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Micro and Macro Models of the Sweet Receptor

Gabriella Morini[1] and Piero A. Temussi[2]

[1]DISMA, Università di Milano, Via Celoria, 2-I 20133 Milano, Italy and [2]Dipartimento di Chimica, Università di Napoli Federico II, via Cinthia, 80126 Napoli, Italy

Correspondence to be sent to: Piero A. Temussi, e-mail: temussi@unina.it

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Introduction

The study of the structure–activity relationship (SAR) of sweet molecules has been traditionally based on indirect analyses that have led to several models of the receptor active site, consistent with the shape of many conformationally rigid sweeteners—see, for example, Temussi *et al.* (1991) and the references therein. Most sweeteners are low mol. wt compounds but a few sweet proteins are also known. Only recently it has been demonstrated that small mol. wt sweeteners and sweet macromolecules interact with the same T1R2–T1R3 receptor (Li *et al.*, 2002), inferring that there is just one sweet taste receptor. However, it is not easy to understand (and show) how low mol. wt sweet compounds and sweet proteins can activate the same receptor.

The three-dimensional structure of the receptor has not yet been elucidated, but it is possible to build homology models to shed light on the SAR of sweet molecules.

The sweet taste receptor (SR) is a G protein coupled receptor similar to the dimeric metabotropic glutamate m1-LBR receptor (Margolskee, 2002). The similarity between the sequences of the two chains of the T1R2–T1R3 receptor and that of the single chain of the homodimer of the m1-LBR mGlu receptor is sufficient to allow homology modelling and to assume that it has the same general features. Existing three-dimensional SR models (Margolskee, 2002; Temussi, 2002) have been built using, as template, the N-terminal domain of mGluR1 whose crystal structure is available (Kunishima *et al.*, 2000).

Sweet fingers

It is conceivable that small mol. wt sweeteners occupy the cavities of the two T1Rs protomers corresponding to the glutamate binding sites of mGluR1. However, we have shown that, even using protruding 'sweet fingers', proteins are not able to interact with these cavities (Tancredi *et al.*, 2004).

SR three-dimensional models show that the active site is located at the bottom of a deep cleft, 20–30 Å from the surface of the protein. Based on the sequences of similar loops consistent with these dimensions, we synthesized three cyclic peptides: c[C^{56}YFDDSGSGIC^{66}] (dubbed T_YI, for thaumatin); c[C^{61}LYVYASDKLFRAC^{73}] (M_LA, for MNEI); and c[C^{37}FYDEKRNLQC^{47}] (B_FQ, for brazzein). None of the cyclic peptides designed to mimic 'sweet fingers' (and that have a similar three-dimensional structure) was able to elicit sweet taste and since there are no obvious alternative choices for putative sweet fingers in the proteins, it is necessary to look for alternative explanations of the high biological activity of the parent proteins (Tancredi *et al.*, 2004).

Macromodel: the Wedge model

If the SR has the same characteristics of the mGluR1, it should exist as a mixture of ligand-free forms in equilibrium (Kunishima *et al.*, 2000): free form I, the 'inactive' conformation with two open protomers and free form II, nearly identical to the 'active' complexed form. Stabilization of the active form may result either from complexation of a small mol. wt sweetener in the glutamate-like pocket or from attachment of a sweet protein to a secondary binding site on the surface of free form II.

The actual feasibility of this binding was checked by docking calculations of brazzein, monellin and thaumatin to a model receptor (Temussi, 2002). All three sweet proteins fit a large cavity of the receptor with wedge-shaped surfaces of their structures.

Micromodels

The receptor model built on the mouse T1R2 and T1R3 sequences was not used to study the SAR of small sweeteners, mainly because species differences would not allow the exploitation of all sweeteners already tested on humans. The availability of the human T1R2 and T1R3 sequences prompted us to build new models to test the SAR of small sweeteners.

The T1R2–T1R3 receptor can bind, among many other molecules, simple hydrophobic aminoacids and synthetic dipeptides, e.g. aspartame. We can hypothesize that the cavity of the T1R2–T1R3 receptor should retain most of the features necessary to build this moiety, i.e. the residues lining the wall of the cavity that hosts the amino and carboxyl groups of aminoacids should retain these binding properties. On the other hand, the residues that line the remaining part of the cavity should change from polar (in the mGluR1 receptor binding the side chain of glutamate) to apolar (in the sweet receptor binding the side chains of hydrophobic aminoacids).

