Hypothesis

Why are sweet proteins sweet? Interaction of brazzein, monellin and thaumatin with the T1R2-T1R3 receptor

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Abstract Sweet tasting proteins interact with the same receptor that binds small molecular weight sweeteners, the T1R2–T1R3 G-protein coupled receptor, but the key groups on the protein surface responsible for the biological activity have not yet been identified. I propose that sweet proteins, contrary to small ligands, do not bind to the 'glutamate-like' pocket but stabilize the free form II of the T1R2–T1R3 receptor by attachment to a secondary binding site. Docking of brazzein, monellin and thaumatin with a model of the T1R2–T1R3 sweet taste receptor shows that the most likely complexes can indeed stabilize the active form of the receptor.

Key words: Sweet protein; Sweet receptor; Sweetener; Docking

1. Glucophores on sweet proteins

Low-calorie sweeteners are important for persons affected by diseases linked to the consumption of sugar, e.g. diabetes, hyperlipemia, caries. Most sweet compounds, including all popular sweeteners, are small molecular weight compounds of widely different chemical nature, but there are also sweet macromolecules, both synthetic [1] and natural, i.e. sweet proteins [2]. Sweet molecules elicit their taste, in humans and other mammals, by interacting with the recently discovered T1R2–T1R3 receptor [3–5]. The sequence of this protein indicates that it is a metabopromic 7 transmembrane G-protein coupled receptor with a high homology to the mGluR subtype 1 [4]. The structure of the N-terminal part of the mGluR has been recently determined by X-ray diffraction and has been used as a template to build a homodimeric T1R3–T1R3 receptor model [4]. It is very likely that small molecular weight sweet molecules occupy a pocket analogous to the glutamate pockets in the mGluR [5], possibly similar to the active site models predicted by indirect receptor mapping studies [6–11].

It is more difficult to envisage the same type of interaction for sweet proteins. If the glucophores on the protein surface are similar to those that account for the taste of small molecules, it might be possible to identify putative 'sweet fingers' that can be accommodated in the cavities of proposed active site models. However, existing models can hardly explain the enormous increase in activity in going from small molecular weight compounds to proteins. For example monellin, one of the best characterized sweet proteins, is 100 000 times sweeter than sucrose on a molar basis [12].

The key groups on the protein surface responsible for the biological activity have not yet been identified with certainty for any of the known sweet proteins [13]. Sweet tasting proteins have different molecular lengths (from the 54 residues of brazzein to the 202 residues of thaumatin), virtually no sequence homology and very little structural homology [14].

It is customary to try to identify corresponding parts of proteins with the same function by comparing their sequences or their 3D structures. I find no sequence homology among miraculin [15], monellin [16], thaumatin [17], curculin [18], mabinlin [19] and brazzein [20]. Attempts to find structural similarity among the 3D structures of monellin, thaumatin and brazzein, were made by means of DALI [21], but yielded only a vague resemblance of three hairpins. Another commonly used strategy is to mutate systematically all residues that are suspected to host potential glucophores or that have a key structural role. Extensive mutagenesis studies on monellin [22] and on SCM, one of its single chain mutants [23], yielded several constructs with substantial loss of activity, but the residues involved are not localized in a single structural element that could be considered a suitable 'sweet finger'. Similarly, point mutations on brazzein show that sweetness determinant sites are located in at least two regions of the protein surface.

2. The T1R2–T1R3 receptor

The easiest way out of this impasse would be to invoke a different receptor for sweet macromolecules with respect to small molecular weight sweeteners, but this would still leave completely open the problem of explaining the commonalities among the sweet proteins. Besides, it has been recently shown [5] that two of the known sweet proteins, i.e. brazzein and thaumatin, elicit a response in the human T1R2–T1R3 receptor, similarly to small molecular weight sweeteners.

I want to show that it is possible to reconcile the interaction of small and macromolecular sweeteners with the same receptor, provided sweet proteins interact with the T1R2–T1R3

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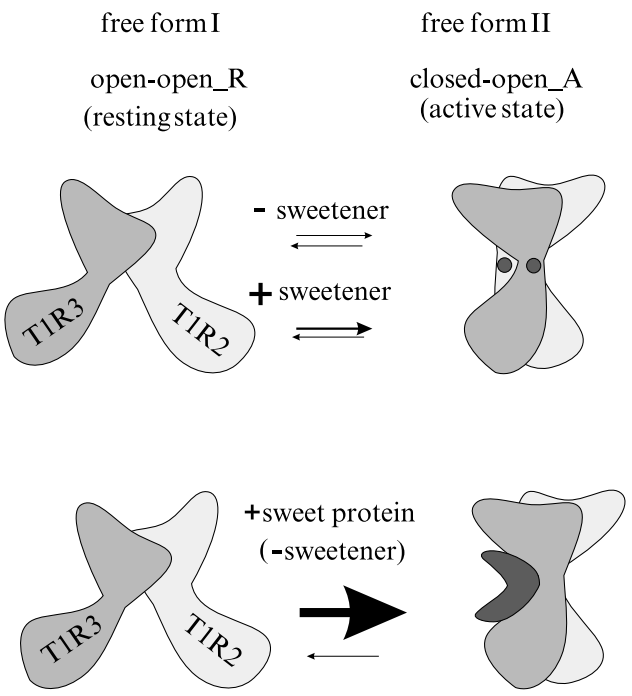

