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Molecular mechanisms of sweet taste 7: The sweet protein, Thaumatin I

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A computer modelling study of the host–guest relationship between the intensely sweet protein named Thaumatin I and our suggested receptor model, an α-helical protein, has revealed a perfect fit within a cleft of the Thaumatin I conformation. The parameters and stereoselective interactions for the initiation of the sweetness sensation are interpreted in accord with our previous stereo-interactive studies on a wide range of low molecular weight sweeteners. Also, the Thaumatin data parallels that of the macromolecular sweet protein Monellin, and their intense sweetness arises from the multiple interactions arising within the docking cleft of the protein receptor. The AH_s/B_s pair of Thaumatin I has been assigned to Lys-97/Asp-98. © 1997 Elsevier Science Ltd

INTRODUCTION

A sweet protein named Thaumatin was originally isolated from fruits of *Thaumatococcus danielli* (Benth), an African plant named in 1839 after Dr W. F. Daniell, who referred to it as 'Katemfe', the Yoruba name, or the 'miraculous fruit of Sudan' (Daniell, 1855; Inglett & May, 1968; Van der Wel, 1974). Van der Wel & Loewe (1972) separated Thaumatins I and II from homogenates of the arils by removing low molecular weight materials. A commercial study of Thaumatin was made by Tate & Lyle under the brand name 'Talin', which has a sweetness of 1600–2700 times that of an 8–10% solution of sucrose (Higginbotham, 1977). A proprietary mixture is commercially available under the brand name of 'San Sweet T-100' in Japan. The discovery, chemistry, chemical modifications and practical applications of Thaumatins have been described in several review articles (Higginbotham, 1979; Lee, 1987; Kim *et al.*, 1991; Van der Wel, 1993; Hough, 1993; Shallenberger, 1993; Witty & Higginbotham, 1994; Zemanek & Wasserman, 1995).

Thaumatins I and II have similar properties, such as amino acid composition, sweetness, molecular weight (22 000) and identical amino acid sequences except for five amino acid residues (Iyengar *et al.*, 1979; Edens *et al.*, 1982). Unlike Monellin, Thaumatin I consists of a single chain peptide of 207 amino acid residues (Fig. 1) with eight intramolecular disulphide linkages, namely 9–204, 56–66, 71–77, 121–193, 126–177, 134–145, 149–158 and 159–164. X-ray diffraction analysis of its crystal at 3.1 Å has revealed the general features of the protein's backbone (De Vos *et al.*, 1985; Kim *et al.*, 1991), and the structural details of Thaumatin I were further refined to a resolution better than 1.65 Å using a combination of energy minimization and stereochemically restrained least-squares methods (Ogata *et al.*, 1992). Structural refinement was attempted by computer-aided molecular modellings (Kim & Weickmann, 1994), using two independent procedures, in one with the XPLOR program (Brünger, 1988), coupled to the PROLSQ program (Hendrickson & Konnert, 1980), and in the other with the TNT program (Tronrud *et al.*, 1987). Recently, Beynon *et al.* (1994) constructed the complete three-dimensional structure of Thaumatin I by using the α-carbon coordinates (De Vos *et al.*, 1985), upon which the side-chains and the disulphide linkages were built, and further refined by molecular dynamics within the molecular mechanics package CHARMm (Correa, 1990).

Circular dichroism studies (Van der Wel *et al.*, 1984) showed very few α-helices, but many β-pleated sheet strands and bends. A flexible region of 28 sequential amino acids (from 93 to 120) was suggested, on the basis of their homology with Monellin, as the most probable site that initiates sweetness with a possible role of Tyr-95, Lys-97 and/or Lys-106 in the chemoreceptive

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1                                   10
Ala-Thr-Phe-Glu-Ile-Val-Asn-Arg-Cys-Ser-Tyr-Thr-Val-Trp-Ala-Ala-Ala-Ser-

    20                                  30
Lys-Gly-Asp-Ala-Ala-Leu-Asp-Ala-Gly-Gly-Arg-Gln-Leu-Asn-Ser-Gly-Glu-Ser-

            40                                  50
Trp-Thr-Ile-Asn-Val-Glu-Pro-Gly-Thr-Asn-Gly-Gly-Lys-Ile-Trp-Ala-Arg-Thr-

