Cloning, Expression, and Characterization of the Aminoethylphosphonate Pathway in Trypanosoma cruzi

Samuel I. Rodriguez^  
*University of Texas at El Paso, srodriguez10@miners.utep.edu

Esteban M. Cordero  
Department of Biological Sciences, University of Texas at El Paso, emcorderoveas@utep.edu

Igor C. Almeida*  
Department of Biological Sciences, University of Texas at El Paso, icalmeida@utep.edu

Follow this and additional works at: http://digitalcommons.utep.edu/couri_abstracts_sum11

Funding Source:  
NIH/Bridges to the Baccalaureate Program (grant#5R25GM049011-12) and NIH grants#1R01AI070655, 3R01AI070655-04S1, 2G12RR008124-16A1, and 2G12RR008124-16A1S1.

Comments:  
BRIDGES TO THE BACCALAUREATE

Recommended Citation  
http://digitalcommons.utep.edu/couri_abstracts_sum11/10

This Article is brought to you for free and open access by the COURI Symposium Abstracts at DigitalCommons@UTEP. It has been accepted for inclusion in COURI Symposium Abstracts, Summer 2011 by an authorized administrator of DigitalCommons@UTEP. For more information, please contact lweber@utep.edu.
Cloning, Expression, and Characterization of the Aminoethylphosphonate Pathway in *Trypanosoma cruzi*

Samuel Rodriguez^, Esteban M. Cordero, and Igor C. Almeida*.

*The Border Biomedical Research Center, Department of Biological Sciences, The University of Texas at El Paso*

*Trypanosoma cruzi*, the etiological agent of Chagas disease, is densely coated with virulence factors, mostly anchored to the plasma membrane by glycosylphosphatidylinositol (GPI). The core of a GPI molecule is conserved among eukaryotes, containing the canonical EtNP-Man₃-GlcN-myoo-Inositol-PO₄-lipid structure. *T. cruzi* is possibly the only eukaryote capable of utilizing either ethanolaminephosphate (EtNP) or aminoethylphosphonate (AEP) as the linker between proteins and the GPI-anchor. Besides the conversion of phosphoenolpyruvate (PEP) into 3-phosphonopyruvate, the *T. cruzi*’s biosynthetic pathway of AEP remains unsolved. Gene survey reveals two enzymes, namely, phosphonopyruvate decarboxylase (PPDC) and aminoethylphosphonate transamidase (AEPT). This study aims to clone, express, and characterize PPDC and AEPT, because their potential chemotherapeutic targets. Specific oligonucleotides were designed to amplify the open-reading frame of PPDC and AEPT genes. The amplicons were cloned into pGEM-T easy vector and sequenced. AEPT and PPDC genes are 1,203 and 1,359 bp in length encoding proteins of 400 and 452 amino acids, respectively. The inserts were sub-cloned into pET28a and pRSET-A expression vectors and transformed into *E. coli* BL-21 strain. The expression was induced for 3h at 37°C with 1 mM IPTG. SDS-PAGE and blotting analyses indicated both proteins were expressed in bacteria. Biochemical characterization of PPDC and AEPT is underway.