VIKRAM DHUNA

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Carrier Objective

Work to get experience in the field of cell biology and immunology for the diagnostic and therapeutic purposes.

Education

Ph.D (Biochemistry) (2006), Guru Nanak Dev University, Amritsar, Punjab, India. (Currently writting Dissertation)

Title of thesis "Studies on the biological properties of some purified phytolectins and identification of amino acid residues essential for their activity.

M.Sc. **Molecular Biology and Biochemistry** (2001): **Ist Div**. Guru Nanak Dev University, Amritsar, INDIA with Immunology, Biochemistry, Molecular Biology, Enzymology and Biomembranes.

B.Sc. **Medical** (1999) **Ist Div.** Guru Nanak Dev University, Amritsar, INDIA with Zoology, Botany, Chemistry, English.

Date of Birth July 4,1979.

Instrumentation Skills

- I have operated common biochemistry laboratory instruments like High performance liquid chromatographic system (HPLC) ELISA reader, Laminar Flow, Cell harvester, Carbon dioxide incubator, Liquid scintillation counter, Densitometer, Gel documentation system, Electrophoretic assembly, UV/Vis spectrophotometer, Fluorescence Spectrophotometer, RC5C refrigerated centrifuge (Sorvall), PCR Thermal cycler, Real Time PCR, DNA sequencer etc.
- Lymphocyte culturing
- Human cell line culturing and microbial culturing

Computation Skills

Windows operating systems; Adobe Photoshop and MS office. Worked on other computer software of various instruments.

Scholarships and Awards

- Qualified Graduate Aptitude Test for Engineering **(GATE)-2002 with 97.45** Percentile conducted by IIT one of premier institutes in India on national level.
- **Senior Research Fellowship** (Sep 2004 onwards) awarded by University Grants Commission (UGC) of INDIA
- **Junior Research Fellowship** (2002-2007) awarded by UGC/CSIR through National Eligibility test (NET) conducted by Council of Scientific and Industrial Research (CSIR) of INDIA in June.
- **Selected in First 20% of the Council of Scientific and Industrial Research (CSIR)-** Junior Research Fellowship (JRF) qualified candidates, which enabled me to appear for the "Shayama Prasad Mukherji" (highest-grade fellowship for young scientists in India) in July 2003.
- **Qualified National Eligibility (NET) test for Lecturership** conducted by University Grant Commission (UGC), INDIA in June 2001.
- **Qualified Graduate Aptitude Test (GRE**) Dec 31, 2002 with Total Score of 1190. Individual Score of Verbal- 530, Quantitative- 660 and Writing- 4.5.
- **Qualified Test of English as a Foreign Language (TOEFL)** 267/300 and 5.5 in writing.
- Received **travel grant from the St. Gallen Oncononferences organizers** to attend 4th International Conference **"Cancer Prevention 2006" at St. Gallen, Switzerland.**

- Received **travel grant from Ministry of Science and Technology**, Department of Biotechnology, Government of India to attend 21st International Lectin Meeting **"Interlec 21" at Japan** during May 23-28, 2004.

Teaching Experience

Taught various practical courses of Immunology and Biochemistry from April 2003-2005 to M.Sc. Molecular Biology and Biochemistry.

Papers Presented/Conferences attended

- Attended **"Advanced Immunology Course**" during March 1-5 conducted by All India Institute of Medical Sciences (AIIMS).
- Presented paper entitled "**In-vitro antiproliferative effect of *Gonatanthus pumilus* lectin on various human cancer cell lines**" in 4th International Conference on "Cancer Prevention 2006" held during February 16-18 **at St. Gallen, Switzerland**.
- Attended 2 days National Workshop on **"Advances in Bioinformatics"** held during February 23-24 at Department if Biotechnology, Guru Nanak Dev University, India.
- Paper presented entitled "**An anti-proliferative lectin from tubers of *Arisaema speciosum*: Purification and characterization**" at National Conference on Immunology "IMMCON-2005 held during at Post Graduate Institute of Madical Education and Research (PGIMER), Chandigarh, India.
- Paper presented entitled "**Purification and characterization of an anticancer lectin from a wild Himalayan Cobra lily, *Arisaema leschenaultii*."** in Plant Sciences-2004" held during, Nov. 25-27 2004, Gandhinagar, Gujrat, India.
- Paper presented entitled **A simple procedure for the isolation of human IgG using immobilized *Sauromatum guttatum* lectin (SGL)** in "Interlec 21- 21st International Lectin Meeting held at **Kanagawa, Japan**.
- Paper presented entitled **"Purification and Characterization of asialofetuin specific lectins from four Himalayan Cobra Lilies"** in "BIOHORIZON 2004" held during March 12-13, 2004 at Indian Institute of Technology, New Delhi.
- Paper presented entitled "**N-acetyl-D-lactosamine specific lectins from genus Arisaema: Purification and characterization."** in 7th Punjab Science congress held at Guru Nanak Dev University, Amritsar, India.

