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Interactions of the Sweet Protein Brazzein with the Sweet Taste Receptor

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Brazzein is a small, potently sweet protein. Homology modeling has been used to construct a model of the ligand-binding domain of the sweet taste receptor, and low-resolution docking has been used to identify potential modes of brazzein−receptor binding. Published brazzein mutation−taste data were then used to select one of these as the most likely brazzein−receptor binding orientation. This orientation places brazzein in contact primarily with the T1R2 subunit of the receptor, and it accounts for 21 of the 23 mutation results examined.

KEYWORDS: Brazzein; sweetener; taste receptor; homology model; docking; *Pentadiplandra brazzeana*

INTRODUCTION

Brazzein is a small protein (54 amino acids) derived from the African plant *Pentadiplandra brazzeana* (*1*). It has sweet taste (potency = 2000 × a 2% sucrose solution on a weight basis, 37500 × a 2% sucrose solution on a molar basis) and is very heat-stable. The three-dimensional structure of brazzein has been determined by NMR spectroscopy (*2*; Protein Data Bank entry 2BRZ). A number of point mutations have been made, and these have identified a number of residues in brazzein that are important for interaction with the sweet taste receptor (*3*, *4*).

Sweet taste has been shown to be mediated by a heterodimeric G protein coupled receptor composed of two proteins, T1R2 and T1R3 (*5*). These proteins have significant homology to a brain metabotropic glutamate receptor, mGluR1, which functions as a homodimer. The extracellular ligand-binding domain of mGluR1 has been crystallized with and without bound glutamate (*6*). The ligand-binding domain has been compared to a clamshell or a Venus flytrap, having two lobes that form the glutamate-binding site. In the absence of ligand, both monomers have very open binding sites. In the ligand-activated form, one mGluR1 chain exhibits a rather closed conformation, and the other has an open binding site.

The ligand-binding domain of the sweet taste receptor has been homology-modeled previously on the basis of the mGluR1 crystal structures (*7*−*9*). Early models dealt only with the T1R3 subunit (*7*, *9*). Temussi has used homology modeling to construct a T1R2+T1R3 model, and he has carried out docking calculations for the sweet proteins brazzein, monellin, and thaumatin (*8*), but he considered only one form of the receptor model (T1R2 closed, T1R3 open). The resulting brazzein−receptor complex model was not described in any detail.

Here we describe the construction of two models of the ligand-binding domain of the sweet taste receptor. We have carried out docking calculations to identify possible binding modes for brazzein to these models. Finally, we have used published brazzein mutation data to evaluate possible binding modes, to choose between two different receptor models, and to propose an atomic level model for the brazzein−receptor complex. This model accounts for most of the known structure−activity relationships of brazzein, and it permits the rational design of new brazzein mutants.

MATERIALS AND METHODS

Homology Modeling of the T1R2 and T1R3 Ligand-Binding Domains. Homology modeling was carried out using Molecular Operating Environment (MOE), version 2005.06 (Chemical Computing Group, Montreal, Canada). The sequences of the human T1R2 and T1R3 ligand-binding domains were used to conduct a FASTA search (*10*) of the sequences in the RCSB Protein Data Bank (*11*). Twelve possible template structures were identified; the most appropriate in terms of sequence similarity (13% identity) were judged to be three structures of the ligand-binding domain of the metabotropic glutamate receptor mGluR1 (*6*). The three structures, all homodimers, are (1) accession code 1EWT, unliganded, with both subunits in an open conformation, resolution = 3.7 Å; (2) accession code 1EWV, unliganded, with one subunit open and one subunit in a more closed conformation, resolution = 4.0 Å; and (3) accession code 1EWK, with two bound glutamate molecules, one subunit open and one subunit in a more closed conformation, resolution = 2.2 Å. We elected to use the liganded structure, 1EWK, as our modeling template, reasoning that this is likely to be an activated conformation because it has agonist bound to both sites. The published mGluR1 structures have a disordered region, residues 125−153, which causes the corresponding region of the homology-modeled taste receptor to be poorly modeled. This region is at the top of the protein structure as depicted in the figures. It would

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Table 1. Brazzein Structure–Taste Data Used To Evaluate Docking Results[a]

brazzein variant	sweetness potency, relative to des-pGlu-1-brazzein	ref
D2N	equal	*3, 4*
C4A	decreased	*3*
K5A	decreased	*4*
K6A	decreased	*4*
K6D	decreased	*4*
Y8A	decreased	*3, 4*
K15A	decreased	*3*
Q17A	equal	*3*
D29A	increased	*4*
D29K	increased	*4*
D29N	increased	*4*
K30D	decreased	*4*
H31A	decreased	*3, 4*
R33A	decreased	*3, 4*
R33D	decreased	*4*
E36A	decreased	*4*
E36K	decreased	*4*
E36Q	decreased	*4*
E41K	increased	*4*
R43A	decreased	*3, 4*
D50A	decreased	*3, 4*
delete Y54	decreased	*3, 4*
add R55, R56	decreased	*3*

[a] Wild type brazzein has pGlu in position 1; all nutants listed lack this residue but retain the wild type numbering.

