INSULIN LIKE ANTIGEN: SOURCES OTHER THAN PANCREAS

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ABSTRACT

Diabetes mellitus is the third largest cause of death after cardiovascular diseases and cancer. The failure to make insulin or insufficiency of insulin, is termed as diabetes mellitus. Insulin therapy is the only effective treatment of Type 1 diabetes mellitus. Insulin is a polypeptide key hormone which controls the level of the glucose in the blood and regulates the carbohydrate metabolism. In addition it also, metabolise fat and proteins and regulate expression of certain genes. Increased medicine’s cost; their pharmacological adverse effects and tremendous increase in diabetic population became a driving force in shifting interest towards development of bio-resource based medicines. Insulin was originally discovered in mammals, but later, insulin-related peptides were also recognized in plants. Insulin like antigen from plant with function, structure and sequence identical to vertebrate insulin are called 'glucokinin'. Studies of our lab showing insulin-like antigens in Spirulina platensis S5 paved the path for in vivo study for anti-diabetic activity. Recently we reported ELISA based screening and western blot of Cyanobacterial strains using anti-human insulin antibody and showed that insulin like antigen was present in Spirulina platensis S5.1-7 This paved the path for confirmation of its antidiabetic activity in-vivo.

Keyword: Diabetes mellitus, Insulin like antigen, Herbal medicines, and Sulphonylureas, biguanides.

INTRODUCTION

Diabetes mellitus is associated with a large variety of complications and a greater risk of all manifestations of atherosclerosis.2 Once diabetes develops, it is a costly disease to manage because of its chronic nature and severity of complications.4 Worldwide estimates of its prevalence are expected to rise from 2.8% (171 million people) in 2000 to 4.4% (366 million people) in 2030.5 Approximately 0.7% of the world’s population suffers from Type 1 diabetes mellitus; while about 90% of the diabetic world’s population suffers from Type 2 diabetes mellitus.3 Type 1 [insulin dependent Diabetes mellitus (IDDM) or juvenile onset of diabetes] is caused by genetic disposition, environmental exposure to virus, toxin, stress, autoimmune reaction where beta cells of pancreas are destroyed by its own immunological system. Type 2 [Non insulin dependent Diabetes mellitus (NIDDM) or adult onset diabetes] is caused by insulin resistance: unable to utilize insulin produced because of cell receptor defect, insufficient production of insulin in response to blood glucose, excess production of glucose from the liver, genetic predisposition and obesity. The third type of diabetes is gestational diabetes mellitus (GDM) and is caused by insulin resistance due to pregnancy.7

Insulin is the primary medication in the treatment of Type 1 diabetes mellitus by directly promoting glucose uptake into muscle and fat cells as regulator of glucose metabolism and is a single hope for diabetic patients. Besides, controlling blood glucose level, insulin also stimulates cell proliferation. Insulin and insulin-like growth factors (IGFs) stimulate a signal transduction pathway that targets the protein synthesis apparatus by phosphorylating the S6 ribosomal protein (rp) on the 40 S ribosomal subunit.7,8 Maize embryonic axis gradually reinitiates protein synthesis, based on translation of stored mRNAs and translation was shown highly regulated.9,10 However, two other related proteins, insulin like growth factor (IGF-I) and IGF-II are more potent cell proliferators.11 Because of greater antigenicity and impurity of animal insulin (bovine and porcine), genetically engineered human insulin was introduced in 1982 by Eli Lilly and Company. Unfortunately, diabetic patients, who received recombinant insulin for long periods developed antibodies to insulin. Developing fruits of Cowpea plant contained proteins of similar mass and amino acid sequence as bovine insulin.12 Its highest concentration was found in the empty pods and seed coats, and not in the embryo, suggesting its involvement in carbohydrate metabolism in facilitating glucose transport across membranes, similar to its role in animals. Therefore, the search for more effective and safer hypoglycaemic agent started as an important area of active research. Plant based products may be one of the alternative for production of natural insulin.

Insulin structure and function

Insulin is a small peptide hormone of about 6 KDa,7 composed of the A chain with 21 amino acids and the B chain with 30 amino acids, produced in vivo in the pancreatic cells (Fig. 1). Both chains are held together by two disulphide bonds between the cystines in CysA7 and CysB7 positions, CysA20 and CysB19 resp. Another disulphide bond is internal (intrachain disulfide bond) (A6 a A11). During insulin synthesis proinsulin is converted to insulin by cleavage of the binding peptide (C-peptide), binding the A and B chains together.

