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July 2, 2002

Mr. James R. Bieschke
Acquisition and Assistance Contracting Officer
Department of Energy
Chicago Operations
9800 South Cass Avenue
Argonne, Illinois 60439

RE: Final Technical Report
DOE Grant Number: DE-FG02-86ER13581

Dear Mr. Bieschke,

On behalf of the University of Florida, I am providing a copy of the final technical report for the above reference grant. I would appreciate it if you would accept my apology on behalf of the University for the delay in forwarding this final technical report to the Department of Energy.

Because the Department of Energy is a valued sponsor, I have asked our Award Administration office, which is the office that receives information on late reports, to let me know when there is a notification of a late report due the Department of Energy so that we can avoid such delays in the future.

Please contact me directly at (352) 392-4802 or by email at sandyg@ufl.edu, if I can provide any additional information or assist with closing out this grant in any other way. Again, we regret the delay in submitting the report.

Sincerely yours,

Sandra Goldstein
Associate Director for University Research
Research and Graduate Programs

cc: T. Walsh, Division of Sponsored Research
C. Anderson, Department of Energy
J. Cole, Institute of Food and Agricultural Sciences
C. Banner, Institute of Food and Agricultural Sciences

DISCLAIMER

Final Report for 1996 - 2000:
Gene-Enzyme Relationships of Aromatic Amino Acid Biosynthesis in Higher Plants

Inhibition studies of amino acids in *Nicotiana silvestris* suspension cells gave clues to the difficulties for obtaining mutants deficient in post prephenate pathway proteins of aromatic amino acid biosynthesis (prephenate aminotransferase, arogenate dehydrogenase and arogenate dehydratase. Such mutants, if successfully obtained, would allow gene-enzyme relationships of aromatic amino acid proteins to be studied. We found that amino acids were inhibitory toward plant cell growth, and thus were unable to rescue analog resistant mutants. Toxicity of all amino acids toward exponentially dividing *Nicotiana silvestris* suspension cultured cells was monitored by following growth rates. Except for *L*-glutamine, all 19 protein amino acids inhibited cell growth. Inhibition of growth progressed to cell deterioration. Electron microscopy showed that amino acids triggered a state of cell shrinkage that eventually degenerated to total cellular disorganization. *L*-glutamine was not only an effective agent for prevention of amino acid toxicity, but enhanced the final growth yield. *L*-glutamine also was able to completely reverse inhibition effects in cells that had been in the slowed exponential phase. Two types of inhibition occurred and we have proposed that any amino acid inhibition that can be completely antagonized by *L*-glutamine be called 'general amino acid inhibition'. 'Specific amino acid inhibition' resulting from particular pathway imbalances caused by certain exogenous amino acids, can be recognized and studied in the presence of *L*-glutamine which can abolishes the complication effects of general amino acid inhibition (1).

The complexity of the regulatory mechanisms that govern amino acid biosynthesis, particularly in multi-branched pathways, frequently results in sensitivity to growth inhibition by exogenous amino acids. Examples of specific amino acid inhibition of growth were demonstrated in *N. silvestris*. In one case *L*-threonine inhibited growth partially in the presence of *L*-glutamine. The residual amino acid inhibition was overcome by the additional presence of *L*-lysine and *L*-methionine, indicating that exogenous *L*-threonine specifically inhibits the biosynthesis of both L-lysine and *L*-methionine. As a second example, the *L*-valine-mediated inhibition of growth that persisted in the presence of *L*-glutamine was overcome by *L*-isoleucine, indicating that exogenous *L*-valine inhibits *L*-isoleucine biosynthesis. The use of amino acid analogs as experimental tools for biochemical-genetic studies in higher plants is also complicated by general amino acid inhibition. Conditions were demonstrated under which *p*-fluorophenylalanine and *m*-fluorotyrosine could be used as specific antimetabolites of *L*-phenylalanine and *L*-tyrosine biosynthesis without interference from general amino acid inhibition (2).

Initial steps to obtain cDNA clones from *A. thaliana* led to several incomplete cDNA genes for arogenate dehydrogenase and arogenate dehydratase. With the recent completion of the sequencing project for *A. thaliana*, it should be possible by PCR to complete the cDNA clones and study gene-enzyme relationships.

Two proteins having quinate dehydrogenase activity have been purified from needle tissue of pine trees (*Pinus taeda* L.) and were shown to also have shikimate dehydrogenase activity (3). These data indicated that each protein having quinate dehydrogenase and shikimate dehydrogenase activities were each catalyzed by a single broad-specificity protein. The purification of an additional quinate dehydrogenase from xylem forming cells has been recently accomplished.

Publications for 1996 - 2000:

(1) CA Bonner, DS Williams, HC Aldrich and RA Jensen (1996) Antagonism by *L*-glutamine of toxicity and growth inhibition caused by other amino acids in suspension cultures of *Nicotiana silvestris*. Plant Science 113: 43-58.

(2) CA Bonner and RA Jensen (1997) Recognition of specific patterns of amino acid inhibition of growth in higher plants, uncomplicated by glutamine-reversible 'general amino acid inhibition'. Plant Science 130:133-143.

(3) V Ossipov, CA Bonner, S Ossipova and RA Jensen (2000) Broad-specificity quinate (shikimate) dehydrogenase from *Pinus taeda* needles. Plant Physiol. Biochem. 38:923-928.