The ins and outs of ring-cleavage dioxygenases: insights from novel spectroscopic approaches

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The aerobic degradation of aromatic compounds, including many environmental pollutants, often proceeds via the oxygenolytic cleavage of a catecholic compound. This ring-cleavage reaction is catalyzed by one of two classes of dioxygenases, differing in regiospecificity: the intradiol dioxygenases use non-heme Fe(III) to cleave the C-C bond of the enediol, while extradiol dioxygenases generally use non-heme Fe(II) to cleave the bond adjacent to the enediol. Despite the very different active sites of these enzymes, both bind catechol in an asymmetric manner, and their respective catalytic mechanisms are thought to proceed via similar bridged iron-alkylperoxo intermediates. We have used ultraviolet resonance Raman (UVRR) and UV-visible (UV-vis) difference spectroscopy to probe anaerobic substrate binding in each class of enzyme. UVRR spectroscopy was enabled using a specialized fiber optic probe assembly which allowed higher laser powers whilst maintaining low laser power densities in the sample. Model compounds and density functional theory (DFT) calculations were used to assign features of the difference spectra. Studies of DHBD, an extradiol dioxygenase, indicate that the asymmetry in the bound catechol is induced by mono-deprotonation of the Fe(II)-bound catechol. By contrast, studies of C12O, an intradiol enzyme, indicate dianionic catechol binding to the ferric ion together with protonation of the displaced tyrosinate ligand. Moreover, the computations suggest that an elevated frequency of the 8b band observed in the C12O:catechol complex is due to increased electron density in the catechol ring as a result of electron donation from a trans tyrosinate ligand. The studies illustrate the power of combining difference UVRR and optical spectroscopies to probe metal ligation in solution.