Table 2. Scoring Results for the 10 Best Brazzein Docking Orientations in (**A**) the T1R2 Closed/T1R3 Open Receptor Model (Form 1) and (**B**) the T1R2 Open/T1R3 Closed Receptor Model (Form 2)[a]

brazzein docking orientation	1	2	3	4	5	6	7	8	9	10
form 1	–4	–9	–3	–1	+9	–11	–5	+7	–7	–9
form 2	+7	–1	–3	+19	+5	+1	–11	–7	–15	–3

[a] See Materials and Methods for a description of the scoring methodology.

appear to be distant from likely binding sites, although we cannot rule out the possibility that brazzein binding might include a portion of this segment.

Alignment of the T1R sequences with the template sequence was based on the alignment available in the Pfam database (*12*). In Pfam, the T1R receptors are part of the ANF receptor family (accession no. PF01094) which, when we accessed it, contained 1268 aligned, homologous sequences.

Because the template structure has two different conformations (one subunit open and one closed), there are two possibilities: T1R2 closed/T1R3 open, which we refer to as form 1, and T1R2 open/T1R3 closed, which we refer to as form 2. For each of the two forms, we adjusted the alignment of the T1R sequences to the mGluR1 sequence so that, to the extent possible, insertions and deletions occur on external loops rather than in the core of the protein. Receptor models were built using the MOE Homology Model module. Final models were evaluated using the MOE Protein Report module to identify bad contacts and unusual bond lengths, bond angles, and torsion angles. When necessary, conformations were modified to alleviate the issues identified in the Protein Report. Finally, the structures were minimized using the CHARMM27 force field (*13*, *14*).

Docking of Brazzein to T1R2:T1R3 Models. Brazzein−receptor docking was carried out using GRAMM, version 1.03 (*15*, *16*), which systematically examines all possible orientations of one protein relative to another, but does not account for conformational flexibility. The program was used in low-resolution docking mode (grid step = 2.5, repulsion = 11.0, attraction = 0.0, potential range = 2.5). This "low resolution" docking can tolerate structural inaccuracy on the order of