Fig. 1: Structure of insulin.
Insulin structure is highly conserved among vertebrates, which is evidenced by its high degree of homology. Porcine insulin has a single amino acid variation from the human variety (alanine substituting threonine in position B30), while bovine insulin has three amino acid variations (alanine instead of threonine in positions A8 and B30, and valine instead of leucine in position A10). The total of six insulin molecules, producing a hexamer, binds to two zinc ions.

Spatial construction including polar and hydrophobic amino acids and relations between the individual amino acids within the chain, affect the molecule’s stability and insulin binding to a receptor. Along with the normal insulin and proinsulin molecules, gene mutations with altered primary structures have also been described.22 These include three types of varied insulin molecules PheB25Leu, PheB24Ser and ValA3Leu. Their biological activity is reduced, resulting in diabetes. Insulin gene mutations may impair release of insulin from the proinsulin molecule, which results in familiar hyperproinsulinemia. These variants include the HisB10Asp mutation. Proinsulin is less effective, has reduced clearance and accumulates in the circulation. The HisB10Asp insulin is released from proinsulin through proteolytic cleavage. Compared to other abnormal insulins, it exhibits increased affinity to the insulin receptor.

Insulin enhances glucose uptake by increasing the number of transporters in the plasma membrane of cells. Insulin is effective in restoring normoglycaemia, suppressing ketogenesis and in delaying or arresting diabetic complications. Insulin promotes the storage of the body’s fuels, facilitating the transport of metabolites and ions (potassium) through cell membranes and stimulating the synthesis of glycogen from glucose, fats from lipids and proteins from amino acids. Insulin also profoundly affects lipid metabolism, increasing lipid synthesis in liver and fat cells, and attenuating fatty acid release from triglycerides in fat and muscle. Insulin resistance occurs when normal circulating concentrations of the hormone are insufficient to regulate these processes appropriately. Thus, by definition, insulin resistance is a defect in signal transduction.

Discovery of insulin

Von Mering and Minkowski15 first noted that removal of the pancreas led to the development of diabetes mellitus in dogs. Feeding pancreas has given harmful results in dogs.16 After that, pancreatic extracts was intravenously given to dogs and found transient reduction in blood glucose levels.17,18,19,20 Schäfer,21 was first to speculate that the antidiabetic hormone, which he calls “insuline,” was from the pancreatic islets. Banting and Best made progress in the extraction of insulin from pancreatic islets. He named the protein “glucokin” instead of insulin because it did not originate from the islets of Langerhans in the pancreas. In the same year Best and Collip22 reported the presence of insulin-like substances in other plant materials like green tops of onions, lettuce leaves, green bean leaves, barley roots, beet-root, yeast and others.

The beef-root extract oral doses to diabetic dogs3 showed blood sugar-lowering effect as rapid as those of animal insulin doses subcutaneously.23 After a long gap, in 1970s Khanna and collaborators27, 31 reported presence of insulin in plants and immediately patented a process for its production from the fruits of Momordica charantia (bitter gourd). The isolated product, which was diversely named v-insulin, polypeptide-p or p-insulin, showed hypoglycaemic activity but differed from insulin in immunoactivity.24 Ng et al.25 utilized the seeds and employed gel filtration and ionic exchange chromatography for isolation of active principle from Momordica charantia seeds and showed properties similar to animal insulin. Again, hypoglycaemic activity of Momordica charantia seeds was confirmed based on SDS-PAGE and amino acid composition.26

In addition, materials resembling insulin were described in spinach, rye and Lemna gibba, which were recognized by broad spectrum anti-pork and anti-chicken insulin antibodies and had molecular weights, chromatographic properties and biological activities similar to those of vertebrate insulin.27 Insulin like proteins was isolated from the leaves or aerial parts of species of mosse, whisk ferns, Selaginella, Equisetum many gymnosperms and angiosperms including monocots and dicots using modified ELISA and Western Blotting.28 They have also shown its presence in red alga Gracilaria, cyanobacterium Spirulina maxima and yeast (Saccharomyces cerevisiae).29 Venâncio et al.30 isolated insulin like proteins from seeds coat of Vigna unguiculata at 16 and 18 days after pollination whose RP-HPLC analysis and amino acid sequence was identical to that of bovine insulin. Insulin potentiating activity was also reported in vitro from common spices such as cinnamon, clove, and bay leaves.31

Bauhinia variegate leaf insulin like protein showed partial sequence identity with bovine insulin that decreased blood glucose in alloxan induced diabetes in mice.32 During immune-histo-chemical and immunocytochemical microscopic observation the protein was found associated with calcium containing crystals in vacuoles of chloroplast. These crystals were absent in the control.33 In addition, the protein from proteolyzed showed 1.2% accumulation of insulin within seeds. Transgenic Safflower, plant derived insulin developed by Semi Biosys insulin is analytically and physiologically similar to human insulin (Eli Lilly’s Humulin). This insulin is cheaper, patient friendly plant sources. One acre of sunflower is expected to yield one Kg insulin, enough to supply 2500 patients for an entire year.