Referees

i) Prof. Sukhdev Singh
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List of Publications

1. **Vikram Dhuna**, Jagmohan Singh, Sukhdev Singh, Jatinder Singh, Shanmugavel, Ajit Kumar Saxena. Purification and characterization of a lectin from *Arisaema tortuosum* Schott having *in-vitro* anticancer activity against human cancer cell lines. Journal of Biochemistry and Molecular Biology. Volume 38, Number 5, 526-532.
2. Jagmohan Singh, **Vikram Dhuna**, Jatinder Singh, Sukhdev Singh Kamboj and Javed N. Agrewala. Novel anticancer and mitogenic lectins from underground tubers of two *Acorus*

species.. . International Immunopharmacology. Volume 5, Issue 9, August 2005, Pages 1470-1478.

3. Urmila Phutela, **Vikram Dhuna**, Shobhna Sandhu and B.S.Chadha (2005) Pectinases and polygalacturonase production by a thermophilic Aspergillus fumigatus isolated from decomposting peels.. Brazilian Journal of Microbiology 36: 63-69.
4. Amandeep Kaur, Sukhdev Singh Kamboj, Jatinder Singh, **Vikram Dhuna** and A.K. Saxena. Isolation of a novel *N*-acetyl-D-lactosamine specific lectin from *Alocasia cucullata* (Schott.). **Biotechnology letters**, Volume 27, Number 22, November 2005, pp. 1815-1820.
5. **Vikram Dhuna**, Sukhdev Singh, Jatinder Singh, Shanmugavel, Ajit Kumar Saxena. Purification and characterization of an anticancer lectin from a wild Himalayan Cobra Lily, *Arisaema leschenaultii*. International Journal of "Bioscience Reporter" 2 (2), 2004, pp. 528-540.
6. **Vikram Dhuna**, Sukhdev Singh Kamboj, Manpreet Kaur, Amandeep Kaur, Jatinder Singh*Further characterization of a monocot tuber lectin from *Gonatanthus pumilus* D. Don and its anti-proliferative effect on various human cancer cell lines. (Submitted to Journal of Molecular Biology and Biochemistry).
7. Navjot Kaur, Sukhdev Singh Kamboj, **Vikram Dhuna** and Jatinder Singh. A novel antiproliferative and antifungal lectin from A*maranthus viridis* seeds (Submitted to Protein and Peptide Letters)
8. Further characterization of Alocasia indica lectin and its anti-proliferative effect on human cancer cell lines. (Manuscript under preparation).
9. Effect of denaturation and Amino acid modification on fluorescence spectrum and Hemagglutination activity of *Sauromatum guttatum* lectin (Manuscript under preparation).
10. Purification and characterization of a novel lectin from Arisaema utile and its mitogenic activity on human lymphocytes. (Manuscript under preparation).

Expertise

- Expert in protein purification using various chromatographic and electrophoretic techniques like affinity chromatography, gel filtration chromatography, ion exchange chromatography, High performance liquid chromatography (HPLC), Native-PAGE, SDS-PAGE, 2-D PAGE, Isoelectric focusing and western blotting. Handled various immunological techniques like ELISA, Immunoblotting, DID etc.
- Very well exposed to techniques of protein crystallography.
- Expert in culturing of Human lymphocytes and various human cancer cell lines. Besides this, skilful in microbial culturing.
- Expert in specific chemical modification of proteins with chemical reagents to explore amino acids involved in its function.
- I had one month extensive training in routinely used molecular biology techniques like DNA isolation, Agarose gel electrophoresis, Restriction Digestion, amplification of DNA with PCR, Southern Blotting, Real Time PCR, DNA sequencing, culturing and expression at Jawaharlal Nehru University, New Delhi.