                        60                                  70
Asp-Cys-Tyr-Phe-Asp-Asp-Ser-Gly-Ser-Gly-Ile-Cys-Lys-Thr-Gly-Asp-Cys-Gly-

                                80                                  90
Gly-Leu-Leu-Arg-Cys-Lys-Arg-Phe-Gly-Arg-Pro-Pro-Thr-Thr-Leu-Ala-Glu-Phe-

                                    100
Ser-Leu-Asn-Gln-Tyr-Gly-Lys-Asp-Tyr-Ile-Asp-Ile-Ser-Asn-Ile-Lys-Gly-Phe-

    110                                 120
Asn-Val-Pro-Met-Asn-Phe-Ser-Pro-Thr-Thr-Arg-Gly-Cys-Arg-Gly-Val-Arg-Cys-

            130                                 140
Ala-Ala-Asp-Ile-Val-Gly-Gln-Cys-Pro-Ala-Lys-Leu-Lys-Ala-Pro-Gly-Gly-Gly-

                        150                                 160
Cys-Asn-Asp-Ala-Cys-Thr-Val-Phe-Gln-Thr-Ser-Glu-Tyr-Cys-Cys-Thr-Thr-Gly-

                                170                                 180
Lys-Cys-Gly-Pro-Thr-Glu-Tyr-Ser-Arg-Phe-Phe-Lys-Arg-Leu-Cys-Pro-Asp-Ala-

                                    190
Phe-Ser-Tyr-Val-Leu-Asp-Lys-Pro-Thr-Thr-Val-Thr-Cys-Pro-Gly-Ser-Ser-Asn-

    200                             207
Tyr-Arg-Val-Thr-Phe-Cys-Pro-Thr-Ala
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Fig. 1. Primary structure of Thaumatin I (Iyengar *et al.*, 1979).

action of Thaumatin (Van der Wel, 1980, 1983; Edens & Van der Wel, 1985).

Either heat denaturation of Thaumatin or cleavage of the disulphide bridges results in loss of sweetness, thus implicating the tertiary structure of the protein in the trigger mechanism, which is therefore a highly stereoselective process (Higginbotham, 1979; Hough, 1993). Variants of Thaumatin have been produced by genetic engineering, and several of these proteins display modified taste profiles (Weickmann *et al.*, 1989). Gradient chromatographic studies on native Thaumatin have revealed at least five variant proteins, with two major components (Thaumatins I and II) (Van der Wel & Loewe, 1972), and three minor components (Thaumatins a, b and c) (Higginbotham & Hough, 1977; Higginbotham, 1979), in different proportions, and separable from one another on the basis of differing charge characteristics.

In developing a radioimmunoassay to determine micro-quantities of Thaumatin in food and drink, antibodies to Thaumatin were found to be of interest in probing the active site of the sweet proteins. The antibody also recognized other high-intensity sweeteners, leading to the suggestion that the antibody could be used to detect the trigger or active site for the sweet sensation (Hough & Edwardson, 1978; Van der Wel & Bel, 1978). The radioimmunoassay method can detect native Thaumatin in solutions with microscopic amounts of cross-reactive protein. Polyclonal and monoclonal antibodies raised against Thaumatin recognized the tertiary structure of the protein but they failed to detect aspartame and sucrose (Weickmann *et al.*, 1989).

Van der Wel & Bel (1976) found that acetylation of the ϵ-amino groups of lysyl residues in Thaumatin I with acetic anhydride–sodium acetate resulted in a

reduction of sweetness. When three of the 11 lysyl residues in Thaumatin I were acetylated, the sweetness was near to zero (Van der Wel, 1994). Unlike Monellin (Morris *et al.*, 1978), progressive reductive *N*-methylation of the lysyl residues, even up to seven residues, had very little effect on its sweetness (Van der Wel & Bel, 1976; Van der Wel *et al.*, 1978, 1994). Treatment of the lysyl residues with succinic acid anhydride had a significant effect on the sweetness of Thaumatin since only one succinyl group decreased it by 50%, whilst two succinyl groups practically quenched its sweet taste (Van der Wel, 1983; 1994). It would appear, therefore, that one or more lysyl residues play an important role as the proton-donating component (AH_s) in the initiation of sweet-taste chemoreception.

Significantly, six of the 12 arginyl residues were derivatized with 1,2-cyclohexanedione without loss of sweetness (Van der Wel, 1981; 1994), and, hence, the reactive arginyl residues were not involved in the interactions with the sweet-taste receptor.

