ORIGINAL ARTICLE

Plant insulin: An *in silico* approach

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Background: Insulin is a hormone traditionally believed not to occur in plants. **Aim:** This study was designed to evaluate whether plant genomes contained sequences similar to animal insulin. **Materials and Methods:** Human insulin amino acid sequence was obtained from UniProt/SwissProt (Accession no. P01308). Plant genomes were obtained from plant genome database (NCBI). Insulin amino acid sequences were blasted with each of the plant sequences. Using these three sequences and that of human insulin, multiple sequence alignment was performed, and phylogenetic tree was constructed. Human insulin sequence was submitted to PFAM and domains obtained; this was followed by submission of the three plant sequences. **Results:** We constructed phylogenetic tree of the different type of insulin, evaluated their domains, and showed insulin like molecules exist in the plant kingdom. In addition, domain common to insulin sequence exist in Cowpea, Jack-bean, and *Bauhinia purpurea*. **Conclusion:** Insulin like proteins may be responsible for plant development and other as yet unknown metabolic functions. In addition, they may be responsible for some of the actions of plant extracts for their antidiabetic properties.

KEY WORDS: Bioinformatics, comparative genomics, sequence

DOI: 10.4103/0973-3930.70858

Introduction

Insulin, the principal glucose regulating hormone was isolated from animal pancreas.[1] Plants do not have pancreatic cells and glucose is not their principal metabolite. It was inferred that insulin was not present in plants.

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Manuscript received: 02.04.2009; Revision accepted: 09.08.2010

Even though early studies showed that chemical substances similar to animal insulin existed in plants and that extracts from these modified the rate of seedling metabolism,[2,3] no further work was undertaken to identify insulin-like peptides in plants until the 1970s when Khanna *et al*. reported that insulin-like material was present in a plant source.[4] Thereafter, scattered publications reported the existence of insulin-like peptides in other life forms such as bacteria and fungi.[5,6]

Following the genomics revolution in the l990s, it was possible to compare the nucleotide and amino acid sequences using bioinformatic processes to identify common proteins that may have existed across life forms.[7,8]

The present *in silico* study was carried out to assess whether insulin-like sequences (glucokinin) were present in plants whose genome sequences are available in public databases. The aim of this study is to use bioinformatic approach to identify homology of glucokinin and its domains, with human insulin as the reference protein.

Materials and Methods

Human insulin amino acid sequence was obtained from UniProt/SwissProt with accession no. P01308 in the FASTA format. Plant genomes were obtained from plant genome database (NCBI). Insulin amino acid sequences were blasted with each of the plant sequences, which brought out the following three with the highest scores: *Bauhinia purpurea*, *Vigna unguiculata*, and *Canavalia ensiformis* [Tables 1–3].

Using these three sequences and that of human insulin, multiple sequence alignment was performed, and phylogenetic tree was constructed.

Human insulin sequence was submitted to PFAM and domains obtained; this was followed by submission of the three plant sequences which showed the domain

Table 1: Comparison of protein sequences

Sequence A	Name of the protein sequences	Length (aa)	Sequence B	Name of the protein sequences	Length (aa)	Score
1	P01308| Human insulin	110	2	gi|7438602| Jack-bean insulin	51	94
1	P01308| Human insulin	110	3	gi|229412| *Bauhinia purpurea* insulin	51	56
1	P01308| Human insulin	110	4	p83770| Cowpea insulin	51	82
2	gi| 7438602| Jack-bean insulin	51	3	gi|229412| *Bauhinia purpurea* insulin	51	56
2	gi| 7438602| Jack-bean insulin	51	4	p83770| Cowpea insulin	51	88
3	gi| 229412| *Bauhinia purpurea* insulin	51	4	p83770| Cowpea insulin	51	52

Table 2: Plants with sequences related to insulin

Scientific name	Common name	Accession number	Protein name	Protein length
Bauhinia purpurea	Purple Orchid-Tree	Gi|229412	Insulin	51
Vigna unguiculata	Cowpea	P83770	Insulin like protein	51
Canavalia ensiformis	Jack-bean	Q7M217	Insulin precursor	51