Research Summary

PhD Research Summary:

Lectins are proteins or glycoproteins having specificity for carbohydrates. These are versatile molecules having various biological properties. No doubt that lectins are plant products but due to their sugar binding property they have variety of effects on various animal cells. Mitogenicity is one of the very important properties of lectins, which has been very useful in increasing our knowledge about the human lymphocytes. Recently, lectins have come out with lot mare properties, which can be exploited for the good of mankind and improvement of crop plants. During my PhD degree I have designed my research to explore new plant lectins, which will have commercially as well as biologically exploitable properties. The findings are hoped to serve as

the opening of new era for the plant lectins, which may build a platform for the commercial use of plant lectins in for various purposes. The properties of plant lectins we have chosen are as follows.

1. Anti-proliferative effect of plant lectins on various human cancer cell lines.
2. Mitogenicity of plant lectins - which enable lectins to make a resting cell proliferate under normal conditions.
3. Purification of Immunoglobulins with the help of lectin affinity chromatography.
4. Antifungal effect of plant lectins.

As an out come of the work we have explored the novel lectins with properties like anti-cancer effect on human cancer cell, mitogenic effect on human lymphocytes and murine splenocytes, IgG purification from human serum and antifungal effect on plant pathogenic fungi.
In addition we have also characterized the sugar binding pocket of the lectin to explore the amino acids involved in its binding with sugar.

Purification of lectins

The first step to work on plat lectins is to obtain them in pure form. For this a various plants were screened for lectin activity. I selected four plants ***Arisaema tortuosum A. utile A. speciosum A. leschenaultii*** were with lectin activity and then explored the sugar specificity with sugar inhibition assay. All the lectins were specific for a desialylated serum glycoprotein **asialofetuin** and a disaccharide **N-acetyl-D-lactosamine**. The lectins were purified by affinity chromatography using asialofetuin linked amino activated silica. The lectins were further characterized for native and subunit molecular masses using various types of electrophoresis. Various physical properties of lectins like effect of temperature, pH, denaturants etc were explored. The secondary structure content of the lectins was also obtained using their CD spectra. Their serological studies by raising hyperimmune serum in rabbit have shown that these lectins have common antigenic determinants.

Mitogenicity of lectins

Resting lymphocytes can be induced to undergo DNA synthesis and subsequently cell division and proliferation by a wide variety of agents, but undoubtedly lectins constitute the most convenient generic group of mitogens. A limited number of lectins, mostly from plants posses the unique ability to induce quiescent lymphocytes and divide, a phenomenon known as mitogenic stimulation. The four purified lectins were studied for mitogenicity on human peripheral blood lymphocytes and murine splenocytes. Interestingly, two ***Arisaema tortuosum and A. utile*** out of the four new lectins were found to have more mitogenic stimulation on human lymphocytes than that of commercially available mitogenic lectins ConA and PHA. Similarly, mitogenic effect of these two lectins ***Arisaema tortuosum and A. utile*** on murine splenocytes was far better than commercially available mitogenic lectins like ConA and PHA.

For the further studies four new lectins ***Arisaema tortuosum A. utile A. speciosum* A. *leschenaultii*** purified by above method and five more lectins ***Sauromatum guttatum Gonatanthus pumilus, Alocasia indica Arisaema consanguineum Arisaema curvatum*** were used to widen the scope of study.

Anti-proliferative effect on human cancer cell lines

I have worked on DU145 (Prostate), PC-3 (Prostate) A549 (Lungs), HCT15 (Colon), 502713 (Colon), KB (Oral), IMR32 (Neuroblastoma) HT-29 (Colon), SiHa (Cervix), OVCAR-5 (Ovary), SNB-78 (CNS) to know the effect of all nine plant lectins on these cancer cell lines. The basis of studying the anti-proliferative effect of these lectins was their specificity for N-acetyl-D-lactosamine, which is one of the very important cancer markers reported in certain type of cancers. Interestingly, we have found that these lectins have very good anti-proliferative effect on many cancer cell lines and we have published. The anti-proliferative effect of these lectins varied on different cancer cell lines. The possible answer to this question may be the difference in the signalling pathway of various cancer cell lines. These lectins can also be used as drug delivery agents.

