoxin (Fd), were measured at the NHMFL using the broadband transmission spectrometer at 406.4 GHz, 14.5 T, and 45 K. The field was calibrated using the EPR signals from Mn^{2+}-doped MgO. DFT studies of PcyA were performed with Gaussian 03 and Orca on various possible intermediate structures, using an X-ray structure determined from dithionite-soaked crystals [2].

Results and Discussion

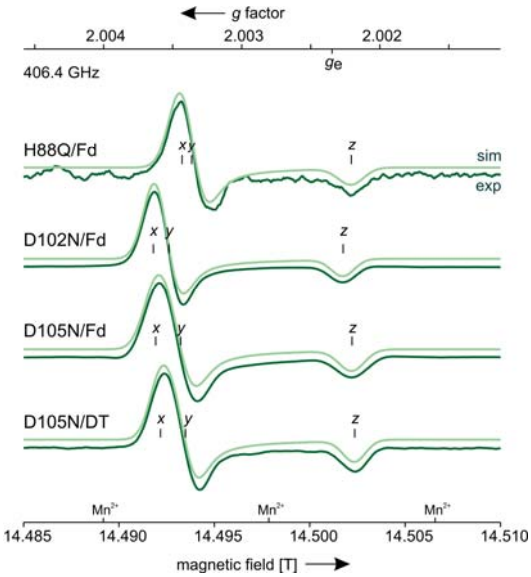

Figure 1: 406.4 GHz cw EPR spectra of frozen solutions of the bound reduced BV substrate radical in PcyA mutants. g_e indicates the free-electron g factor 2.002319. Mn^{2+} indicates the position of three low-field lines of MgO:Mn^{2+}. The field positions corresponding to the three principal directions of the g tensor are indicated by x, y and z.

The spectra for various mutants and reducing agents are shown in Fig. 1. The principal g values of the mutant D105N reduced with DT are 2.00395(5), 2.00341(5) and 2.00218(5). All g tensors are similarly axial and have a similar very small anisotropy. DFT calculations on several dozen protonation states of the biliverdin radical show that this high axiality and small anisotropy is only consistent with a structure where both carbonyl oxygens are protonated [2]. A similar radical is expected to be observable in the wild-type enzyme.

References

[1] Tu, S.-L., et al., J. Am. Chem. Soc., 126(28), 8682–8693 (2004).
[2] Stoll, S., et al., J. Am. Chem. Soc., submitted.