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From oligopeptides to sweet proteins‡

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NMR OF DILUTE PEPTIDE SOLUTIONS WITHOUT A CAT

The first time I worked with Murray Goodman was in 1970. Since 1959, with the publication of a pioneering work on [L-Glu(OMe)]n (OMe, methoxy) homo-oligomers [1], Murray Goodman had been considered worldwide the most prominent figure in the field of oligopeptides. In particular, he had promoted detailed chemical and physicochemical studies on oligopeptides [2] with the goal of determining the type and stability of the basic conformations, such as the α-helix and the β-sheet, that they were supposed to share with proteins. During the same period, I had been involved in the NMR study of polydisperse polypeptides that, in spite of the merely statistical significance of limiting conformations, indicated well-defined helix-coil transitions [3,4]. In 1969 I asked Murray Goodman if I could spend a short time in his laboratory to see whether the NMR behavior of polyaminoacids could be also found in pure monodisperse oligopeptides.

When I managed to visit his laboratory in the Brooklyn Poly, in the fall of 1970, I found plenty of well-characterized oligopeptides and limited NMR time on the 220 MHz spectrometer located in the Rockfeller University. This spectrometer had the highest field available at the time and was equipped, in principle, with a rudimentary computer to add spectra, the CAT. However, at the time I was using it, the CAT was not working, so I had to work long shifts at night to run spectra at very low speed, an inexpensive, albeit tedious, way with scanning spectrometers to record spectra of dilute solutions. The results were satisfactory and led to a publication of which I was very proud, showing that indeed it was possible to monitor a helix-coil transition also in oligopeptide solutions [5]. Whenever Murray referred to our work he used to say that the spectra had been recorded by a human CAT.

The main result of my short stay in Brooklyn, however, was not the publication in PNAS [5] but the understanding, through many discussions with Murray and with Fred Naider, a newly acquired friend, of the importance of bioactive peptides.

In fact, as a consequence of my stay in Brooklyn, I did change my main research interest from polyaminoacids to bioactive peptides.

SMALL MOLECULAR WEIGHT SWEETENERS

During my stay, I also heard for the first time of the interesting problem of the interaction of sweet molecules with their receptor. Murray was just beginning to study sweet peptides, but I did not pay too much attention at the time. The interest in sweet compounds and that in conformational studies of bioactive peptides came together thanks to Claudio Toniolo, a former post-doc with Murray Goodman, who, in 1973, asked me whether I was interested in studying by NMR the conformation of aspartame that he had independently synthesized in Padova. Aspartame was the first peptide sweetener that would soon dominate the market of artificial sweeteners, but its mechanism of action was unknown. Claudio's proposal was going to influence not only my research for many years but also my relationship with Murray, since from then on our collaboration and competition were centred on the problem of sweetness (and bitterness). We managed to propose a likely solution conformation for aspartame [6] and discussed it with Murray, who had just moved to La Jolla.

In the following years, while Murray Goodman continued his efforts in determining the structure-activity relationship of sweet dipeptides by chemical synthesis [7 and references therein], the group in Naples (Lelj, Tancredi and Temussi), in collaboration with Claudio Toniolo (Figure 1) in Padova, the other Italian city most linked to Murray Goodman, proposed a model of the active site of the sweet receptor [8,9].

At first, this model was not accepted by Murray, who understandably thought that cartoon models had too many limitations and preferred to rely on solid experimental evidence. Therefore, I decided to go and work with him again in order to compare our views. These two approaches, i.e. the synthetic one and the indirect model building, were compared during a second

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Figure 1 The Italian connection. La Jolla 1984: from left to right, Murray Goodman, Piero Temussi and Claudio Toniolo (Courtesy of P.A. Temussi).

(longer) visit to Murray's laboratory in San Diego, during the second half of 1984.

The comparison led to a substantial agreement on the models of receptors for sweet and bitter tastes [10,11]. Later on, Murray developed his own model that, while incorporating most of the features of our model [12], differed in the detailed steric aspects [13].

To put it in a nutshell, our model was consistent with an extended conformation of aspartame, whereas the Goodman model favored a folded conformer. X-ray studies were not sufficient to give an unequivocal answer, since the conformer found in the crystal structure of aspartame [14] was consistent with Murray's model but that of the more rigid and sweeter [(L-α-Me)Phe[2]] aspartame [15] was consistent with ours.

