A REVIEW ON SOMATOSTATIN & THAUMATIN: THE TWO PROTEINS- A GROWTH ATTENUATOR AND A SWEETNER

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ABSTRACT

Somatostatin and its receptors have a critical role in mammalian growth through their control pattern of secretion of growth hormone, but the evolutionary history of somatostatin and somatostatin receptors are ill defined. To date, we have identified a minimum of two genes of somatostatin and five somatostatin receptor genes in mammalian species with variable forms. Its clinical application has been limited by its very short half-life, necessitating continuous intravenous infusion. Octreotide is an 8 amino acid synthetic analogue of somatostatin that possesses similar pharmacological effects. It has a much longer duration of action, however, and can be given subcutaneously. Both the intravenous and subcutaneous routes of injection of octreotide are well tolerated. Octreotide inhibits gastroenteropancreatic secretion, especially of insulin, glucagon, pancreatic polypeptide, gastric inhibitory polypeptide, and gastrin. Thaumatin, a mixture of protein first isolated from the Katemfe fruit. Thaumatin which is about 100,000 times sweeter than sucrose on a molar basis. Thaumatin has potential use a low calorie sweeter for industrial application. Thaumatin is highly digestible protein. Thaumatin has been crystallized in four different forms: orthorhombic (1.75 Å), monoclinic (2.60 Å), tetragonal (1.75 Å) and hexagonal (1.60 Å).

KEYWORDS: Somatostatin, Octreotide, Thaumatin, Thaumatin like protein, Microgravity, crystallization

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INTRODUCTION

Somatostatin (abbreviated as SST) can also be termed as SRIF (somatotropin release inhibiting factor) is a polypeptide secretory hormone first isolated by Brazeau et al. in 1973 from the tissue of mammalian hypothalamus. Basically the secretory hormone is somatostatin 14, structurally a tetradecapeptide. It has been found out accidentally while searching for growth hormone releasing factor. The hormone was found to inhibit growth hormone (GH) secretion. In mammals, the growth hormone inhibiting hormone-SRIF was found to be multifunctional polypeptide that is commonly distributed throughout central nervous system and peripheral tissues. Originally the molecule is having cyclic arrangement joined by two intramolecular disulphide bonds between the two cysteine residues (Figure). Reduced sequence is linear, however, has got same biological activity as that of cyclic form in vitro – that is cyclic form cannot be recognised by specific somatostatin receptors.

**Somatostatin (Somatostatin 1)**, in mammals is present in two predominant biologically-active forms: SRIF-14 & SRIF-28. The later one, is having NH$_2$- terminal extension of 14 amino acids as compared to somatostatin 14. Both forms are encoded by a common gene and obtained from a single precursor. Second native form has been found in 1980, SRIF-28 either may act as a precursor or works as the hormone in its own right, with a bit different mode of action in other tissues. Somatostatin 28 supresses the GH secretion for longer duration but not in case of gastric acid. Somatostatin 28 virtually does not exist in stomach & duodenum, but shows dominance in lower gut. Somatostatin secretion, in neural and peripheral secreting cells can be activated by depolarization and increasing Ca$^{2+}$ concentration. SST secretion can be stimulated by number of hormones, neuropeptides, neurotransmitters, cytokines, growth factors and nutrients. Taken into consideration, growth hormone-releasing hormone (GHRH), neurotensin and corticotrophin-releasing hormone (CRH) are all potent stimulators of SST secretion in several tissues. In contrast to that, neurotransmittersopiates and γ aminobutiric acid (GABA) are inhibitors SST secretion. SST action can be mediated by of G protein-coupled receptors’ (GPCR’s) with somatostatin receptors (SSTRs) with six subtypes, encoded by separate genes except SSTR2A. On the basis of structural characteristics and pharmacological properties, and keeping in view with their sequence homology, the receptor subtypes can be classified into two subgroups, SRIF1 and SRIF2. SRIF 1 is having receptors like SSTR2, SSTR3 and SSTR5 whereas SSTR1 and SSTR4 are included inSRIF2 group.

