CHAPTER 1

INTRODUCTION

Understanding the mechanism of sweetness has been a challenging problem. It is necessary to understand this in order to design safer sweet molecules, for in many diseases (like diabetes, hyperlipidemia) a low calorie sweetener is needed and thus it is a very pressing need. However, a wide range of molecules belonging to divergent chemical classes taste sweet [1-8], thus posing a challenge to understand mechanism of sweetness.

Many natural sweet proteins that have been discovered are more than 4000 times sweeter as compared to sucrose [9-10]. But unresolved enigma remains why they are sweet? What could be the reasons for their heightened sweetness? What do these proteins have in common, if at all? Do they have a homologous region at any level of structure- be it primary or higher order? How does the sweet taste receptor recognizes them?

Designing newer sweet molecules desires the knowledge of pharmacophores (also called as glucophores for sweet molecules like glucose). Whereas in case of small sweet molecules, identification of glucophores evolved over the years and presently accepted glucophore is AH-B-X; where A, a hydrogen atom donor approximately 2.6 Å away from a hydrogen atom acceptor (B) and 3.4 Å from an apolar group (X), while B and X are separated by a distance of 5.5 Å. Identification of the same for sweet proteins has not been attempted because of the complexity involved. However, even in the case of small molecules, the identified glucophores may not comprehensively explain sweetness. This is to say that, identified glucophores may not be sufficient condition to explain sweetness. For instance, Tancredi et al. [11] tried to design a peptide based on AH-B-X glucophores. Though the peptide contained the glucophore proposed by three point attachment theory, still the synthesized peptide turned out to be tasteless. This forced us to rethink, whether AH-B-X glucophores proposed by Kier [12] the sufficient/essential condition for sweet molecules?

The story gets complicated in case of sweet proteins. There are huge variations in their size and structure. Comparison of primary structure as well as crystal structure of these sweet proteins
yielded no similarity [11]. Also, no universal results could be obtained about determinants for sweetness from point mutation studies. Understanding their structure-function relationship can boost the performance of small molecular weight sweeteners and thus help in the design of safe sweeteners.

To identify glucophores in proteins, the knowledge and understanding of structure of human sweet taste receptor (hSTR) is desirable. hSTR is a heterodimer of T1R2-T1R3 subunits, each being ~800 amino acid long. Each subunit comprises of three distinct domains- amino terminal domain (ATD), cysteine rich domain (CRD) and a transmembrane domain (TMD) [13]. This makes it a formidable task to find a reliable structure experimentally (crystallography, NMR) or by computational modeling. Till date, most of the modeling studies are reported on either amino terminal or transmembrane domain of T1R2-T1R3 subunits of hSTR but none on complete hSTR. In addition to this, the identified site of interaction of a few of the sweet proteins is CRD that is completely different from identified sites for other small sweet molecules as ATD or TMD.

Since human sweet taste receptor has multiple ligand binding sites, it is likely that different domains have different glucophores recognized by different types of sweeteners. Therefore, it would not be incorrect to assume that proteins or sweet molecules binding to different domains may have different glucophores. Thus, we set out to identify different glucophores (if any), binding sites on receptor and functional groups or crucial residues of receptor.

**Motivation**

People affected by diseases like diabetes, obesity, hyperlipidemia, caries etc. which are linked to the consumption of carbohydrates are increasing tremendously. An estimated 346 million people worldwide are suffering from diabetes alone. Left untreated or poorly controlled, diabetes increases the risk of cardiovascular diseases, blindness, kidney failure, eye problems, nerve damage and amputations. In addition to other measures like healthy diet, regular exercise etc., decreased sugar and saturated fat intake is recommended to prevent or delay the onset of the disease. Hence search of alternate sweeteners is necessitated. Efforts in this direction led to low calorie sweeteners like aspartame, saccharin, sucralose, acesulfame-K etc that have gained wide popularity. However, there are contradictory reports regarding prolonged usage of these and
associated side effects like dizziness, nausea, lung cancer, chronic respiratory disease, hallucination, hypersensitivity, bladder cancer, heart failure and brain tumour etc [14-16]. In view of above facts there is a need for alternate safer sweeteners with less side effects and which is easily metabolized. Naturally occurring sweet proteins like Brazzein [17-18], Thaumatin [10,20], Monellin [21-26], Mabinlin [27], Miraculin [28], Curculin [29-30] and Neoculin [31-32] that are many fold sweeter than sucrose, can be an alternate. Besides, they have negligible side effects and do not require insulin for their metabolism. However, in many diseases like hyperlipidemia where high protein diet cannot be administered, these proteins cannot be given. Hence there is a dire need to understand the mechanism of sweetness and the involved pharmacophores, to help design safer sweeteners.

Objective of the present work is to investigate determinants of sweetness. To achieve the above goal we have adopted two different approaches for identification of functional groups or pharmacophores responsible for sweetness – 1) ligand based and 2) receptor based approach. Former being the conventional approach widely used to find pharmacophores using ligands or in other words common substructures in sweet molecules responsible for eliciting sweetness. Since sweet molecules belong to diverse chemical class and structures, we have adopted as an alternative receptor based approach to identify crucial residues of receptors responsible for sweetness. The specific objectives are

1) Classification of available sweet molecules based on active site(s) of interaction.

2) Identification of crucial/essential residues of the receptor involved in interaction.

3) If possible pinpoint the source of stability and specificity.

Thesis is structured as Introduction (Chapter 1) followed by Review of Literature (Chapter 2) and the Methodology (Chapter 3) used in the present work and finally Results and Discussion is presented in Chapter 4. Thesis is concluded with presentation of important results and summary in Chapter 5 Conclusion. Plan or design of study is presented below.
Chapter 1 presents the perspective, motivation or need of the present study in view of the lacunae of the existing artificial sweeteners. This is followed by Chapter 2 presenting a detailed review of the literature of artificial (synthetic) as well as natural sweeteners, their features and associated controversies. In addition to this, experimental studies involving point mutations and chimeras (human and mouse sweet taste receptor chimeras) [33-42], and effect of inorganic salts on sweetness [43] of sweet molecules and proteins and computational studies to identify pharmacophores [44] have been reviewed.

Chapter 3 gives a brief description of the methodology followed by us to identify substructures in sweet molecules as well as in human sweet taste receptor (hSTR) responsible for conferring sweetness. The strategy followed in the present work can be subdivided into four major sections — 1) Optimization of structure of sweet molecules and sweet inhibitors, 2) Pharmacophore analysis, 3) Structure prediction of hSTR and 4) Docking of sweet molecules and proteins with hSTR for identification of site of interaction of sweet molecules, crucial residues involved in interaction and responsible for eliciting sweetness and stability to the receptor structure.

To identify the residues important for stability and specificity of the receptor, we took two different approaches first is mutational study and another one is docking of sweet molecules in presence of ions based on corresponding experimental studies. Single point mutations were
done to identify the changes occurring on the receptor due to mutation, affecting sweetness. In another study, sweet molecules were docked in presence of ions $[\text{Zn}^{2+}, \text{Na}^+ \text{ and } \text{Mg}^{2+}]$ and the effect of ions on the activity of molecules was evaluated.

**Results and Discussion** is presented in Chapter 4 followed by summary and conclusion in chapter 5.