SWEET PROTEINS

From 1984 on, the work on sweet compounds by the Naples group was less intense but the collaboration with Murray Goodman continued indirectly through the organization of the Capri workshops that were generously supported by him. In addition to creating a nice international melting pot, I could use these meetings to discuss with Murray the latest aspects of sweet compounds, particularly when I began to work on monellin [16].

During the past few years we have resumed work on sweet compounds but exclusively on sweet proteins. The renewed interest in sweeteners came mainly from the identification [17] of receptors for sweet taste. Since it has been demonstrated that small molecular weight sweeteners and sweet macromolecules interact with the same T1R2-T1R3 receptor [18] we have tried to find a common mechanism and possibly to validate old models of the active site [12,13]. It is not easy to understand how low molecular weight sweet compounds and sweet proteins can activate the same receptor. The mentioned indirect models of the active site [12,13] based on the shape of small sweeteners would also be compatible with the interaction of proteins if, on the surface of the proteins, there are thin protruding features that can probe the active site, i.e. 'sweet fingers' chemically similar to small sweeteners.

Among the known sweet proteins there is no sequence homology. There is also little similarity among the tertiary folds of brazzein, monellin and thaumatin, the three proteins of known 3D-structure. The only common elements among the three proteins are short segments of secondary structure, β-sheet loops. All three loops host residues consistent with glucophores already identified in small sweeteners [18]. Based on the sequences of these loops we have recently synthesized three cyclic peptides, but none of them was able to elicit a sweet taste. Since there are no obvious alternative choices for putative 'sweet fingers', it is necessary to look for different explanations of the high biological activity of the parent proteins [19].

The presence of 'sweet fingers' is not the only possible explanation of the sweet taste of proteins. The sweet taste receptor is a G-protein coupled receptor similar to the dimeric metabotropic glutamate m1-LBR receptor [20]. 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 model building and to assume that it has the same general features. Existing sweet receptor (SR) models [20,21] have been built from the mouse sequence, using the crystal structure of the *N*-terminal domain of mGluR1 [22] as a template.

If the SR has the same characteristics as mGluR1, it should exist as a mixture of ligand-free forms in equilibrium [22]: free form I, the 'inactive' conformation with two open protomers (dubbed **Roo**) and the free form II (active, open-closed: **Aoc**), nearly identical to the 'active' complexed form. Stabilization of the active form may result either from complexation of a small molecular weight sweetener in the glutamate-like pocket (depicted by old models) or from the 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 built from the mouse sequence, using the active open-closed (**Aoc**) form of mGluR1 as a template [21]. All three sweet proteins fit a large cavity of the receptor with the wedge-shaped surfaces of their structures.

Unfortunately, I could not discuss these findings with Murray since I could not attend the last two Capri meetings, but I do hope he would have appreciated this

last effort that, like all our work on sweet molecules, benefited enormously from his seminal work on sweet molecules.

Recently, we have also modelled the active site of the T1R2-T1R3 receptor that accepts aspartame [23]. The bottom of the cavity is very similar to old indirect models [12,13] but the resolution is not sufficient to discriminate between the two models. However, we are close to knowing the correct answer since there is little doubt that the 3D-structure of the receptor, and possibly also of its complex with aspartame, will soon be available.

