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An immunological approach to the structural basis of the sweet taste

Murray Goodman[a], Darin Kent[a], Maurizio Zanetti[b], Mara Gerloni[b], Masanori Kohmura[c], and Yasuo Ariyoshi[c]

[a]*Department of Chemistry and Biochemistry, Univ. of Calif, San Diego, CA 92093, USA.*
[b]*Department of Medicine, Univ. of Calif., San Diego, CA 92093, USA.*
[c]*Ajinomoto Co., Inc., Central Research Laboratories, 1-1 Suzuki-Cho, Kawasaki 210 Japan*

Abstract: Identification of the sweet taste receptor has become a challenging problem for those interested in understanding the structural basis of taste. Our laboratories have been interested in this issue for many years. This manuscript summarizes our recent work and briefly describes future directions of our research efforts.

For many years we have synthesized peptide and peptidomimetic taste ligands (ref. 1). Our work together with results from other laboratories (ref. 2-5) have allowed us to develop a model to explain sweet taste-structure relationships for these molecules. This model commences with the AH, B and X sites as proposed by Shallenberger and Acree (ref. 6) and Kier (ref. 7).

In our model, sweet peptide derivatives assume a coplanar L shape in which the zwitterionic ring of the N-terminal residue is the stem of the L and the group X is the hydrophobic base of the L. Our work has shown that x-ray structures, in general, cannot be used to define the molecular basis of the sweet taste since the crystal structures are dominated by packing forces (ref. 1). We established our model for sweet taste using NMR studies in solution followed by extensive computer simulations of the peptide-based structures (ref. 1).

It has become clear to us that studies of ligands do not yield fundamental information on the nature and structure of taste receptors. Therefore, we undertook a novel immunological approach to attempt to characterize and perhaps isolate sweet taste receptors.

Originally, our working strategy derived from the observations made almost twenty years ago showing that rabbit polyclonal antibodies generated against the sweet proteins thaumatin and monellin cross-react immunologically (ref. 8 and 9). Those findings were important because, for the first time, it was possible tentatively to delineate a correspondence between a function (sweetness) and the discriminating power of the antigen-binding sites of antibodies. Moreover, implicit in those reports was the fact that they indicated an approach to the identification of sweet taste receptors which could be based on proteins with sweet taste rather than on sugars themselves. The advantage was immediately evident since it was well known that proteins can generate antibodies much easier than sugars.

We commenced the immunological studies of the sweet taste using a two fold reasoning. First, the antigen binding site of antibodies has an infinite spectrum of specificities, making it possible to identify virtually any antigenic determinant, including those dictated by the tertiary and quaternary structure of the protein. Second, it was already known that the antigen binding sites of antibodies are themselves templates for complementary antigen-binding sites and that these are sometimes the molecular mimics of antigens (ref. 10). Thus we operated on the premise that an antibody-based approach can be used to understand the molecular basis of the sweet taste. We viewed our efforts as a viable alternative to conventional approaches to isolate sweet receptors based on chemical purification and analysis which to date have proven difficult if not impossible.

We generated a series of monoclonal antibodies against two sweet proteins, monellin and brazzein, and searched for cross-reactivity among monellin (ref. 11), thaumatin (ref. 12), and brazzein (ref. 13), (Fig.1). Surprisingly, it was relatively easy to generate cross-reactive antibodies, i.e., anti-monellin antibodies

reacted not only with monellin but also with brazzein and thaumatin (ref. 14) (Fig. 2). It was even more surprising that these antibodies bound the protein against which they had not been generated more avidly (Fig. 2). Antibodies of this type are generally referred to as heteroclytic (ref. 15).