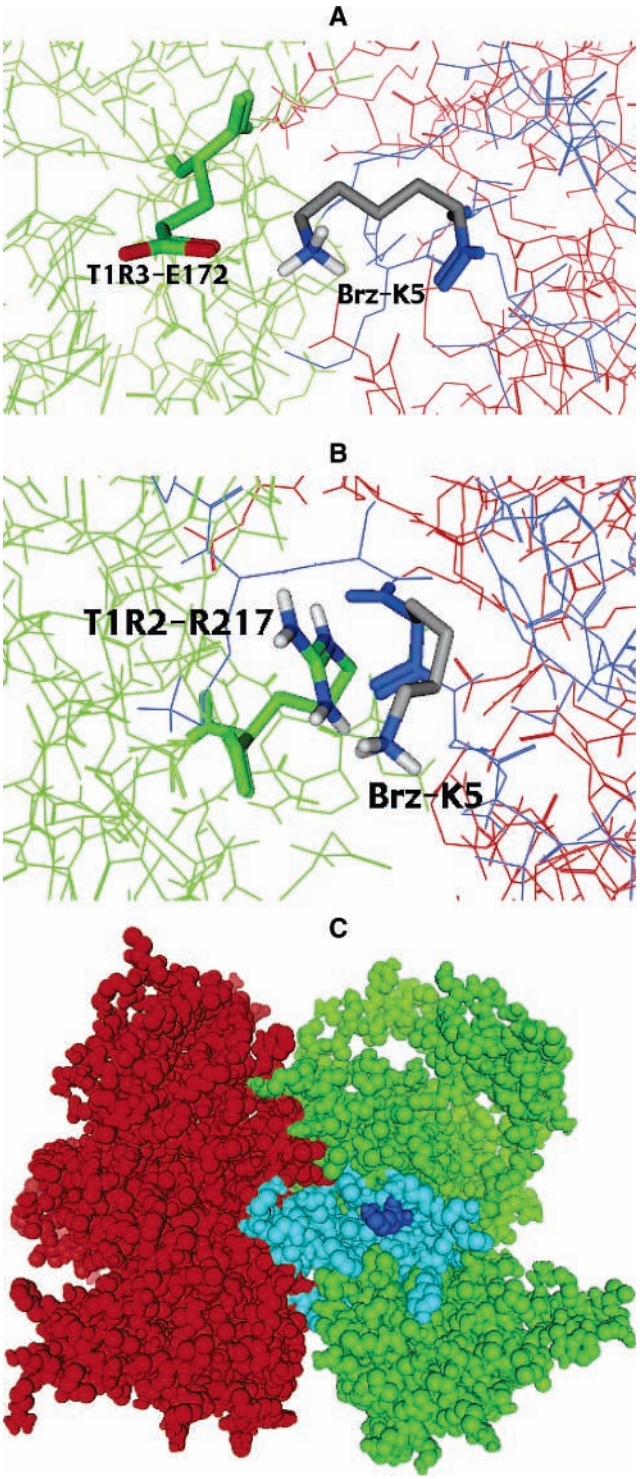

Figure 1. Representative examples of the way in which brazzein–receptor docking orientations were scored. These three examples illustrate scoring with respect to the K5A mutant, which was shown experimentally to have significantly decreased potency relative to brazzein. (**A**) In this orientation, mutation of K5 would remove a favorable interaction; because this is consistent with the experimental result, this position was scored +1. (**B**) In this orientation, mutation of K5 would alleviate an unfavorable interaction; because this is not consistent with the experimental result, this position was scored –1. (**C**) In this illustration, the receptor proteins are shown in red and green, and brazzein is shown in light blue with K5 highlighted in dark blue. In this orientation, K5 is exposed to solvent, and mutation should have no effect on potency; because this is not consistent with the experimental result, this position was scored –1.

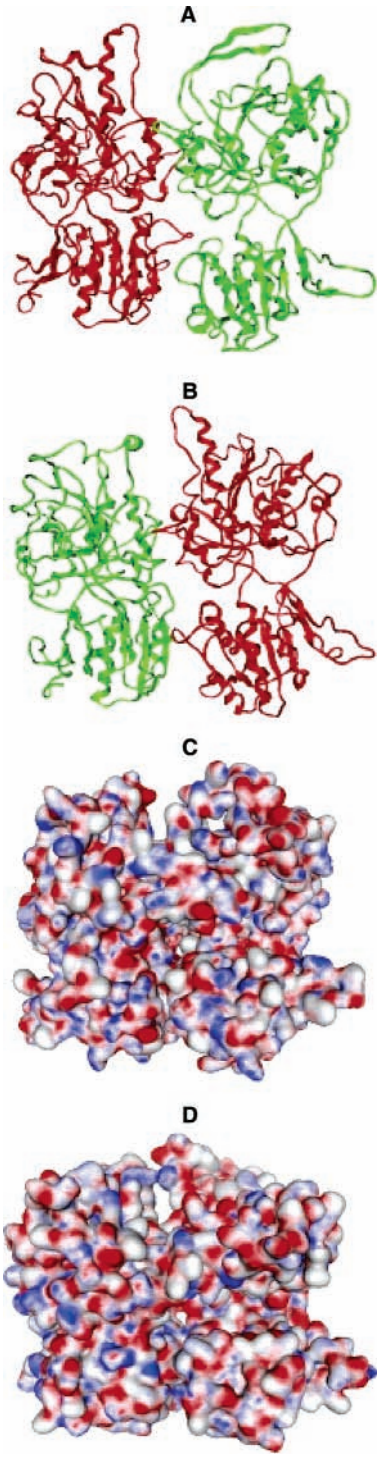

Figure 2. Homology-modeled structures of the ligand binding domain of the T1R2+T1R3 receptor: (**A**) form 1, T1R2 closed, shown as red backbone ribbon, T1R3 open, shown as green backbone ribbon; (**B**) form 2, T1R2 open, shown as red backbone ribbon, T1R3 closed, shown as green backbone ribbon; (**C**) form 1 and (**D**) form 2, surface representations colored according to partial atomic charge, where red = negative charge, white = neutral, and blue = positive charge.

