Kinetic and Spectroscopic Characterization of Cyano-Tyrosinate Adducts of Soluble Δ9-Desaturase

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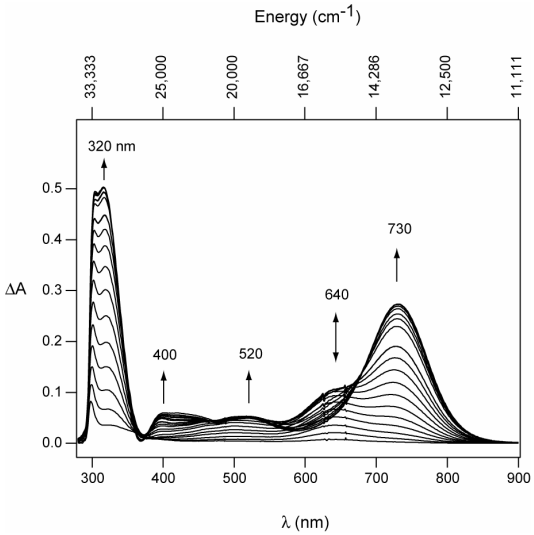
Previous studies have shown that the diferric active site of soluble Δ9 desaturase from *Ricinus communis* (castor bean plant) binds cyanide and, under basic conditions, generates an intense LMCT band observed in the visible spectrum at 730 nm. The coordination responsible for the intense absorption band of the cyano-Δ9D adduct was proposed to involve the simultaneous ligation of CN and a nearby tyrosine residue (Y236). This tyrosine is of particular interest since it has been implicated in a putative electron pathway between the diiron center and the bound ferredoxin (Fd). In this work the cyano-Δ9D adduct is characterized by a variety of spectroscopic techniques and a mechanistic model is proposed.



As observed in **Figure 1**, addition of excess cyanide at pH 9.5 produces several absorption bands in the UV/visible spectrum. The 640 nm peak appears to be a kinetic precursor of the cyano-Δ9D species observed at 730 nm. Formation of the 730 nm species is linearly dependent on CN concentration with a pseudo-first order rate constant of $k_{obs} = 5.6 \times 10^{-3}$ s^{-1}. Additionally, by using the CN ion as a kinetic probe for O_2 binding an increase in the rate cyano-Δ9D species formation was observed in the presence of 18:0 ACP. These results support previous kinetic and spectroscopic experiments in which substrate binding to the reduced enzyme affects both the geometry of the active site and the rate of autooxidation. Thus, substrate binding appears to perturb the active site diiron center and facilitate binding of the oxygen analogue. This work was supported by a grant by NIH GM 50853 to BGF.