**THE SOMATOSTATIN RECEPTOR FAMILY**

SST works on the multiple cell targets by a family of six receptors that originally arises from five genes: SSTR1, SSTR2a, SSTR2b, SSTR3, SSTR4, and SSTR5. Upon splicing SSTR2 on C-terminus produces SSTR2a & SSTR2b variants having a bit different tissue distribution.Humans are having only one
Somatostatin gene, SST. Somatostatin exerts control over insulin and glucagon.

Table 1

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligand</th>
<th>Tissue distribution</th>
<th>Antibodies</th>
<th>Epitopes</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSTR1</td>
<td>SST-4, SST-28 CST</td>
<td>Brain, Pancreas (β cells), GI tract, Several human tumors.</td>
<td>Anti-SSTR1 (AASR-001) Extracellular</td>
<td>Epitope 20-40</td>
</tr>
<tr>
<td>SSTR2a</td>
<td>SST-4, SST-28 CST</td>
<td>Brain, Pancreas (α cells), GI tract, Adrenal gland, Immune cells, Several human tumors.</td>
<td>Anti-SSTR2 (AASR-004) Extracellular</td>
<td>Epitope 30-34</td>
</tr>
<tr>
<td>SSTR2b</td>
<td>SST-4, SST-28 CST</td>
<td>Brain, Pancreas (β cells), GI tract, Several human tumors.</td>
<td>Anti-SSTR3 (AASR-003) Intracellular</td>
<td>Epitope 22-34</td>
</tr>
<tr>
<td>SSTR3</td>
<td>SST-4, SST-28 CST</td>
<td>Brain, GI tract, Liver, Spleen, Several human tumors.</td>
<td>Anti-SSTR4 (AASR-004) Extracellular</td>
<td>Epitope 91-134</td>
</tr>
<tr>
<td>SSTR5</td>
<td>SST-28, SST-14 CST</td>
<td>Brain, Pancreas (β and T cells), GI tract, Several human tumors.</td>
<td>Planned</td>
<td>G(31H)</td>
</tr>
</tbody>
</table>

SSTR: Somatostatin Receptor; SST: Somatostatin; GI tract: Gastrointestinal tract.

**LOCALISATION OF SOMATOSTATIN**

Large amounts of somatostatin are found in the gastrointestinal system, including the pancreas, visceral autonomic nervous system, endocrine cells, and gut lumen. Immuno cytochemical studies show that somatostatin localised to the D cells of the pancreas and gut mucosa, particularly in the gastric fundus, antrum, and duodenum. D cells are situated in the lower third of the crypts, from where they extend cytoplasmic processes along the basal membranes to the basal pole of neighbouring glands. SSTR expression can be modulated by several factors. First, as in most GPCRs, ligand binding to the receptor induces either receptor internalization and/or uncoupling of the receptor from the G-proteins resulting in receptor desensitization. Second, several hormones such as estrogen and thyroid hormone can regulate SSTRs expression in several tissues at the transcriptional level. Besides their expression in normal tissues, SSTRs have been identified in tumor cell lines of different etiology including pituitary, pancreatic, breast and hematopoietic. Moreover, the majority of human tumors do express SSTRs, often more than one receptor subtype. In general, SSTR2 is the most common SSTR subtype found in human tumors followed by SSTR1 with SSTR3 and SSTR4 being less common. SSTR5 appears to be more tumor specific with strong expression in some tumors (i.e. breast) and complete absence in others (i.e. pancreatic).

**ACTIONS**

Somatostatin has long recognised inhibitory effects on pancreatic endocrine and exocrine secretion - that is, on insulin, glucagon, and...
pancreatic polypeptide and also on pancreatic enzyme and bicarbonate responses to cholecystokinin and secretin. It also inhibits the secretion of a wide variety of stimulatory gastrointestinal hormones and decreases gastrointestinal motility and blood flow.