Immunoglobulin Purification

All the nine lectins were tested to have affinity with the human immunoglobulins by a self-designed ELISA method where we immobized our lectin at the bottom of plate. After blocking the remaining site of the plate we

added pure Immunoglobulin and then identified with the anti-immunoglobulin tagged with HRP. *Sauromatum guttatum*, one of the nine lectins gave positive binding with human IgG. The lectin was then immobilized on affinity column to purify the IgG from human whole serum. Interestingly the lectins picked only IgG from whole serum so this was the single step purification of human IgG from whole serum. We had presented this finding in Interlec 21, May 23-28, 2004 at Kanagawa, Japan. Protein A or protein G affinity chromatography is among the most popular for IgG purification. Yet, even these gels require harsh elution conditions and have the disadvantages of ligand leakage, low gel capacity and low-pressure capability. Our new method for the purification of IgG has cost advantage over the Protein A or protein G method because our lectin can be purified by simple affinity chromatography and can be used for the purification of IgG.

Antifungal effect on plant pathogenic fungi

Various plants have been infected by pathogenic fungi, which reduce their yield. Commercially available antifungal agents are hazardous chemical, which have very deleterious effects on the food chain. So, It has become necessity to look for the new harmless alternative. Lectins being plant products and proteinaceous in nature are easy to incorporate in the commercial plants with the help of DNA technology. So, we decided to study the nine lectins for antifungal properties. Two lectins ***Gonatanthus pumilus*** and ***Alocasia indica*** gave promising results in antifungal studies and were effective even at 50µg/ml concentration.

Characterization of sugar binding site of lectins

All most all of the lectin properties are due to their sugar binding property of the lectins. So, studying the amino acid involved in sugar binding will give us first hand knowledge about the interior of sugar binding pocket. So, we decide to modify the lectin amino acid with specific modifying agents to elucidate the sugar-binding site of lectins. We modified tryptophan, tyrosine, histidine, cysteine, arginine and serine to know which of these amino acids of lectin are involved in the sugar binding. We have found that Tryptophan is the most important amino acid and if we modify this amino acid all the lectins loose their complete activity. It shows that tryptophan is present is required for the sugar binding of lectins and is present in the sugar binding picket or near to it. Tyrosine is also important for the sugar binding because with its modification lectins lost 50-100% of the activity. Rest four amino acid were not present in the sugar-binding site of the lectins so not requited for the biological activity of lectins.

Research work in M.Sc. (Lab Rotation Advanced Practicals)

Molecular Biology and Biochemistry department where I completed my M.Sc. was having Lab Rotation Advanced Practicals, which were designed to train the students in various fields of Molecular Biology and Biochemistry. Our department is having three major research laboratories namely, Molecular Biology laboratory, Protein Laboratory and Membrane Laboratory. Students were divided in to groups to carry out experiments at these laboratories.

Molecular Biology Laboratory

In molecular biology laboratory I had practical hand on training for DNA isolation from human blood, plasmid isolation from bacteria, Agarose gel electrophoresis, Restriction digestion and PCR amplification.

Protein Laboratory

In protein laboratory I purified and characterized lectin from Glycine max. To purify the lectin the Glycine max seeds were soaked and crushed to make the extract. The lectin activity of the crude extract was checked with hemagglutination. The extract was then subjected different to ammonium sulphate precipitation 0-20%, 20-40%, 40-60%, 60-80%. The fraction having maximum lectin activity was passed through affinity column. The purity of lectin was checked with SDS-PAGE. The purified lectin was characterized for molecular mass with gel filtration and effect of various temperature and pH. Techniques handled during the stay in protein laboratory are SDS-PAGE, Native PAGE at pH 4.5 and 8.3 and Isoelectric focusing. Besides this hyperimmune serum was also raised in rabbit against the *Glycine max* lectin.

Membrane Laboratory

Membrane laboratory focuses on the yeast culturing and tolerance in yeast. I learned culturing yeast cells and estimation of cholesterol and High-density lipoproteins from human serum.