Fig. 1. Cartoon representation of the equilibrium between hypothetical free form I and free form II (equivalent to the complexed form) of the T1R2–T1R3 receptor showing possible stabilization from attachment of a sweet protein to a secondary binding site on the surface of free form II. The dark spots in the complex on the upper right-hand side represent small molecular weight sweeteners in pockets corresponding to the glutamate pockets. The sweet protein is represented as a darker wedge on the left flank of free form II, preventing it to revert to form I.

receptor with a different mechanism with respect to small molecular weight compounds.

Let us assume that the T1R2–T1R3 receptor, apart from obvious differences in the active site, shares the general characteristics of the mGluR. The most striking feature of the N-terminal domain of the mGluR, highlighted by the beautiful structural work of Kunishima et al. [24,25] is the large conformational change induced by glutamate complexation. Kunishima et al. [24] have determined the crystal structures of the N-terminal ligand-binding region of m1-LBR, the mGluR subtype 1, both free and complexed with glutamate. m1-LBR is a flexible homodimer whose 'active' and 'resting' conformations are modulated through the dimer interface. Each protomer is composed of two domains, LB1 and LB2, that can form 'open' or 'closed' conformations. Ligand binding increases the population of the 'active' conformer, i.e. the so-called 'closed-open_A'. The ligand-free receptor exists as two different structures, free form I (open-open_R), the 'resting' conformation with two open protomers and free form II (closed-open_A), nearly identical to the complexed form. The mechanism suggested by these structures is that the receptor is in dynamic equilibrium, and that ligand binding stabilizes the 'active' dimer.

There are thus two ways, in principle, to activate the receptor: the most obvious one is to complexate form I with the proper ligand (glutamate for the mGluR, aspartame or any other small molecular weight sweetener for the T1R2-T1R3 receptor) and, secondly, by shifting the equilibrium between free form I and free form II in favor of free form II. I propose that sweet proteins interact with the free form II of the T1R2–T1R3 receptor and stabilize it.

Fig. 1 shows a scheme (adapted from [24]), which explains how stabilization may result from attachment of a sweet protein to a secondary binding site on the surface of free form II.

In order to substantiate this hypothesis I have built a heterodimeric model of the T1R2–T1R3 receptor using the SWISS MODEL tool of EXPASY in the oligomeric mode [26–28] and simulated complexation in silico with representative models of the three known sweet protein structures by means of docking calculations with the free form II of the receptor.

The T1R2–T1R3 model was built using the coordinates of the free form II of m1-LBR (1ewv.pdb). This is the only possible template, as judged from a search of databases, but the homology of both protomers is high enough (>40%) to yield a reliable model. The main missing residues in the crystal structure, from 125 to 153, are located in a region that is not critical for the conformational transition from free form I to free form II.

3. Docking

Docking was performed using the program GRAMM in the low-resolution mode [29,30]. This docking method has been optimized to circumvent the multiple minima problems and is best suited to cases in which there is no hint of likely binding sites. The essential point of this procedure is to reduce protein structures to digitized images on a 3D grid. Structural elements smaller than the step of the grid are not present in the docking, thus allowing tolerance to structural inaccuracies and making it possible the study of the role of the low-resolution recognition in protein complexes. Tolerance is essential in a case where at least one of the partners (the receptor model) is not a high-resolution structure. The search for the possible external binding site was first performed with MNEI (1fa3.pdb), a single chain monellin since in this case the structure–function relationship has been thoroughly investigated and it is possible to use a wealth of information from closely related proteins, i.e. native monellin and another single chain monellin [14,31].

Among the first 40 preferred solutions found by GRAMM, none is on the 'wrong' side of the receptor model, i.e. the side facing the membrane; all of them are in fact on the same side of the receptor and most of them are centered on a large cavity of the T1R3 protomer. I selected the best candidates on the basis of the possibility of inhibiting the conformational rearrangement that leads to free form I; the most obvious candidates are those that 'bridge' the two protomers (T1R2 and T1R3), possibly inserting crucial residues in the cleft between them.

The face of MNEI interacting with the receptor includes most residues previously identified by mutagenesis and surface survey experiments [14], particularly the crucial N-terminal residues (I6, D7, G9) and C-terminal residues, with the putative sweet finger (63–68) bridging the protomers. It is also very interesting that the electrostatic potentials of the T1R3 protomer cavity and MNEI have largely complementary charges, with a prevalently negative T1R3 cavity and a prevalently positive MNEI surface.