Esterification of the carboxyl groups of Thaumatin eliminated the sweet taste, whereas amidation of the carboxyl groups with ammonium chloride and a carbodiimide derivative dramatically increased the sweetness of Thaumatin six-fold, to some 12000 times that of sucrose. However, derivatization of the single methionyl residue (Met-112) with iodoacetic acid did not cause sweetness loss. In contrast, derivatization of all three tryptophanyl residues (Try-14, Try-37 and Try-51) by Koshland's reagent (2-hydroxy-5-nitrobenzyl bromide) resulted in complete loss of taste, which was attributed to a conformational change (Van der Wel, 1982; 1994; Higginbotham, 1983).

Whilst Monellin and Thaumatin are both intensely sweet, there appears to be little structural similarity between them (Ogata *et al.*, 1987). Despite the difference in amino acid sequences, antibodies against Thaumatin also compete for Monellin (Hough & Edwardson, 1978). There are, however, homologous tripeptide sequences within these sweet proteins (De Vos *et al.*, 1985), with five regions of Thaumatin having tripeptide homologies with five regions of Monellin. The five tripeptides in Monellin have their homologous counterparts in Thaumatin at the amino acid residues of 94–96, 100–102, 101–103, 118–120 and 128–130 (Iyengar *et al.*, 1979). All of these regions are well exposed; hence, they are probably responsible for the immunological cross-reactivity, and their combined conformations could well play a significant role in the stereoselective sweet-receptor process (De Vos *et al.*, 1985). Significantly our study of Monellin (Suami *et al.*, 1996) revealed that all five of the homologous tripeptides interacted with our proposed receptor model.

Kang (1988) determined the cross-reacting antigenic sites of Monellin and Thaumatin using polyclonal and monoclonal antibodies on tryptic peptides and endoprotease Glu-C peptides, and he concluded that Arg-76 (AH_s) and Asp-60 (B_s) and Leu-74, Leu-75 and Phe-58 (X_s components) made triangular structures ($AH_s/B_s/X_s$), which could account for the sweetness of Thaumatin (Van der Heijden, 1993).

Recently, Slootstra *et al.* (1995) postulated, by a study of antibodies, that the two regions of 19–29 and 77–84 in Thaumatin are important sweet-taste determinants. These regions contain an aspartame-like site which is formed by the Asp-21 and Phe-80 residues spatially positioned next to each other.

These data, considered together with our findings on the sweet protein Monellin (Suami *et al.*, 1996), stimulated us to investigate the molecular interactions between the Thaumatin I molecule and our proposed α-helical receptor model (Suami & Hough, 1991) using three-dimensional computer modelling.

MATERIALS AND METHODS

The present work was performed using a Silicon Graphics workstation. Initial α-carbon coordinates of the structure of Thaumatin I were generated from the Brookhaven Protein Data Bank, and its conformation including main chain and side-chains was generated by the BIOCES (NEC Co. Ltd., Tokyo, Japan). An energy of the Thaumatin I conformation was minimized by the molecular mechanics program MAXIMIN 2 with Prof. Kollman's all-atom force field and charges (Weiner *et al.*, 1984) in the SYBYL 6.1a system (TRIPOS Inc., St Louis, MO, USA, 1995). Initial coordinates for the receptor model were generated for an idealized α-helical protein having L-asparaginyl and L-prolyl residues at the N-terminus and adjacent sites, respectively, and L-leucyl residues arranged in a right-handed α-helix, which was composed of a total of 33 amino acid residues in nine helical turns. An energy of the conformation was minimized by the molecular mechanics program MAXIMIN 2 with Prof. Kollman's all-atom force field and charges (Weiner *et al.*, 1984) in the SYBYL 6.1a system. The initial model for the Thaumatin I–receptor model complex within the main cleft of the Thaumatin I conformation was constructed with: (1) 1:1 stoichiometry, (2) a hydrogen bond between an NH_3^+ group of the N-terminal asparaginyl residue of the receptor and a β-COO^- group of Asp-98 (see Table 1), (3) a hydrogen bond between a $CONH_2$ group of the N-terminal asparaginyl residue of the receptor and an $\epsilon - NH_3^+$ group of Lys-97 (see Table 1). Each of the above-mentioned hydrogen bonds was rigidly maintained at the correct bond angles and defined lengths, as described by Taylor *et al.* (1984) and Jeffrey & Saenger (1991).

