REDOX GATING OF THE STEAROYL-ACP DESATURASE CATALYTIC CYCLE

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Stearoyl-acyl carrier protein (ACP) Δ^9-desaturase (Des) belongs to the class of binuclear non-heme iron proteins and is involved in plant fatty acid synthesis. The enzyme catalyzes cis double bond introduction between carbons 9 and 10 of stearoyl-ACP that results in oleoyl-ACP [1]. It contains two μ-oxo bridged diiron centers arranged in a homodimer. The exact catalytic mechanism is still debated, however it is established that substrate-bound Des is reduced by Spinach Ferredoxin (SpFd) in the initial stages of the cycle. Several studies clearly demonstrated that chemical $4e^-$ reduction using non-physiological agents does not result in a catalytically competent Des [2]. To provide insight into the specific role of SpFd and substrate in defining the desaturase catalytic mode we have studied their effect on Des formal potential. Spectroelectrochemical titrations were conducted in a thin layer cell [3] using nanocrystalline Sb doped tin oxide electrodes. Enzyme oxidation state was determined from diiron chromophore absorbance. While substrate-free enzyme was 2 electron reduced around -0.03V, addition of SpFd resulted in appearance of another 1electron reduction wave at –0.32V. Formal potential of this redox process shifted to –0.21 V upon 18:ACP substrate binding to Des, suggesting thermodynamic redox gating of the enzymatic cycle. There is also evidence to suggest that substrate binding induces one versus two-electron reduction occurring in the substrate-free protein.

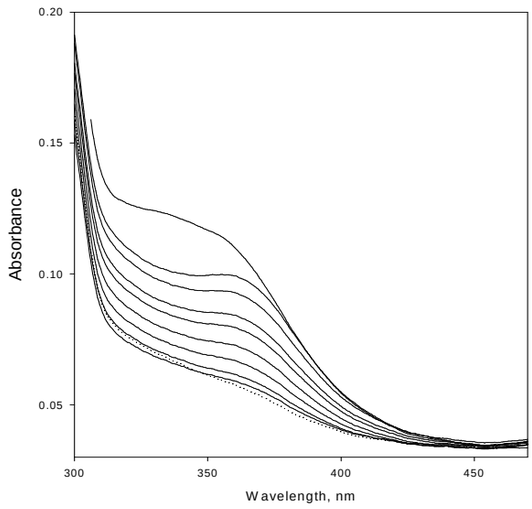

Figure 1. Desaturase absorbance variation during the potential scan in the thin-layer cell

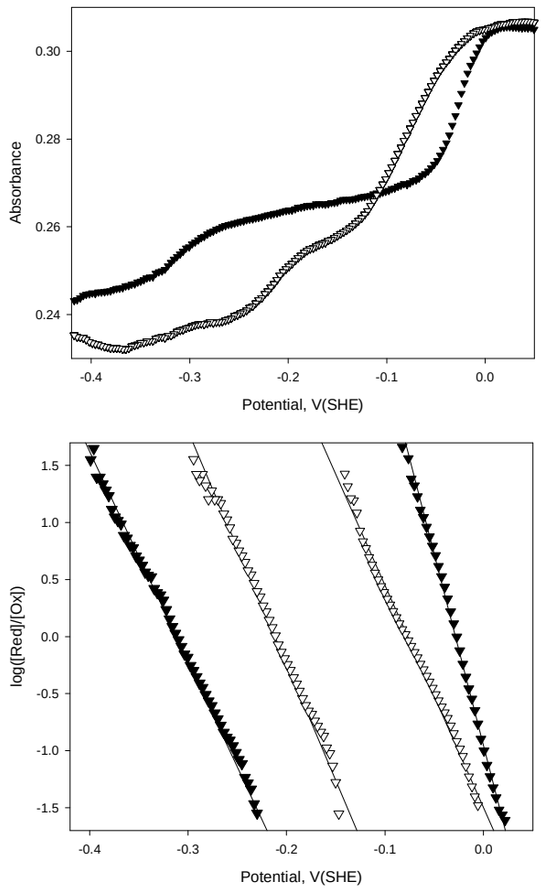

Figure 2. 340 nm absorption responses (upper graph) and corresponding Nernst plots (lower graph) of the substrate free (▼) and bound (∇) Des during the 0.05mV/s potential scan.

References

1. B. G. Fox, J. Shanklin, C. Somerville, and E. Münck *Proc. Natl. Acad. Sci. USA* 90, 1993, 2486-2490.
2. J. A. Broadwater, J. Ai, T. M. Loehr, J. Sanders-Loehr, and B. G. Fox *Biochemistry* 37, 1998, 14664-14671.
3. V. Reipa, M. P. Mayhew, M. J. Holden, and V. Vilker *Chem. Comm.* 2002, 318-319.