I selected analogous complexes for brazzein (1brz.pdb) and thaumatin (1thw.pdb); also in these two cases the preferred

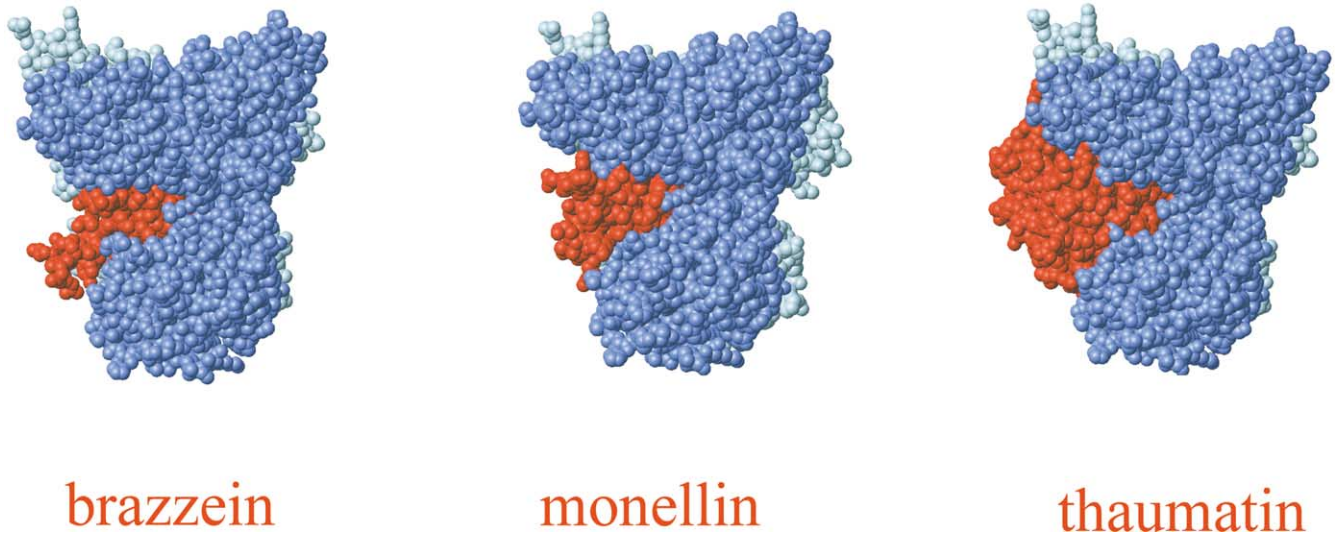

Fig. 2. Representative models of the complexes of the T1R2–T1R3 receptor with brazzein, monellin (MNEI) and thaumatin. All proteins are shown as space-filling models, with the T1R3 protomer colored in blue and the T1R2 in a paler shade of blue. The sweet proteins are colored in red.

solutions included several complexes that would prevent the conformational switch from free form II to free form I. Also in these cases the surface of interaction of the protein is prevalently positive. Fig. 2 shows representative models of the three complexes.

The proposed mode of interaction of sweet proteins is consistent with the difficulty of finding consensus sweet fingers (i.e. a small structural feature protruding from the surface) common to the three of them; in fact, with a large interacting surface, it is easier to explain a stronger binding and it is not necessary to have high sequence homology (only the shape of the interacting surface needs to be conserved). Such an interaction is consistent with a more efficient and long-lasting stabilization of the activated form of the receptor, with respect to the conformational transition induced by small sweeteners entering the cavity analogous to the glutamate pocket.

4. Potential experiments

Can this hypothesis be tested? The most obvious experiment would be to perform an X-ray study of the receptor complexed with one of the sweet proteins. So far there is no crystal structure of the receptor itself but the close similarity with the mGluR suggests that such a structure may be available in a reasonable time. Then, it ought to be relatively easy to obtain crystals of a complex with a sweet protein since the very high sweetening power of sweet proteins implies a large binding constant. This view is strongly supported by the model emerging from the docking study, since the interacting area is very large and with good electrostatic complementarity.

Other experimental approaches that come to mind are competition experiments and fluorescence resonant energy transfer [32]. In this respect, using the complex of MNEI, it can be suggested that suitable chromophores could be attached to residues not directly involved in the interaction, e.g. Lys25 that sits at the bottom of the helix of monellin and Lys127 of the T1R2 protomer of the receptor model that is located at the beginning of a long helix. Another obvious experiment could be to predict tasteless functional mutants different from those described in [22,23]. For instance, Arg39 of monellin, that is located on the interacting surface of MNEI, could be as crucial as Asp7, particularly if one considers the electrostatic complementarity. Additional survey of recent literature indeed shows that the R39E mutant is devoid of sweet taste [33]. An interesting residue of MNEI is Tyr65, since it is part of both the sweet finger and of the interacting surface. Whereas mutant Y65G (corresponding to YA13G) proved sweet [22], I foresee, on the basis of electrostatic complementarity, that Y65D ought to be tasteless.

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