STRUCTURE-FUNCTION RELATIONS OF SOMATOSTATIN

The action of native somatostatin is brief and rapid, and is followed by rebound secretion, all of which suggests that it inhibits release rather than synthesis, perhaps by depressing exocytosis. Somatostatin receptors have been identified in the exocrine pancreas, on islet cells secreting insulin, glucagon and somatostatin, and on gastric cells. Intracellularly, somatostatin achieves its effects by cyclic AMP dependent and independent mechanisms and may act by affecting calcium transport through the cell membrane. Because somatostatin inhibits many aspects of cellular function, including endocrine secretion, muscular contraction, and cellular growth, it must antagonise a variety of intracellular signalling programmes. The dephosphorylation processes crucial for secretory activity intimately participate in inhibition by somatostatin at the molecular level. After binding to its receptor, somatostatin probably acts as a dephosphorylator to inhibit secretory processes, but whether the hormone also interacts with DNA to affect transcription is disputed.

THAUMATIN

Thaumatin is intensely sweet tasting protein produced in the arils of the fruit of the Africa shrub. In the rainforests of West Africa the herbaceous plant of Thaumatococcus daniellii grows. In West Africa the fruit of Thaumatococcus daniellii has been traditionally used as a sweetener of palm, wine, corn, bread, and sour fruit. Thaumatin which is, about 100,000 times sweeter than sucrose on a molar basis. Thaumatin has powerful efficacious use as a low-calorie sweetener for industrial applications and could be useful in clarifying the mechanisms of the perception of sweet taste. Plant Thaumatin consists of five extremely sweet forms, with two major group components of Thaumatin I and Thaumatin II and other three are minor group of components like Thaumatin a, b and c.

BIOCHEMICAL AND PHYSICAL PROPERTIES OF THAUMATIN

Thaumatin I and Thaumatin II, both contain a single polypeptide with 207 amino acid create an entire new field in oncology; peptide therapy. More than fifteen years ago a synthetic radiolabeled SST analogue was used to localize neuroendocrine tumors and its metastasis in vivo by scintigraphy, a technique where binding of the radiolabeled peptide to the SSTR could be detected as hot spots by γ camera scan. The technique is still considered the most accurate for the diagnosis of cancers of neuroendocrine origin. SST analogues have also been used in direct tumour reduction with 90Y radiolabeled analogues and in the symptomatic treatment of hormone secreting tumours. Somatostatin inhibits many aspects of cellular function, including endocrine secretion, muscular contraction, and cellular growth, it must antagonise a variety of intracellular signalling programmes. The dephosphorylation processes crucial for secretory activity intimately participate in inhibition by somatostatin at the molecular level. After binding to its receptor, somatostatin probably acts as a dephosphorylator to inhibit secretory processes, but whether the hormone also interacts with DNA to affect transcription is disputed.
residues and have a basic character as manifested by isoelectric points above 11.5. The amino acid sequence for Thaumatin II has been deduced from its nucleotide sequence and a gene for Thaumatin II has been cloned from messenger RNA derived cDNA. The five amino acids in the Thaumatin II sequence which differ from the Thaumatin I sequence (N46K, S63R, K67R, R76Q, and N113D). The presence of eight disulfide bridges which is stabilized, for tertiary structure of Thaumatin also which makes it unusually stable with respect to heat and pH. Molecular weight of Thaumatin is approximately 22 kilo Daltons. Thaumatin is very much soluble in water (up to 20%) and is also fairly soluble in ethanol/water mixture. It is however insoluble in most common organic solvent such as acetone.

**CRYSTALLIZATION OF SWEET PROTEIN THAUMATIN**

The Thaumatin has been crystallized in four different forms like (1) orthorhombic (1.75 Å), (2) monoclinic (2.60 Å), (3) tetragonal (1.75 Å) and (4) hexagonal (1.60 Å). By using hanging drop vapour diffusion method from polyethylene glycol as a precipitating agent which form a crystal forms of Orthorhombic and monoclinic. By vapor diffusion method in the presence of 1 M sodium, potassium tartrate containing 0.1 MADA (sodium-N-2-acetamido-iminodiacetic acid) at pH 6.5 forms a tetragonal crystal. (McPherson 1999). These crystals were similar to one grown from ammonium sulphate solution. Thaumatin has also been crystallized in hexagonal crystal form from a tartrate and glycerol containing solution after shifting temperature from 293 K to 277 K.

**Expression of recombinant Thaumatin**
The limited availability of Thaumatin from natural plant sources, due to regional cultivation restrictions, has encouraged alternative production method of Thaumatin. So far, recombinant Thaumatin I and II have been expressed see below in table 2.

