Characterization of methionine 160 axial ligand mutants from a soluble Cu_A domain from cytochrome ba_3 of *thermus thermophilus*

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The thermostable *Thermus* Cu_A fragment has enabled us to isolate and characterize all possible amino acid substitutions in the M160 position. Importantly, this site-saturation generates a series of binuclear, valence delocalized [Cu(1.5)Cu(1.5)] centers that yield a broad range of absorption spectra. X-band EPR characterization[1] of the M160Q and M160E mutants have shown increases in $A_{\parallel}$ ($^{63,65}Cu$), 4.2 mT in these mutants compared to 3.1 mT in the native center. Pulsed EPR/ENDOR studies[2] have revealed other subtle perturbations of the ground state in these mutants. Preliminary electrochemical results indicate that the reduction potential of M160Q [75 mV vs. normal hydrogen electrode (NHE)] is significantly lower than the native center [240 mV vs. NHE]. We wish to present complete X-band EPR, electrochemistry and absorption data sets defining the range seen in these axial *Thermus* Cu_A mutants. We hope that one (or several) of these mutants may prove to be an instructive structural analog to the low-lying excited state of this center seen in the paramagnetic NMR of *Thermus* Cu_A[3].

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Direct electrochemical characterization of hyperthermophilic *t. celer* metalloenzymes involved in hydrogen production from pyruvate

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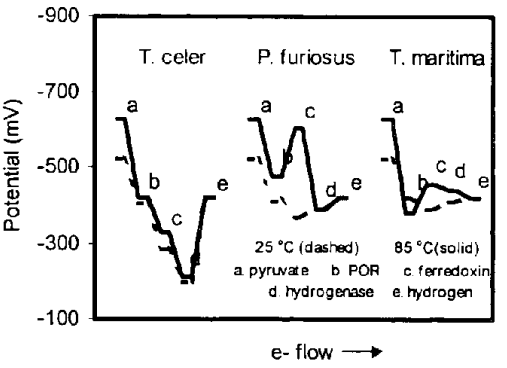
Redox potentials of pyruvate ferredoxin oxidoreductase (POR), ferredoxin and hydrogenase isolated from hyperthermophilic *Thermococcus celer* (T_{opt} = 88°C) were determined as a function of temperature from 10 to 85°C. Voltammetry experiments were carried out on 15 µL samples directly at a pyrolytic graphite electrode using $MgCl_2$ as a promoter. POR exhibited two voltammetric waves with peaks at -280 and -403 mV at 25°C, indicating multiple redox centers, and a single wave at -420 mV at 85°C. These waves displayed different temperature-dependent peak positions and peak heights, indicating that these redox centers have different thermodynamic and kinetic properties. Ferredoxin displayed a single linear temperature-dependent wave at -280 mV at 25°C and -327 mV at 85°C. Hydrogenase displayed a single biphasic temperature-dependent wave at -197 mV at 25°C and -211 mV at 85°C. Thermodynamic parameters for electron transfer are reported.



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