A refinement procedure was carried out to align side-chains of amino acid residues of the receptor with those of the wall of the main cleft of Thaumatin I conformation and to facilitate a good fit. The stepwise protocol for structure refinements used 300 steps of conjugate

Table 1. Distance[a] (Å) between possible proton donors (AH_s candidates) and possible proton acceptors (B_s candidates) in Thaumatin I

B_s component	AH_s component				
	Lys-19	Lys-97	Lys-137	Lys-163	Lys-187
Glu-4	25.48	20.82	38.96	38.09	32.38
Asp-21	10.18	29.57	36.49	32.31	35.74
Asp-25	13.96	24.29	36.25	34.91	37.21
Glu-35	20.91	28.44	38.76	39.65	36.88
Glu-42	20.16	15.51	45.98	37.63	34.45
Asp-55	26.26	39.35	27.55	34.68	34.76
Asp-59	25.75	42.09	37.99	42.81	43.71
Asp-60	21.15	37.01	40.67	41.33	43.64
Asp70	11.95	30.23	26.57	27.58	28.30
Glu-89	19.19	20.74	25.86	25.83	23.53
Asp-98	23.05	**2.54**	41.39	31.94	23.87
Asp-101	18.07	12.34	33.06	27.76	20.38
Asp-129	30.84	35.76	20.23	27.09	25.74
Asp147	14.08	31.20	14.07	13.44	17.76
Glu-156	23.51	36.49	12.83	1.94	13.51
Glu-168	30.66	38.57	8.89	10.46	15.02
Asp-179	24.85	21.27	27.24	23.97	15.79
Asp186	19.53	24.64	22.34	12.07	6.41
C-terminal COO^-	32.22	28.34	39.16	40.44	32.75

[a] Distance is measured from the AH_s proton to the B_s (Shallenberger & Acree, 1967).

gradient minimization to minimize initial undesirable interactions.

RESULTS AND DISCUSSION

The initiation of sweetness by a diverse group of organic compounds is generally attributed to a molecular arrangement consisting of a hydrophilic bifunctional unit with AH_s (a proton donor) and B_s (a proton acceptor) components, each in close proximity and separated by only 2.5–4.0 Å (Shallenberger & Acree, 1967; Shallenberger, 1978), which acts in concert with strategically placed hydrophobic X_s components (Kier, 1972; Suami & Hough, 1992).

In the Thaumatin I molecule, there are 12 proton-donating groups with potential as AH_s candidates: namely, the N-terminal NH_3^+ group, the $\epsilon - NH_3^+$ groups of Lys-19, 49, 67, 78, 97, 106, 137, 139, 163, 174 and 187 residues. The N-terminal NH_3^+ group can be excluded from the candidates, because substitution of the N-terminus has no effect on taste activity (Lee *et al.*, 1988; Kim & Weickmann, 1994). Considering the taste data from chemical modifications with the homology to Monellin (Van der Wel, 1980, 1983; Edens & Van der Wel, 1985) makes one of the $\epsilon - NH_3^+$ groups of Lys-19, 97, 137, 163 and 187 residues the preferred position for the AH_s component. As we have seen, amidation of the carboxylic acids increased the sweetness markedly (Van der Wel, 1982, 1994; Higginbotham, 1983), indicating that modification of the Asp, Glu and C-terminal residues introduces a more powerful proton-accepting function into the B_s component. Consequently, we considered 19 candidates for the B_s component, namely the β-COO^- groups of Asp-21, 25, 55, 59, 60, 70, 98, 101, 129, 147, 179 and 186 residues, the γ-COO^- groups of Glu-4, 35, 42, 89, 156 and 168 residues, and the C-terminal COO^- group. In all, there are 95 different combinations of potential AH_s/B_s pairs to be investigated.

Then the three-dimensional structure of Thaumatin I was constructed, which was practically superimposable on that described by Beynon *et al.* (1994), and the distance between the two components of each pair of the potential AH_s/B_s was determined on the Thaumatin I molecule (Table 1).

