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2001 Program
Investigator Initiated (2-year project)

Project Title: Directed evolution of pyridoxal phosphate-dependent proteins to acquire aminolevulinate synthase activity

Project Summary: Cardiovascular disease takes more lives in the United States than cancer, accidents and the next five leading causes of death combined. Hemoglobin is the major heart protein and heme is the essential functional group of hemoglobin. Defects in heme biosynthesis can lead ultimately to heart diseases; thus heme biosynthesis needs to be finely regulated so that the perfect balance and functioning of the heart is maintained. The funded research focused on 5-aminolevulinate synthase (ALAS), the enzyme responsible for the first and regulatory step of heme biosynthesis. The primary objective of this research was to study the scaffold of ALAS and related-enzymes in order to unravel how ALAS functions.

Project Successes: Research findings include 1) circular permutation of ALAS does not prevent folding of the polypeptide chain into a structure amenable to binding of the PLP cofactor and assembly of the two subunits into a functional enzyme, 2) ALAS and circularly permuted variants folded reversibly; the equilibrium unfolding/refolding profiles were biphasic and followed a unimolecular process with the presence of at least one stable intermediate; 3) the formation of this intermediate was preceded by the disruption of the dimeric interface or dissociation of the dimer without significant change in the secondary structural content of the subunits; 4) linking the two subunits into a single polypeptide chain dimer yielded an enzyme with a ~7-fold greater turnover number than that of wild-type ALAS; 5) aspartate 279 has an important role in the modulation of the cofactor chemistry of ALAS. These findings are relevant in understanding how the two subunits assemble so that the cofactor can be nested at the inter-subunit interface and yield a functional (ALAS) enzyme.

Selected publications from BRP funded research in Peer Reviewed Journals:

Ferreira GC. Heme Synthesis. In: Lennarz W, Lane MD, eds. *Encyclopedia of Biological Chemistry.* San Diego, CA: Academic Press; 2004: in press.

Zhang J, **Ferreira GC.** Transient state kinetic investigation of 5-aminolevulinate synthase reaction mechanism. *J Biol Chem.* 2002;277:44660-44669.

Ferreira GC, Cheltsov AV. Circular permutation of 5-aminolevulinate synthase as a tool to evaluate folding, structure and function. *Cell Mol Biol.* 2002;48:11-16.

Cheltsov AV, Guida WC, **Ferreira GC.** Circular permutation of 5-aminolevulinate synthase: effect on folding, conformational stability and structure. *J Biol Chem.* 2002;278:27945-27955.

Ferreira GC, Zhang, J. Mechanism of 5-aminolevulinate synthase and the role of the protein Environment in controlling the cofactor chemistry. *Cell Mol Biol.* 2002;48:827-833.

Selected presentations from BRP funded research:
Ferreira GC. Department of Molecular Biophysics, Center for Chemistry and Chemical Engineering, University of Lund, Sweden, November 2002.

Ferreira GC. *Aminolevulinate Synthase and Heme Biosynthesis.* Saint Petersburg, Moscow, Russia: Engelhardt Conference; June 29 – July 5, 2003.

Ferreira GC. Porphyrins and Porphyrias 2003, Charles University–Carolinum, Prague, Czech Republic, September 21 – 24, 2003.

New grants based in part on BRP-funded work:
NIH
Title: (#DK63191) Aminolevulinate synthase and heme biosynthesis
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Award amount: $603,967