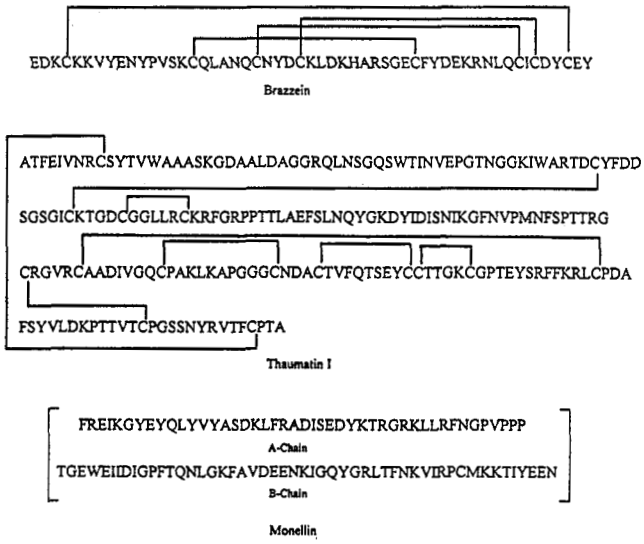

Fig. 1 Primary Structures of Sweet Proteins

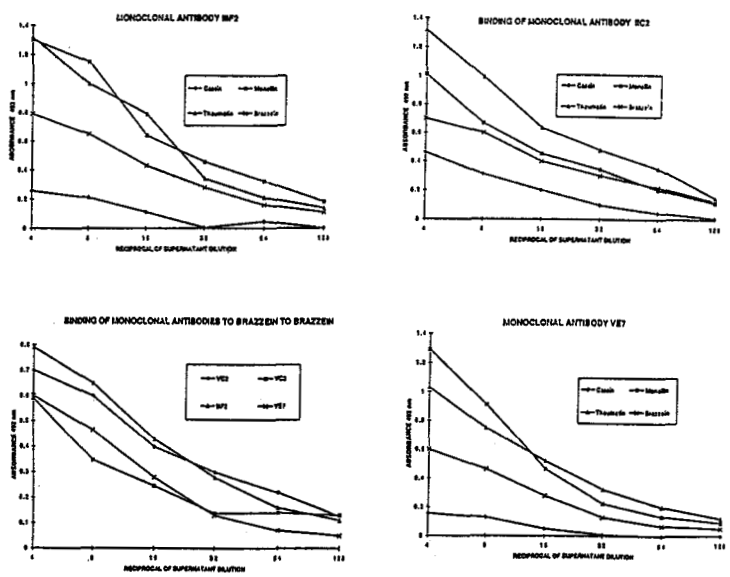

Fig. 2 Monoclonal Antibodies to Brazzein Crossreact with Monellin and Thaumatin

While we were pursuing the above studies other groups reported that monoclonal antibodies generated against thaumatin cross-react with monellin (ref. 16). Collectively the picture that emerges is that there exists considerable immunological cross-reactivity among these three sweet proteins (Fig. 3).

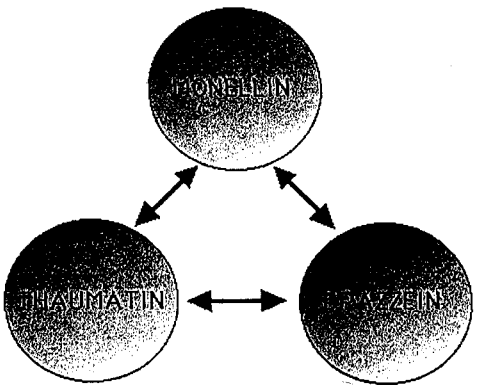

Fig. 3 Cross Reactivity for Three Sweet Proteins

Recently we devised a system that may accelerate the process of resolving the enigma: we developed a system that allows us to engineer *in vitro* antibody molecules with structural complementarity for cross-reacting antibodies to sweet proteins (ref. 18 and 19). The approach is exemplified in Fig. 4. Briefly, antibodies are modified genetically to encode in the complementarity determining loops discrete stretches from the sequences of monellin. The amino acid sequences of the sweet protein are grafted into the antibody variable domain and expressed in an constrained manner at the surface of the molecule. Studies performed by our group on other model systems already verified that this method of peptide expression is efficient and in most instances maintain accessibility of the grafted peptide, with antigenicity and immunogenicity (ref. 18 and 19). Therefore, an entirely programmable *in vitro* process exploiting the discriminating power of the antibody binding site and concept of molecular complementarity between antibodies may soon provide us with additional new information on the structural basis of sweet taste.