By rejecting those pairs of the AH_s and B_s candidates that fell outside the restricted internal dimension of 2.5–4.0 Å (Shallenberger & Acree, 1967), only one pair remained for further consideration, namely Lys-97(AH_s)/Asp-98(B_s), with the correct distance of 2.54 Å. By taking into account the clockwise arrangement of an orientation of the bound $AH_s/B_s/X_s$ components in the complex with the receptor, when viewed from the position of the receptor (James *et al.*, 1989; Hough & Khan, 1993), this pair also qualified. The final selection of this pair was then made by computer analysis of the interactions between the cleft (or trough) of the conformation of the Thaumatin I macromolecule and the helical receptor model.

On the other hand, little is known about the structure of the receptor, except that it is proteinaceous in character and its active site(s) must recognize the chirality of sweet compounds, such as aspartame and Monellin, which have a wide variance in molecular structure and weight. An α-helix seemed to be the most relevant and, by using empirical amino acid preferences for specific positions in α-helices (Richardson & Richardson, 1988; Presta & Rose, 1988), we have postulated that it is a right-handed α-helical poly-L-leucine with L-asparagine

at the N-terminus, linked to L-proline, the penultimate residue (Suami & Hough, 1991).

Since our receptor model occupied most of the space available in the large binding cavity of the sweet protein Monellin (Suami *et al.*, 1996), we examined the conformation of Thaumatin I for a similar cavity or cleft on its surface. A major cleft was clearly observed which was contained by a number of hydrophobic amino acid side-chains, including Gly-81, Phe-90, Tyr-95, Gly-96, Ile-100, Ile-102, Phe-181, Val-184 and Leu-185, and its diameter of 13.9 Å between the side-chain of Ile-100 and that of Val-184 (15.0 Å between these two α-carbons) is similar to that found in Monellin for docking perfectly with our proposed receptor model.

Using the above-mentioned energy-minimized conformation of the Thaumatin I molecule, we constructed the two primary intermolecular hydrogen bonds, with the correct distances and acceptable bond angles, between the N-terminal asparaginyl residue of the proposed receptor model (Suami & Hough, 1991) and the Thaumatin I molecule: namely, $AH_r(NH_3^+)\cdots B_s(\beta\text{-}COO^-$ of Asp-98) (1.84 Å, 163.6°; Taylor *et al.*, 1984; Jeffrey & Saenger, 1991) and $B_r(CONH_2)\cdots AH_s$ ($\epsilon - NH_3^+$ of Lys-97) (1.89 Å, 160.0°; Taylor *et al.*, 1984; Jeffrey & Saenger, 1991). The receptor model was accommodated comfortably in an optimum position within the cleft of the Thaumatin I conformation. The Thaumatin I–receptor complex revealed a variety of possible van der Waals and hydrogen-bonding attractions, similar to Monellin (Suami *et al.*, 1996), after energy minimizations of the complex were performed by the molecular mechanics program. The receptor model dovetailed into the cleft (or trough) of the Thaumatin I conformation, along the two antiparallel β-strands (87–93 and 99–105), where it was intercalated precisely within the long cleft on the surface of the Thaumatin I molecule (Fig. 2).

Contrary to our expectation from the Monellin study, the fourth glutamyl residue of the receptor could not form an additional hydrogen bond to the stimulus molecule. Even so, the intimate binding of the receptor helix to the Thaumatin I molecule undoubtedly strengthens the binding between the host and the guest macromolecule in accord with intensification and prolonged sweetness of this protein. The threshold value of 10^{-8} mol litre^{-1} for the binding to the taste-cell membrane supports its strong affinity for the receptor (Van der Wel, 1993). The potency of a particular sweetener is dependent upon the number of its potential binding sites and varies according to an affinity of each binding site for the receptor (Nofre & Tinti, 1993).

The stereoviews of the structures of the Thaumatin I molecule before and after the intercalation with the