<table>
<thead>
<tr>
<th>Host</th>
<th>Promoter*</th>
<th>Secretion</th>
<th>Yield</th>
<th>Sweet phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.coli</td>
<td>Trp/Lac</td>
<td>No</td>
<td>Very low</td>
<td>No</td>
<td>34</td>
</tr>
<tr>
<td>S.cerevisiae</td>
<td>Pgk</td>
<td>No</td>
<td>Low</td>
<td>No</td>
<td>38</td>
</tr>
<tr>
<td>B.subtilis</td>
<td>α-amy</td>
<td>Yes</td>
<td>1mg L⁻¹</td>
<td>Yes</td>
<td>40</td>
</tr>
<tr>
<td>S.lividans</td>
<td>β-gal</td>
<td>Yes</td>
<td>0.2 mg L⁻¹</td>
<td>?</td>
<td>41</td>
</tr>
<tr>
<td>A.awamori</td>
<td>Gla</td>
<td>Yes</td>
<td>5-7 mg L⁻¹</td>
<td>Yes</td>
<td>42</td>
</tr>
</tbody>
</table>


The Thaumatin II gene has been transferred to apple in an endeavour to improve their taste quality and phytopathogen resistance. Also the expression of a Thaumatin II gene under control of the CaMV 35S promoter has been shown to improve plant resistance against pathogen. Produced transgenic cucumber plant expressing Thaumatin II protein that showed an enhance resistance against the pathogenic fungus like *Pseudoperonospora cubensis*. Thaumatin II gene introduce into strawberry produced transgenic line which has significantly higher level of resistance to gray mold like *Botrytis cinerea*. Thaumatin II was also expressed in transgenic potato, tomato, pear, barley and tobacco plants.

**FUNCTIONAL PROPERTIES**

Thaumatin can be used as a sweetener or a taste-modifying protein; Flavour Enhancer and Mouth feel Improvement, Masking Bitterness and off-Flavours.

**THAUMATIN LIKE PROTEIN**

Originally, based on amino acid sequence characteristics PR (pathogenesis related) protein were divided into five families. Now, this group of proteins has been
extended into 17 families based on amino acid sequence characteristics. Multiple isoforms of Thaumatin like Protein, which belong to the PR-5 family, have been found in the crop species barley (Hordeum vulgare L.) , rice (Oryza sativa) and wheat (Avena sativa). They are named after their amino acid sequence and structural similarities to the sweet tasting protein Thaumatin. TLP’s (Thaumatin like protein) known as osmotins and permatins. The TLP’s plays a variety of roles in the development of seed, fruit and flowers of tissues, and specific TLP’s have been shown to protect plants against osmotic stress, pathogen attack and the deleterious effects of freezing. The expression of some TLP’s is induced by biotic and abiotic factors, such as microbial infection, osmotic stress, abscisic acid (ABA), ethylene, SA, MJ and elicitor (Kitajima and Sato 1999).

CONCLUSION

Since its discovery more than thirty years ago, much progress has been made in understanding the pathological and physiological function of SST and its receptors. The variety of target tissues and cell pathways involved in the biological functions of SST, promise to keep scientists engaged in this field for many years to come. A better elucidation of the different signalling pathways engaged by the different SSTR subtypes should be attempted, as well as a better understanding of the SSTR subtype tissue distribution in normal and diseased states. For this endeavour highly specific antibodies to both intra- and extracellular epitopes of the different SSTR subtypes will be indispensable. The new insights gained from these studies are eagerly awaited. Thaumatin is sweet testing protein found in herbaceous plant of Thaumatococcus daniellii. Thaumatin consist of five extremely sweet protein like Thaumatin I and II, a, b and c, out this two major group Thaumatin I and II both differ from their amino acid sequences. Also the Thaumatin has been formed four different crystal by hanging drop vapour diffusion method. Now a day’s limited availability of Thaumatin can be increased by using recombinant technology and expression of Thaumatin. Today’s TLP’s used for plays a variety of roles in the development of seed, fruit and flower of tissues, protect plants against osmotic stress, pathogen, deleterious effects of freezing.

REFERENCES


