Engineering Soybean for Enhanced Sulfur Amino Acid Content

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Executive Summary

Soybeans are a key component in high-protein feed ingredient in livestock production; however, corn- and soybean-based rations do not contain sufficient methionine to meet the nutritional demands of rapidly growing swine or poultry. Genetic engineering of soybeans for high sulfur amino acid content will have a significant impact on livestock and poultry industry. The primary objective was to obtain a through understanding of sulfur assimilatory pathway in soybean and utilize the knowledge gained to increase the overall sulfur amino acid content of soybeans by genetic engineering. Specific aims are to: 1) Express ATP sulfurylase, the entry point enzyme of sulfur assimilation, in soybean to provide increased metabolic flux through the pathway; 2.) Determine the three-dimensional structure of this enzyme to understand its metabolic regulation. Significant progress has been made for both aims. First, we have carried out detail biochemical characterization of key enzymes of sulfur assimilatory pathway. Additionally, several transgenic Arabidopsis and few soybean plants overexpressing ATP sulfurylase have been generated. Substantial progress has also been made on growing crystals of soybean ATP sulfurylase in complex with APS that diffracted to 3.5 Å resolution on a home x-ray source. The results obtained from this investigation will greatly aid our understanding of sulfur assimilation in soybean and provide insights as to how to increase the accumulation of sulfur-containing amino acids by manipulating rate-limiting enzymes in this biochemical pathway. Soybeans accumulating high amounts sulfur amino acids will enable the soybean farmers to expand their market share. Further, generation of such superior soybean varieties would obviate adding synthetic amino acids to poultry and livestock rations which cost producers million of dollars per year.

Project Highlights

We have expressed, purified, and kinetically characterized the ATP sulfurylase from soybean (Phartiyal et al., 2006). In addition, the biochemical assays developed for measuring activity of the purified protein were adapted to determining ATP sulfurylase activity in plant extracts. These studies showed that enzyme activity was highest in soybean root tissue and seeds at the earliest development stages (Phartiyal et al., 2006). We have also biochemically characterized two other enzymes (APS reductase and APS kinase) in the sulfur assimilation pathway of soybean. We used a truncated version of soybean APS reductase lacking the first 80 amino acids for protein expression and

purification. The steady-state kinetic parameters and product inhibition effects of purified recombinant soybean APS reductase were determined. In addition, the activity of the enzyme was determined in soybean tissue extracts. As shown for ATP sulfurylase, the reductase activity is highest in young soybean seeds and is induced by low-temperature stress (Phartiyal et al., 2008). Utilizing transgenic Arabidopsis we have demonstrated overexpresion of ATP sulfurylase provide protection against cold stress.

We have optimized the protein crystallization conditions to obtain diffraction quality crystals of soybean ATP sulfurylase. Crystallization of the enzyme required the formation of a complex with the reaction product, 5'-adenylsulfate (APS) that diffracted to 3.5 Å resolution on a home x-ray source. Initial characterization of the x-ray diffraction data indicates that the protein crystallizes in a tetragonal lattice group; however, the quality of the data is not sufficient for determination of the space group. Currently, we are screening for modified crystallization conditions and are exploring the effect of additives on improving the quality of the diffraction data.

Publication and Presentations

Phartiyal, P., Kim, W-S., Cahoon, R.E., Jez, J.M., and Krishnan, H.B. (2006). Soybean ATP sulfurylase, a homodimeric enzyme involved in sulfur assimilation, is abundantly expressed in roots and induced by cold treatment. Arch. Biochim. Biophys. 450: 20-29.

Phartiyal, P., Kim, W-S., Cahoon, R.E., Jez, J.M., and Krishnan, H.B**.** 2008. The role of 5'-adenylylsulfate reductase in the sulfur assimilation pathway of soybean: molecular cloning, kinetic characterization, and gene expression. Phytochemistry 69: 356-364.

Kumaran, S., Francois. J.A., Krishnan, H.B., and Jez, J.M. 2008. Regulatory protein-protein interactions in primary metabolism: the case of the cysteine synthase complex In Sulfur Assimilation and Abiotic Stress in Plants (NA Khan, S Singh, S Umar, Eds.) pp. 97-109, Springer-Verlag, NY

Jez, J.M. and Fukagawa, N.K. 2008. Plant sulfur compounds and human health In Sulfur: A Missing Link Between Soils, Crops, and Nutrition (Jez JM, Ed.) pp. 281-292, ASA-CSSA-SSSA Publishing, Madison, WI

Jez, J.M. and Krishnan, H.B. 2009. Sulfur assimilation and cysteine biosynthesis in soybean seeds: towards engineering sulfur amino acid content In Modification of Seed Composition to Promote Health and Nutrition (Krishnan HB, Ed.) ASA-CSSA-SSSA Publishing, Madison, WI (in press)

The data from this project has been presented in several invited seminars and lectures including:

Dr. Krishnan was an invited speaker at the 23rd Annual Mid-Atlantic Plant Molecular Biology Society Meetings, Maryland and presented a talk on “Genetic modification of soybean seed composition”. 2006.

Dr. Krishnan was an invited speaker at the 11th Biennial Conference on the cellular and Molecular Biology of Soybean (Soy2006), University of Nebraska-Lincoln and presented a talk on “Genetic engineering of the sulfur assimilatory pathway in soybean”. 2006.

Dr. Krishnan presented an invited seminar at Seoul National University, Korea on “Modification of soybean seed composition to promote health and nutrition”. 2007.

Dr. Krishnan presented an invited seminar at Kobe University, Japan on “Genetic modification of soybean seed composition”. 2007.