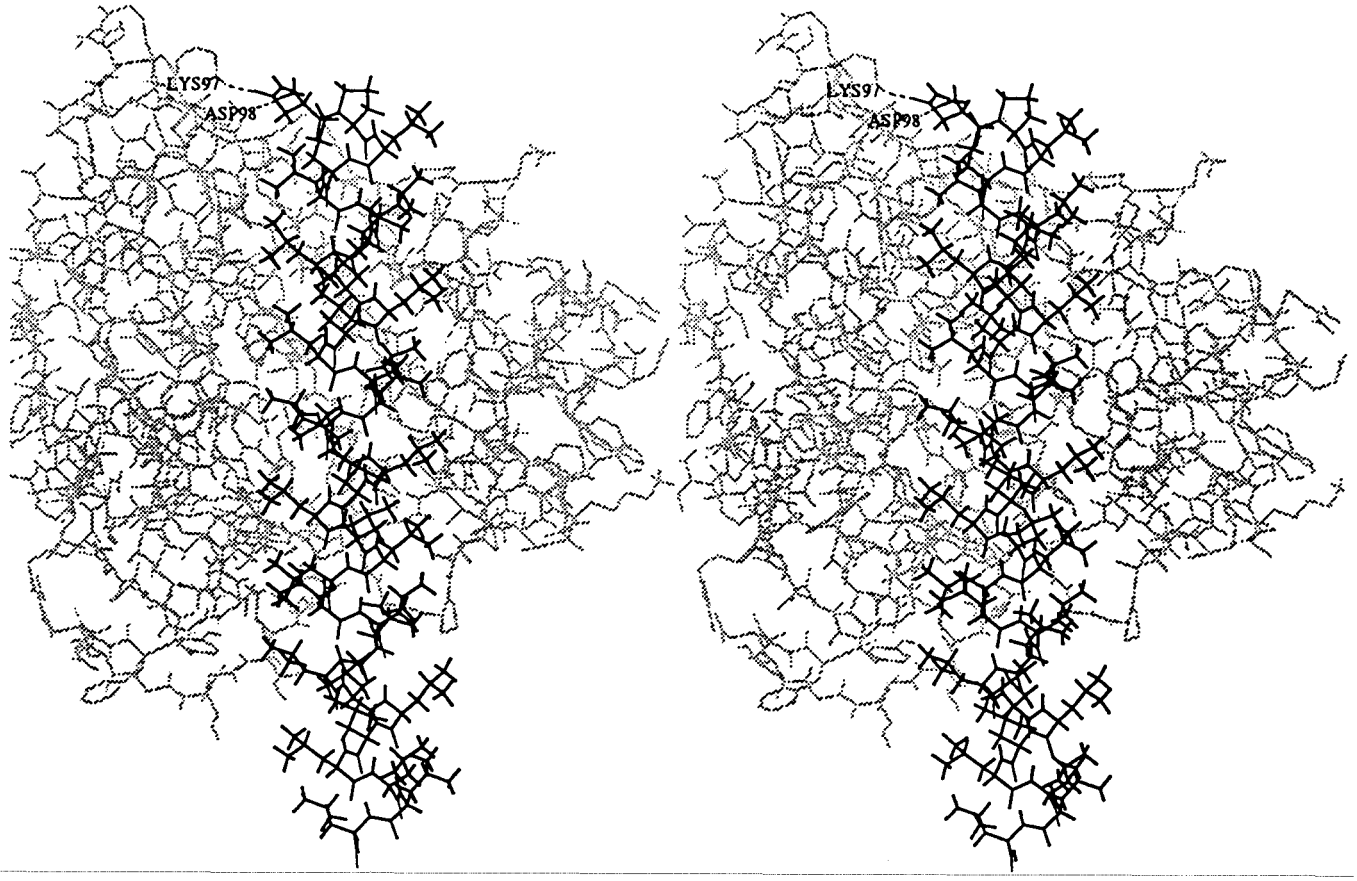

Fig. 2. Stereoviews of the Thaumatin I-α-helical receptor complex. The intermolecular H-bonds are shown with dotted lines, and the receptor model is highlighted.