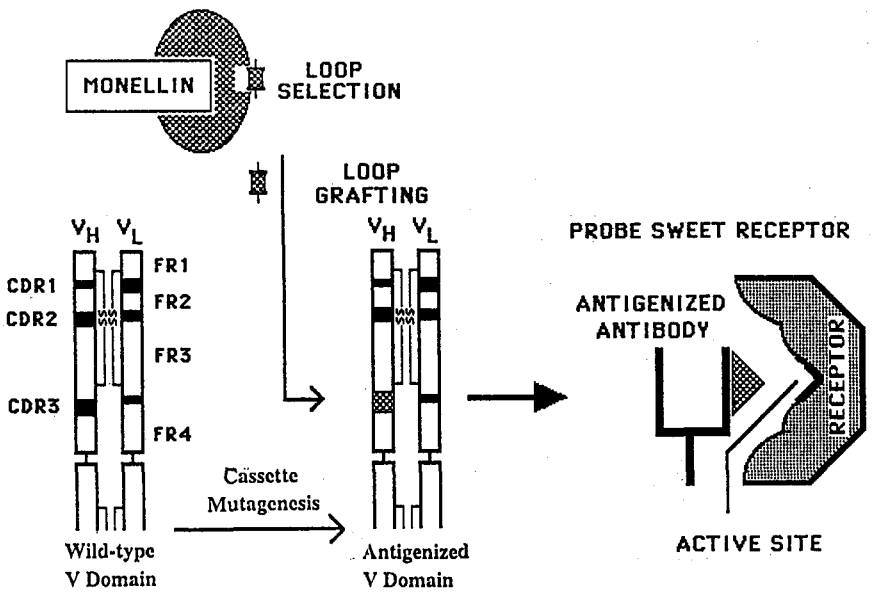

Fig. 4 Antigenized Antibodies and Receptor Recognition

Why do sweet protein cross-react immunologically? What is the structural basis for this phenomenon? It is obviously impossible at this time to know why proteins that possess very different primary structures (Fig. 1) share immunological cross-reactivity. We have speculated that this may have arisen as a consequence of selective pressures on the immune repertoire by viruses (ref. 14). High homology was reported between the tobacco mosaic virus and thaumatin (ref. 17).

We recognized a greater challenge to identify the structural basis for immunological cross-reactivity. Since the binding site of an antibody is approximately 700 Å wide, it is clear that the cross-reactivity is a function of a discrete portion in each sweet protein. Moreover since the sweet taste is lost upon denaturation, we suspect that immunological cross-reactivity is a function of a similar topochemical configuration existing in sweet proteins.

We have available a series of chemically synthesized monellin analogs (ref. 20). It is our intention to examine these molecules for cross reactivity with the above antibodies and hopefully to correlate features of immunologic behavior to their taste potency. It is interesting to note that alterations in composition of monellin in B chain between residues 5 and 10, essentially destroy all sweet taste. Following our molecular modeling studies on monellin, we predicted that [pipecolic acid^{B10}] monellin and [NMeAla^{B10}] monellin would substantially retain sweet potency (ref. 21). These analogs have been synthesized and their taste potency measured (Table 1).

TABLE 1. Taste Potency of Designed Monellin Analogs

Protein	Sweetness Potency (x sucrose)
Monellin	4000
[Pipecolic Acid^{B10}] Monellin	3500
[NMeAla^{B10}] Monellin	2000

We will explore the immunochemical properties of these analogs and relate them to the essentially tasteless other analogs with altered residues in the B^5 to B^{10} region of the protein.

In our future research we intend to exploit the study of antigenized antibodies as molecular probes for the sweet receptors. We intend to select a loop from monellin or its natural amino acid containing analogs as likely regions that interact with receptor. The coding region for the selected loop will be cloned into the hypervariable loop of the immunoglobulin and expressed at the surface of the molecule. From these structures, a panel of antigenized antibodies will be created which can be viewed as anti-receptor antibodies with which we will probe for sweet receptors.

This paper is devoted to an immunological approach to characterize sweet receptors. The observations by Hough and van der Wel and Bel (ref. 8) and Edwardson (ref. 9) can now be carried forward. From such studies, it is hoped that sweet receptors can be isolated and characterized.

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