Silva et al.34, found insulin like antigen from red alga Gracilaria, green alga Acetabularia and the cyanobacterium protozoite (Cyanobacterium) Spirulina maximum and by adopting ELISA. Reverse phase chromatography and N-terminal amino acid sequence of Smaxima protein showed homology with sequence of proinsulin. During cyanobacterial screening we found insulin like antigen in Spirulina platensis S5 in molecular weights, chromatographic behaviour, and immunological properties, isolated protein was similar to those of bovine insulin.1,2 This paved the path for in-vivo studied for confirming its hypoglycaemic activity. Our findings with Splatensis S5 crude extract as well as purified insulin like antigen also confirmed their blood glucose lowering property (Anwer et al, communicated 2012). Antihyperglycemic effect of Spirulina was also observed on streptozotocin induced diabetes in male albino wistar rats Layam and Reddy.44 In a human clinical study involving 15 diabetic patients 2 g/day Spirulina supplementation showed, a considerable decrease
in the fasting blood sugar level after 21 days. 44 *Spirulina maxima* effect on Streptozotocin induced diabetic male wistar rats orally showed significant decrease in blood sugar level. At a dose of 15 mg/kg body weight a higher level of significance than the doses of 5 and 10 mg/kg body weight.45

**Insulin like antigen in non-photosynthetic organisms**

Glucokin was also detected in prokaryotic bacteria (*Escherichia coli*) and eukaryotic organism protozoa (*Tetrahymena*), fungi (*Neurospora crassa* and *Aspergillus fumigates*).39, 47, 48

Insulin like antigen was also reported from extra-pancreatic tissues of rodents and human brain. 49, 50, 51

**Amino acid sequence of insulin**

Amino acid sequencing (MALDI-TOF) of *Spirulina S5* insulin like antigen when blasted with bovine insulin (N-terminal sequence) it showed 100% identity (3 residues) with one blast hit (Fig2) with human insulin (N-terminal sequence) only 1% identity (3 residues) with one blast hit could be detected (Fig3). Our result suggests only partial identity with bovine and human insulin. This also indicates that 3 residues (44 GER 46) which were matched with bovine and human insulin might be responsible for immunoactivity in ELISA and Western Blotting.

Insulin like protein extracted from *Bauhinia variegata* leaves (cow’s foot) and *Vigna unguiculata* (cow pea) also showed only partial amino acid sequences identical with bovine insulin (Table.1).13, 40

Momordica charantia (which is reported to have hypoglycaemic effect) insulin like protein, sequences was not identical with bovine insulin.35, 52 Its amino acid composition had methionine residues unlike animal insulin. Our *Spirulina S5*, hypothetical protein also had methionine residue (Fig.4). Among the plant insulin protein only that insulin isolated from seed coat of *Canavalis ensiformis* (Jack bean) showed sequence equal to that of bovine insulin (α and β chain) (Table. 1).53

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**Fig. 2: S. platensis S5 insulin like antigen sequences producing significant alignments with bovine insulin.**

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**Fig. 3: S. platensis S5 insulin like antigen sequences producing significant alignments with human insulin.**

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<td>Bovine insulin (β-chain)</td>
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<tr>
<td>Spirulina platensis S5</td>
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**Table 1: Comparison of plant insulin sequences with bovine insulin sequences.**

**CONCLUSION**
The insulin like antigen reported in eubacteria, fungi, protocista and plantae support wider distribution of insulin and their conserved nature during evolution in all organisms from unicellular bacteria to multicellular vertebrate. Presence of insulin like antigen in plant system shows parallelism between plant and animal physiology for glucose metabolism.

The success in isolation of insulin like antigen from plant sources their less toxic and less cross reactive effect and low cost provides an alternative/substitute to human/animal based insulin. Not only this use of transgenic photosynthetic prokaryotic and eukaryotic systems, may appear to be revolutionary in future.

_Spirulina_ extract has ability to lower blood glucose level in mice and humans (Mani et al, 1998). Therefore, search for plant based antihyperglycemic medicines became indispensible.

Immuno-recognition with anti-bovine or human insulin antibodies in prokaryote and eukaryote it was suggested that the insulin-like peptide is present many organisms, other than animal pancreas.

REFERENCES


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