The two active sites of mGluR1, although very similar in the residues that bind the glutamate molecule, have different dimensions, with site A smaller than site B. In modelling the human T1R2–T1R3 receptor on the mGluR1 template, we can have either T1R2(A)–T1R3(B) or T1R2(B)–T1R3(A). Since a smaller site, in a low-resolution model, can exclude some sweet molecules on the basis of dimensions only, we decided to concentrate our efforts mainly on the active sites of T1R2(B) and T1R3(B).

Table 1 shows corresponding key residues of the active site in the structure of mGluR1 and in the T1R2 and T1R3 models obtained using different alignment programs. It can be seen that in all different alignments the residues corresponding to those binding the aminoacidic moiety of glutamate are well conserved in the SR models, as expected. On the other hand, residues lining the wall of the active site in correspondence to the glutamate side chain are changed, on average, to less polar or uncharged residues.

Once having obtained the three-dimensional models of both T1R2(B) and T1R3(B), the active sites were identified. We have chosen model No. 1 of T1R3(B) for docking representative sweet molecules. The active site considered consisted of 66 amino acids. Docking was first done manually, also to allow for the fitting of flexible ligands in different conformations. Subsequently, 22 sweet molecules were inserted in the active site and their fit optimised by means of PrGen (Vedani *et al.*, 1995), a program that allows a

Table 1 Alignments of key residues of the active site in the structure of mGluR1 and in the T1R2 and T1R3 models obtained using different alignment programs[a]

hT1R2													
mGluR1 (A)	Y74	R78	S164*	**S165***	**S186***	T188*	D208*	**Y236***	E292	G293	**D318***	R323	K409
No. 1	N83	S87	N143	**S144**	**S165**	I167	A187	**Y215**	P277	D278	**E302**	D307	A394
No. 2	I67	L71	N143	S144	S165	I167	A187	Y215	P277	D278	E302	I306	S380
No. 3	I67	L71	N143	S144	S165	I167	A187	Y215	P277	D278	E302	–	S380
No. 4	I67	L71	N143	S144	S165	I167	A187	Y215	P277	D278	E302	–	R383
hT1R3													
mGluR1 (B)	Y74	R78	**S164***	**S165***	S186*	**T188***	**D208***	**Y236***	E292	G293	**D318***	R323	K409
No. 1	F65	W72	**S146**	**S147**	G168	**S170**	**D190**	**Y218**	S276	V277	**E301**	S306	T390
No. 2	F65	W72	S146	S147	G168	S170	D190	Y218	S276	V277	E301	S306	N386
No. 3	N68	W72	S146	S147	G168	S170	D190	Y218	A275	S276	E301	S306	S392
No. 4	F65	W72	S146	S147	G168	S170	D190	Y218	S276	V277	E301	S306	S392

[a]All alignments were obtained from programs available in the EXPASY Molecular Biology Server, using default parameters: No. 1, SwissPDB; No. 2, CLUSTAL W 1.74; No. 3, T-COFFEE; No. 4, ALIGN.
*Residues binding the amino acidic moiety of glutamate in the mGluR1. Conservation is highlighted with bold characters.

semiquantitative measurement of binding affinity and a comparison with the biological activity, i.e. sweetness. In all cases we obtained a good fit with the shape of the cavity that hosts the active site. The binding site model of the sweet taste receptor identified is very reliable, since it is based on the amino acids present in the 'real' binding protein.

Methods

The heterodimeric models of the T1R2–T1R3 receptor were built by the SWISS MODEL tool of EXPASY in the oligomeric mode (Guex and Peitsch, 1997) using, as template, the coordinates of the complexed form of m1-LBR (1ewk.pdb). Different methods of alignment, all available in the EXPASY Molecular Biology Server, were used. Once obtained a model and defined the active site, some compounds were docked manually using MolMol (Koradi *et al.*, 1996) to manipulate the molecules. To insert and evaluate semiquantitatively free energies of binding of the ligands the program PrGen 2.1 (Vedani *et al.*, 1995) was used.

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