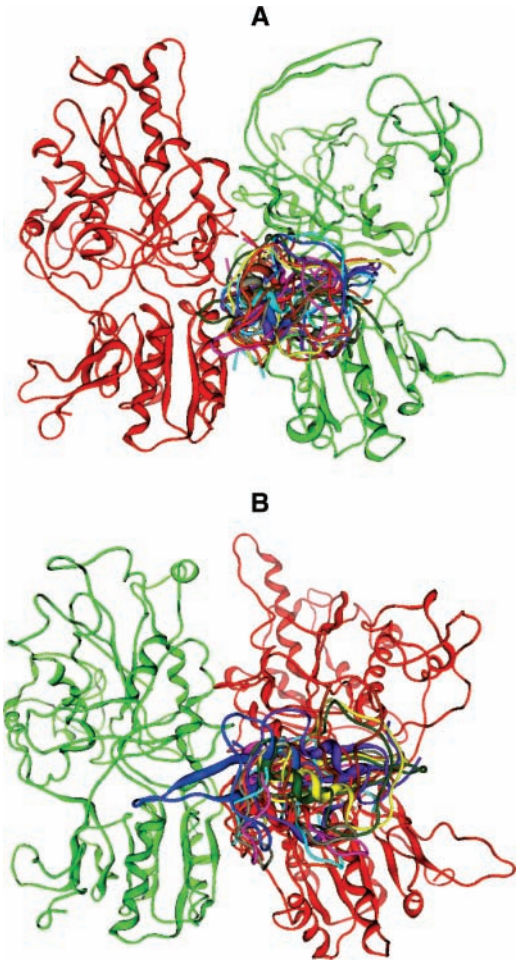

Figure 3. Ten best orientations found for brazzein in each form of the receptor model. All structures are represented as backbone ribbons; red = T1R2, green = T1R3. (**A**) In form 1, brazzein was consistently docked into the open T1R3 subunit. (**B**) In form 2, brazzein was consistently docked into the open T1R2 subunit.

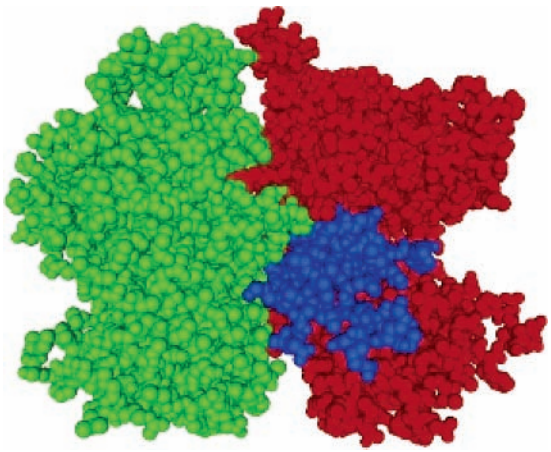

Figure 4. Final model of brazzein complexed with the modeled T1R2/T1R3 ligand binding domain. Red = T1R2; green = T1R3; blue = brazzein.

7 Å, but it can predict only gross features of a complex. Thus, it is necessary to critically evaluate the best docking orientations found. Fortunately, many single point mutants have been prepared and tasted (*3*, *4*), so that we can use the known structure−taste relationships to objectively assess whether or not each docking orientation is reasonable. In the present study we evaluated the top 10 docking orientations for brazzein in each of the two forms of the receptor model (total of 20 docking orientations). Each orientation was scored with respect to 23 different brazzein variants (listed in **Table 1**).

Each docking orientation was evaluated according to the following scoring scheme. At each brazzein mutation position, the nearby receptor model residues were rated with respect to possible interactions with wild type and mutant brazzein side chains. If the mutation−sweetness potency experimental results were consistent with the model at this

position, the docking orientation score was increased by one point; if the experimental results were not consistent with the model at this position, the docking orientation score was decreased by one point. Representative examples in **Figure 1** illustrate how this scheme works. The K5A brazzein mutant is significantly less sweet than brazzein (*4*). In the docking orientation shown in **Figure 1A**, brazzein K5 is placed near E172 of T1R3, producing a favorable binding interaction (ion pair). The K5A mutation would remove this favorable interaction and should decrease potency. Because this docking orientation is consistent with the experimental mutation−sweetness result, this orientation received a +1 score at this position. A second docking orientation, shown in **Figure 1B**, places K5 of brazzein near R217 of T1R2, producing a repulsive interaction between the two positively charged side chains. Here, the K5A mutation would remove an unfavorable interaction and should *increase* potency. Because this prediction is inconsistent with the experimental result, this docking orientation received a −1 score at this position. A third docking orientation, shown in **Figure 1C**, places K5 in a solvent-exposed position where it should have no interaction with the receptor. In this situation, a mutation should result in equal potency. Because this prediction is inconsistent with the experimental result, this docking orientation received a −1 score at this position. Each of the 20 docking orientations was scored in this manner with respect to each of the 23 mutants listed in **Table 1**. The resulting scores are shown in **Table 2**.

