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**MOLECULAR ENGINEERING AND CHARACTERIZATION OF  
RECOMBINANT GAMMA ZEIN FOR THE DIETARY TREATMENT  
OF PHENYLKETONURIA (PKU)**

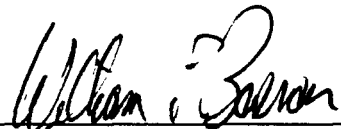
**Stephanie C. Ems-McClung**

**Submitted to the faculty of the University Graduate School  
in partial fulfillment of the requirements  
for the degree  
Doctor of Philosophy  
in the Department of Medical and Molecular Genetics,  
Indiana University**

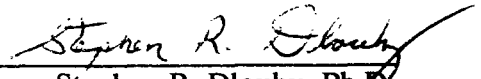
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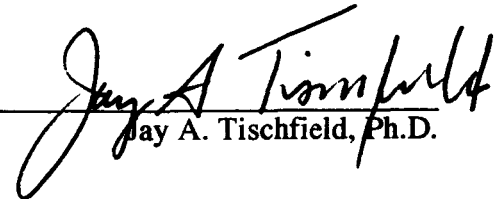
  
Bryan E. Hainline, M.D., Ph.D.

  
William F. Bosron, Ph.D.

Doctoral Committee

  
Stephen R. Dlouhy, Ph.D.

July 9, 1998

  
Jay A. Tischfield, Ph.D.

## ABSTRACT

Stephanie C. Ems-McClung

### MOLECULAR ENGINEERING AND CHARACTERIZATION OF RECOMBINANT GAMMA ZEIN FOR THE DIETARY TREATMENT OF PHENYLKETONURIA (PKU)

Phenylketonuria is the accumulation of phenylalanine in all bodily fluids and results in severe mental retardation. Life-long treatment requires the restriction of dietary phenylalanine by limiting protein intake. Treatment with a medical formula containing crystalline amino acids provides the necessary essential amino acids for normal growth and development. Adolescents and adults do not tolerate the formula due to poor odor and taste. An intact phenylalanine-free protein may alleviate dietary noncompliance.

Gamma zein, a corn prolamin, that contains only two phenylalanine residues was identified. A thioredoxin-based bacterial expression system was developed for gamma zein and resembled gamma zein expression in *Xenopus* oocytes and transgenic *Arabidopsis*. Expression of intact gamma zein or the repetitive N-terminal domain in *Escherichia coli* resulted in protein insolubility, low yield, and retention within the bacterium after osmotic shock. In contrast, the C-terminal domain with or without the Pro-X region expressed in the presence of thioredoxin was soluble, accumulated to high levels, and was released by osmotic shock. Thioredoxin-induced solubility suggested that the gamma zein C-terminal domain likely contained stabilizing intramolecular disulfide bonds between the nine cysteine residues (C117, C128, C136, C144, C148, C155, C156, C205, and C213).

Results from reduction and alkylation of the thioredoxin-linked C-terminal domain and site-directed mutagenesis of several cysteines (C117, C128, C136, and C156) was

consistent with the presence of at least three intramolecular disulfide bonds. Based on these results and cysteine conservation with other prolamins, the following intramolecular disulfide bond map was proposed: C128-C155, C136-C148, C144-C213, and C156-C205.

Modification of the two phenylalanine codons (F131 and F172) to isoleucine or valine resulted in C-terminal insolubility, whereas modification of both residues to tyrosine maintained native solubility. The aromatic ring of tyrosine probably provided the proper spatial orientation, which allowed native folding. Osmotic shock release was independent of thioredoxin fusion and was correlated with fusion protein solubility ( $r^2=0.95$ ). This suggested a dependence on proper folding and an innate association with the bacterial cytoplasmic membrane. These findings will aid the development of a nutritionally enhanced phenylalanine-free recombinant protein for the dietary treatment of phenylketonuria.

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Bryan E. Hainline, M.D., Ph.D., Chairman

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William F. Bosron, Ph.D.

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Stephen R. Dlouhy, Ph.D.

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Jay A. Tischfield, Ph.D.

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