Cryogenic Transient Raman Studies on Mononuclear non-Heme Iron-Oxygen Species of TauD.

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Continuous flow resonance Raman (cf-RR) spectroscopy is a powerful tool for studying fine structural events at the active sites of metalloenzymes during catalytic turnover. We have been developing a new experimental setup to overcome the major drawback of traditional cf-RR—demand for large amounts of sample—by using cryogenic temperatures to slow the reaction. Traditional mixing relies on spontaneous turbulence when linear velocity exceeds a critical value, which is directly proportional to sample viscosity. Utilization of cryoprotectants increases the critical velocity by increasing sample viscosity (x4 for 50% ethylene glycol at 20^{o}C). Lowering of the temperature further escalates the viscosity and raises the critical flow rate. Our setup uses an externally driven rotor to induce local turbulence, thus eliminating the lower limit on the flow rate. In combination with its miniature geometry, our current design allows us to probe time delays as short as 70 ms at flow rates of less than 1 ml/min/reactant in aqueous samples at -38^{o}C. This corresponds to a delay of 1-10 ms for a typical reaction at ambient temperature.

We applied this methodology to study transient oxygen species in the reaction of taurine/α-ketoglutarate dioxygenase (TauD) from *E. coli*. TauD is a non-heme, mononuclear iron enzyme which binds Fe(II) and α-ketoglutarate and, in the presence of taurine, reacts with molecular oxygen. We carried out continuous flow measurements at -38^{o}C in 50% ethylene glycol. Using an optical fiber spectrometer and in-situ absorption probe we recorded changes in transient absorption spectra to correlate the reaction profile with room-temperature data. Subsequent 16O/18O isotope difference RR measurement revealed an oxygen vibration at 821/787 cm^{-1}. The frequency and the magnitude of this shift allowed us to assign it to the Fe=O stretching mode. At least one more oxygen-isotope sensitive model was detected at 583/555 cm^{-1} (single Fe-O bonds range). Our data suggest that a third mode may be present between 850 and 1100 cm^{-1} (O—O or O=O range), although interference from solvent modes hinders its identification. Corresponding vibrations have been detected at 3x longer delay time when ^{2}H-taurine was used, which has been shown to extend the lifetime of Fe=O. Implications of these observations for the catalytic mechanisms of TauD will be discussed.