RESULTS

Homology modeling produced two models of the ligand-binding domain of the T1R2+T1R3 complex, shown in **Figure 2**. In form 1 (**Figure 2A**,**C**), T1R2 exists in the closed conformation and T1R3 in the open conformation. In form 2 (**Figure 2B**,**D**), T1R2 is open and T1R3 is closed.

Brazzein was docked to each of the two forms of the receptor model. The top 100 docking orientations were visually inspected for each form, and brazzein was consistently placed in the binding site of the open subunit. **Figure 3** shows the top 10 brazzein orientations for each form of the receptor. Each of these orientations was examined in detail and scored as described above.

Overall, scores were somewhat higher for brazzein docked into form 2 (T1R2 open), with four positive scores compared to only two positive scores for form 1. One docking orientation scored much higher than all of the others. Orientation 4 in form 2 scored +19 (21 mutations consistent, 2 not); the next highest score was +9. Thus, we consider orientation 4 in form 2 to be the most likely binding mode for brazzein.

This orientation is shown in **Figure 4**. In this orientation, shape complementarity is quite good. Brazzein binds primarily to T1R2, but also makes some favorable contacts with T1R3, which may help to stabilize the activated conformation of the receptor. Among the 23 mutations examined, 21 are consistent with published structure−taste data. One that is not is the Y54 deletion, which experimentally decreases sweetness. In the docking model, this residue makes unfavorable steric contact, and its removal would be predicted to improve the fit. However, it is known from the NMR structure determination of brazzein (*2*) that the C-terminal residues (*52*−*54*) are poorly defined and probably quite flexible. It is likely that, when brazzein binds to the receptor, the C-terminal portion adopts a conformation in which Y54 can interact favorably, and when Y54 is deleted, this interaction is lost. The other mutation that is not explained by this docking orientation is Q17A, which maintains sweetness. In the model, the glutamine side chain can hydrogen bond to an arginine side chain of T1R2, and mutation to alanine should result in loss of a hydrogen bond. It is possible that the arginine side chain is long and flexible enough to hydrogen bond to the backbone C═O of the Q17A mutant.

DISCUSSION

The GRAMM docking consistently places brazzein into the apparent binding site of the open subunit. This indicates good shape complementarity between the brazzein molecule and the receptor; in cases when there is not shape complementarity, GRAMM often produces an almost random-looking distribution of one protein relative to another. Low-resolution docking with a program such as GRAMM can identify likely binding sites, but the scoring is not sufficiently accurate to determine the precise orientation of one molecule relative to another. In this case, we were able to make use of a significant body of structure−activity data for brazzein mutations. This allowed us to identify one of the 20 candidate orientations as being significantly better than the others. The docking orientation we identified differs significantly from that proposed previously by Temussi (*8*). Our best docking orientation placed brazzein in contact with the T1R2 subunit rather than the T1R3. Whereas GRAMM can identify the open form of T1R3 as having shape complementarity with brazzein, the mutation data show that the fit is not as good on the atomic level.

We note that Jiang et al. have demonstrated that point mutations in the cysteine-rich region of T1R3 can affect response to brazzein (*17*). This domain couples the large domain modeled in this paper to the seven-helix transmembrane domain. Although it is possible that the mutants could directly affect binding, we consider it more likely that they affect the conformational change required to activate second messenger signaling inside the cell. Consistent with this, the two mutations shown to inhibit brazzein response are ones that are known to limit conformational flexibility (A537T increases steric bulk and introduces β-branching; F540P introduces a cyclic side chain that prevents rotation around the C-α−CO bond). Furthermore, these mutations significantly decrease receptor response to sucrose and several other sweeteners. Finally, these authors report (without showing data) that human T1R2 is better able to respond to brazzein than mouse T1R2, consistent with our modeling results.

Future research will focus on experimental evaluation of this result, using the final brazzein plus receptor model to design new mutants of brazzein. The model has identified a number of brazzein residues that should interact with the receptor and which have not yet been mutated. For example, in our model, K3 of brazzein can interact with an aspartate side chain on T1R2; mutation of this residue is predicted to decrease sweetness. Similarly, E9 of brazzein is interacting with a lysine in our model, so mutation to a neutral or positive side chain should decrease sweetness.

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