

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 09/24/93

Project No. G-33-E15 _____ Center No. 10/24-6-R7610-0A0_
Project Director IKEDA R A _____ School/Lab CHEMISTRY _____
Sponsor DHHS/PHS/NIH/NATL INSTITUTES OF HEALTH _____
Contract/Grant No. 5 R01 GM36610-08 _____ Contract Entity GTRC
Prime Contract No. _____
Title HIGH RESOLUTION STRUCTURES OF PISUM SATIVUM LECTIN _____
Effective Completion Date 930731 (Performance) 931031 (Reports)

Closeout Actions Required:	Y/N	Date Submitted
Final Invoice or Copy of Final Invoice	Y	_____
Final Report of Inventions and/or Subcontracts	Y	_____
Government Property Inventory & Related Certificate	N	_____
Classified Material Certificate	N	_____
Release and Assignment	N	_____
Other _____	N	_____

Comments EFFECTIVE DATE 8-1-92. CONTRACT VALUE \$166,308. _____

Subproject Under Main Project No. _____

Continues Project No. _____

Distribution Required:

Project Director	Y
Administrative Network Representative	Y
GTRI Accounting/Grants and Contracts	Y
Procurement/Supply Services	Y
Research Property Management	Y
Research Security Services	N
Reports Coordinator (OCA)	Y
GTRC	Y
Project File	Y
Other CARL BAXTER-FMD _____	Y
FRED CAIN-00D _____	Y

NOTE: Final Patent Questionnaire sent to PDPI.

Final Progress Report

Grant Number: 5R01 GM36610-08
Principal Investigator: Dr. F. L. Suddath (Note: Due to the death of Dr. Suddath, Dr. Richard Ikeda was designated as PI for the final year of the grant to close out the research in an orderly fashion)
Grantee Institution: Georgia Tech Research Corporation
Project Title: "High Resolution Structures of Pisum Sativum Lectin"
Period of the Project: 08/01/85 through 07/31/93

Summary Statement

Specific Aims

A very common family of proteins that selectively binds carbohydrates is the lectins, a family of proteins that can be isolated from peas. These proteins have been crystallized and the structure of a number of lectins has been reported, but although the lectins are known to selectively bind different sugars, the structural basis for carbohydrate binding to lectins is not well characterized. It has been hypothesized that the selective binding of carbohydrates to lectins is a result of specific contacts between the sugars and the amino acid side chains of the lectins. To determine the specific nature of these protein/sugar contacts:

- (1) the structure of pisum sativum lectin will be solved to high resolution,
- (2) pisum sativum lectin with different sugars bound to the protein will be crystallized and the structure of the protein/sugar complexes will be determined, and
- (3) recombinant pisum sativum lectin with altered sugar binding specificities will be produced by genetic engineering, and the altered lectins will be biochemically characterized, the mutant proteins crystallized, and the structure of the mutant proteins analyzed.

Results

(Note: Due to the death of the original PI, Dr. F. L. Suddath, Dr. Richard A. Ikeda was named as a replacement PI to oversee the orderly completion of the research grant and the disbanding of the laboratory. The research summarized below is the work that Dr. Richard Ikeda has been able to document with the records available. Other research may be described in previous progress reports that were not available to Dr. Ikeda.)

During the duration of the grant the following specific aims were achieved:

- (1) The structure of the pea lectin dimer has been determined to 1.7 Å resolution. The 1.7 Å model gives a crystallographic R value of 17.7 and an RMS deviation from ideal bond distances of 0.027 Å. The electron density has also suggested positions for a number of bound water molecules, and 294 of these have been added to the refinement model. Eight of the water molecules are ligands to the two Mn^{2+} and Ca^{2+}

ions bound in the pea lectin dimer, completing the octahedral geometry around the ions. Surprisingly, nine water molecules were found to lie in the interface between the two monomers. The remainder of the water molecules form hydrogen bonds to polar and charged side chains on the surface of the pea lectin dimer, and many are related by the two-fold symmetry of the dimer. Some of these surface water molecules lie near the putative carbohydrate binding site. Presumably, upon binding of a carbohydrate, the hydrogen bonds to water would be displaced by new hydrogen bonds to the hydroxyl groups of the carbohydrate molecule.

Submission of the manuscript detailing this structure was delayed because of the death of Dr. Suddath. It is anticipated that a collaborative effort with Dr. Howard Einspahr at Upjohn and Dr. Ed Meehan at Alabama-Huntsville will result in the submission of this structure for publication in the future.

(2) Pea lectin was cocrystallized with mannose, α -methyl mannose, trehalose, α -methyl glucose, α -benzyl mannose, α -methyl,3-O-methyl mannose, and phenyl- α -D-pyranoside. Preliminary characterization of the crystals showed that the cocrystals are of high quality, and the space groups of the unit cells within many of the cocrystals was determined (Masters Thesis of Mi Li, Georgia Institute of Technology, 1992). Due to the termination of the grant collection of x-ray diffraction data from the crystals was deferred. Instead, crystallization conditions were reconfirmed and recorded and cocrystals were cataloged and stored.

(3) In a collaboration with Dr. Jeff Engler, Alabama-Birmingham, pea lectin cDNA was cloned into a bacterial expression plasmid and active pea lectin protein was expressed in *E. coli*. Cloning of pea lectin into *E. coli* also allowed for mutagenesis of the protein. The following mutants were produced and their binding capability was measured (PhD Thesis, Thomas Prasthofer, Alabama-Birmingham, 1990).

<u>Pea Lectin</u>	<u>Sugar Binding Activity</u>
Native	+
Recominant	+
Asn39 to Asp	+
Asp81 to Pro	+
Asp81 to Asn	+
Asn125 to Asp	-
Phe123 to Thr	-
Arg at 99 and 100	+

The molecular interpretation of these results will require the structural analysis of the proteins.

Publications

- 1) Phillips, S., Einspahr, H., Meehan, E., and Suddath, F. L., "The Crystal Structure of Pea Lectin at 1.7 A Resolution." In preparation.

- 2) Prasthoffer, T., Phillips, S. R., Suddath, F. L., and Engler, J. A., "Design Expression, and Crystallization of Recombinant Lectin from the Garden Pea (*Pisum sativum*).²" *J. Biol. Chem.*, **264**, 6793-6796 (1989).
- 3) Basu, D., Delucas, L., Parks, E. H., and Suddath, F. L., "Preliminary Crystallographic Study of the α -D-Galactose-specific Lectin from Jack Fruit (*Artocarpus integra*) Seeds." *J. Mol. Biol.*, **201**, 661-662 (1988).
- 4) Einspahr, H., Parks, E. H., Suguna, K., Subramanian, E., and Suddath, F. L., "The Crystal Structure of Pea Lectin at 3.0 A Resolution." *J. Biol. Chem.*, **261**, 16518-16527 (1986).
- 5) Suddath, F. L., Parks, E. H., Suguna, K., Subramanian, E., and Einspahr, H., "The Crystal Structure of Pea Lectin at 3.0 A Resolution." *Mol. Biol. of Seed Storage Proteins and Lectins*, pp 29-44, (1986).
- 6) Einspahr, H., Suguna, K., Suddath, F. L., Ellis, G., Helliwell, J. R., and Papiz, M., "The Location of the Manganese and Calcium Ion Cofactors in Pea Lectin Crystals by use of Anomalous Dispersion and Tuneable Synchrotron X-Radiation." *Acta. Cryst.*, **B41**, 336-341 (1985).