REFERENCES

1. Goodman M, Schmitt EE. Conformational transitions in polypeptides. *J. Am. Chem. Soc.* 1959; **81**: 5507–5508.
2. Goodman M, Verdini AS, Toniolo C, Phillips WD, Bovey FA. Sensitive criteria for the critical size for helix formation in oligopeptides. *Proc. Natl Acad. Sci. USA* 1969; **64**: 444–450.
3. Paolillo L, Temussi PA, Trivellone E, Bradbury EM, Crane-Robinson C. NMR and optical spectroscopic studies of block copolymers of polypeptides. 1. Block copoly (benzyl-L-glutamate)$_n$:(benzyl-L-aspartate)$_m$; *Biopolymers* 1971; **10**: 2555–2568.
4. Bradbury EM, Cary P, Crane-Robinson C, Paolillo L, Tancredi T, Temussi PA. Experimental evidence for the assignment of α-CH peaks in the NMR spectra of polypeptides. *J. Am. Chem. Soc.* 1971; **93**: 5916–5917.
5. Temussi PA, Goodman M. Conformational transitions in oligopeptides. A PMR study. *Proc. Natl Acad. Sci. USA* 1971; **68**: 1767–1772.
6. Lelj F, Tancredi T, Temussi PA, Toniolo C. Interaction of aspartame with the receptor site of the sweet taste bud. *J. Am. Chem. Soc.* 1976; **98**: 6669–6674.
7. Tsang JW, Schmied B, Nyfeler R, Goodman M. Peptide sweeteners. 6. Structural studies on the *C*-terminal amino acid of L-aspartyl dipeptide sweeteners. *J. Med. Chem.* 1984; **27**: 1663–1668.
8. Temussi PA, Lelj F, Tancredi T. Three-dimensional mapping of the sweet taste receptor site. *J. Med. Chem.* 1978; **21**: 1154–1158.
9. Lelj F, Tancredi T, Temussi PA, Toniolo C. Interaction of α-L-aspartyl-D-phenylalanine methyl ester with the receptor site of the bitter taste. *Il Farmaco* 1980; **35**: 988–996.
10. Goodman M, Temussi PA. Structure-activity relationship of a bitter diketopiperazine revisited. *Biopolymers* 1985; **23**: 2349–2360.
11. Goodman M, Bland J, Tsang J, Coddington J, Temussi PA, Tancredi T, Lelj F, Fuller WD, Verlander M. Molecular correlations of taste including a new class of aminoacid based sweeteners. In *Peptides: Structure and Function*, Deber CM, Hruby VJ, Kopple KD (eds). Pierce Chemical Company: Rockford, IL, 1985; 725–728.
12. Kamphuis J, Lelj F, Tancredi T, Toniolo C, Temussi PA. SAR of sweet molecules: conformational analysis of two hypersweet and two conformationally restricted aspartame analogues. *QSAR* 1992; **11**: 486–491.
13. Yamazaki T, Benedetti E, Kent D, Goodman M. Conformational requirements for sweet-tasting peptides and peptidomimetics. *Angew. Chem. Int. Ed.* 1994; **33**: 1437–1451.
14. Hatada M, Jancarik J, Graves B, Kim S-H. Crystal structure of aspartame, a peptide sweetener. *J. Am. Chem. Soc.* 1985; **107**: 4279–4282.
15. Polinelli S, Broxterman QB, Schoemaker HE, Boesten WHJ, Crisma M, Valle G, Toniolo C, Kamphuis J. New aspartame-like sweeteners containing L-(αMe)Phe. *Bioorg. Med. Chem. Lett.* 1992; **2**: 453–456.
16. Tancredi T, Iijima H, Saviano G, Amodeo P, Temussi PA. Structural determination of the active site of a sweet protein. A ^{1}H NMR investigation of pMNEI. *FEBS Lett.* 1992; **310**: 27–30.
17. Kitagawa M, Kusakabe Y, Miura H, Ninomiya Y, Hino A. Molecular genetic identification of a candidate receptor gene for sweet taste. *Biochem. Biophys. Res. Commun.* 2001; **283**: 236–242.
18. Li X, Staszewski L, Xu H, Durick K, Zoller M, Adler E. Human receptors for sweet and umami taste. *Proc. Natl Acad. Sci. USA* 2002; **99**: 4692–4696.
19. Tancredi T, Pastore A, Salvadori S, Esposito V, Temussi PA. Interaction of sweet proteins with their receptor. A conformational study of peptides corresponding to loops of brazzein, monellin and thaumatin. *Eur. J. Biochem.* 2004; **271**: 2231–2240.
20. Margolskee RF. Molecular mechanisms of bitter and sweet taste transduction. *J. Biol. Chem.* 2002; **277**: 1–4.
21. Temussi PA. Why are sweet proteins sweet? Interaction of brazzein, monellin and thaumatin with the T1R2-T1R3 receptor. *FEBS Lett.* 2002; **526**: 1–3.
22. Kunishima N, Shimada Y, Tsuji Y, Sato T, Yamamoto M, Kumasaka T, Nakanishi S, Jingami H, Morikawa K. Structural basis of glutamate recognition by a dimeric metabotropic glutamate receptor. *Nature* 2000; **407**: 971–977.
23. Morini G, Temussi PA. Micro and macro models of the sweet receptor *Chem. Senses* 2005; **30**: i86–i87.