Table 3: IIGF a like domain, domain length, starting–ending location and E-value

Species name	Protein name	Accession number	Domain length	Domain starts–ends	E-value
Homo sapiens	Insulin	P01308	82	28–109	1.56e–35
Bauhinia purpurea	insulin	Gi|229412	26	25–50	0.00613
Vigna unguiculata	Insulin-like protein	P83770	47	4–50	3.81e–07
Canavalia ensiformis	Insulin precursor	Gi|7438602	47	4–50	1.34e–25

[a]Insulin like growth factor.

common to insulin sequence. The amino acid sequences for domain were collected from SMART database, multiple sequence alignment was performed, and phylogenetic tree was constructed.

Results

Comparison of the protein sequences is given in Table 1.

The Phylogenetic tree was obtained as shown in Figure 1.

Discussion

In this study, we have shown that plant species have sequences that are similar to that of animal insulin. It was postulated that plants do not possess insulin structure as there is no pancreatic tissue present in plants, and plant-metabolism does not need insulin.[9]

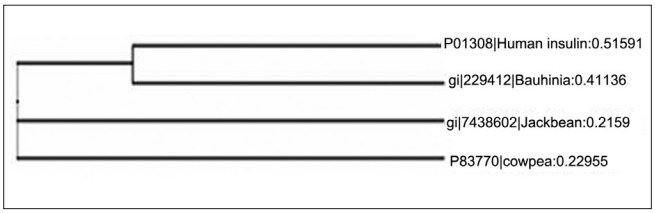

Figure 1: Phylogenetic tree of four sequences

However, *in vivo* empirical studies as well as historical studies have shown that plant sources were used effectively in the management of diabetes; similarly extracts resembling insulin affected the growth rate of plants. A recent report has shown that transgenic expression and recovery of biologically active recombinant human insulin were possible from plant seeds.[10]

Availability of genomic sequences and bioinformatic tools to compare them made it possible to perform comparative genomics and identify similar protein sequences across life forms.[8]

In 1987, material that resembled insulin was isolated in two plants, spinach and lemea.[11] This was followed by the identification of insulin-like antigens in a variety of other species including leaves and aerial parts of green plants, red alga, cyanobacterium, and fungi.[12]

These observations, together with the isolation of insulin-like protein from the chloroplasts of *Bauhinia variegata* plant leaves suggested that insulin signaling pathway was conserved through evolution.[13] Similarly, developing fruits of Cowpea plant contained proteins of similar mass and amino acid sequence as bovine

insulin.[14] The 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.

The occurrence of insulin adjacent to calcium-containing crystals in chloroplasts suggests that the crystals may protect insulin from degradation when ingested as decoctions.[13]

The existence of insulin-like material across life forms indicates a possible conserved role for insulin in protein transcription and glucose transport, but does not throw light on its origin, whether it was inserted into plants from animal source, or whether later convergent development of the insulin family occurred.[11] Since the concentration of insulin in plants is low, it may be responsible for the control of different metabolic processes such as growth and development.

Multiple lines of evidence have shown that unicellular green algae have insulin receptors in plasma membrane, and that extracts of red alga tested positive for insulin-like antigens. Pseudogene for preproinsulin in fungi was cloned, followed by the isolation of insulin-like protein in *Aspergillus fumigatus*.

In summary, we have demonstrated using *in silico* approach that insulin-like sequences including domain structures occur in plant species also. This could both give a biological basis for the effectiveness of plant extracts and decoctions in the management of hyperglycemia. Despite the known divergence between insulin amino acid sequences and cladistic analysis among dissimilar taxa,[15] it is possible to perform iterative *in silico* and *in vitro* studies[16] and infer the role of insulin and related peptides in other metabolic processes such as growth and development, or even other as yet unidentified pathways.[17]

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Source of Support: Nil, **Conflict of Interest:** None declared