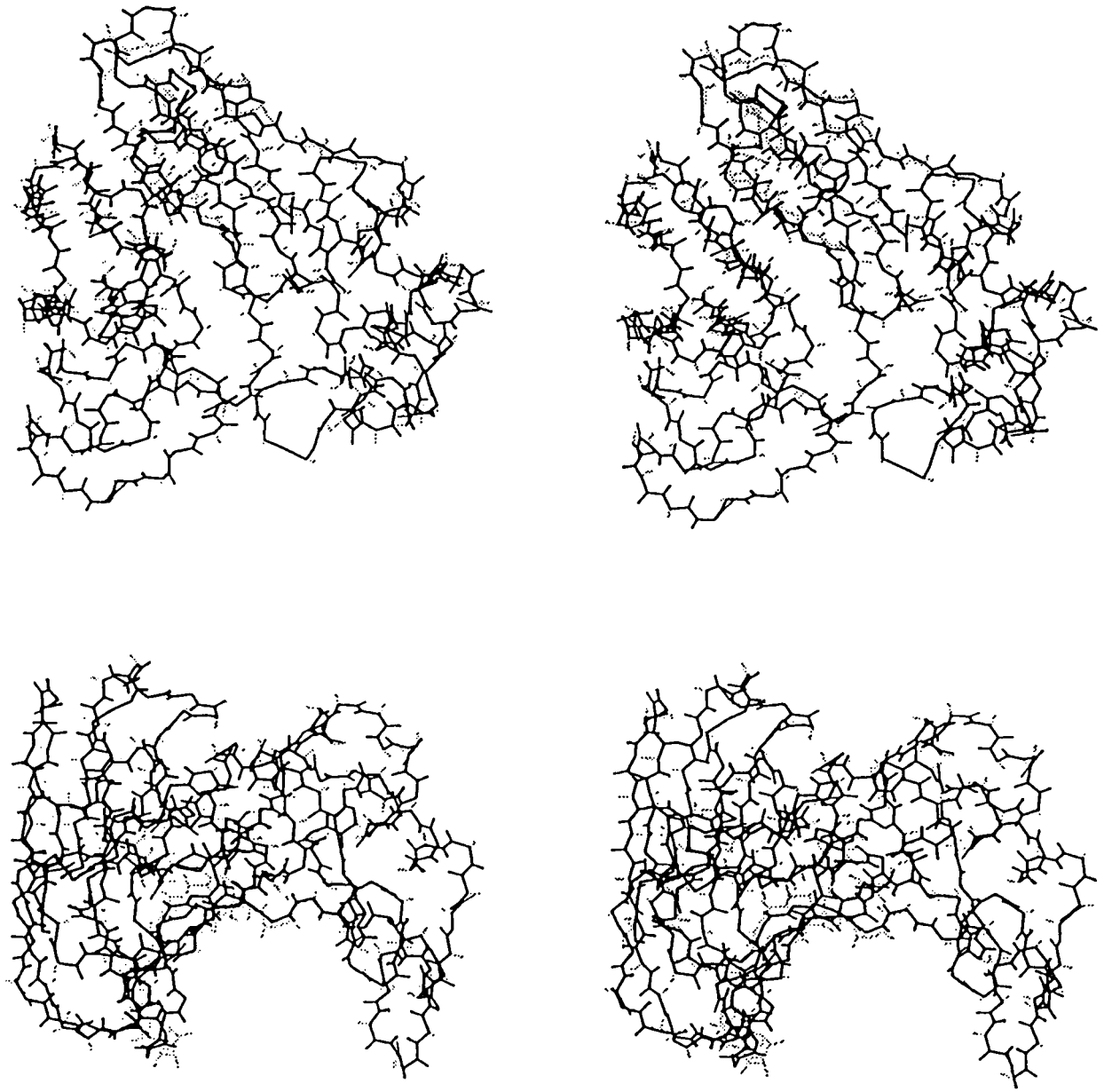

Fig. 3. Side and top stereoviews of the main-chain structures of Thaumatin I, before and after (highlighted) the intercalation with the receptor model.

α-helical receptor model are shown in Fig. 3. The two skeletal structures are practically superimposable, indicating that the Thaumatin I molecule can be intercalated with the receptor model without appreciable deformation of the main-chain structure of the molecule.

The structure of the Thaumatin I–receptor complex conformed to the traditional shape of lock-and-key motif of enzyme binding (Fig. 4), as it did in the case of Monellin, indicating that our receptor model must be very close to the dimensions of the active site of the native receptor. Such molecular precision seems to be more than coincidental, and accordingly the isolation and characterization of the natural receptor is eagerly awaited for confirmation of our findings and further development of our studies.

The interactions described above for Thaumatin I provide a rationale of several previous observations on its structure–sweetness relationships:

1. The intense sweetness and a prolonged period of sweet taste perception of Thaumatin I can be interpreted by the multiple interactions within the intimacy of the cleft of the Thaumatin I molecule that envelops the proposed receptor model. The cleft interactive docking feature of the Thaumatin I–receptor complex resembles that of the Monellin–receptor complex, and the lock-and-key similarity is reflected in similar sweetness characteristics.
2. Both the heat denaturation of Thaumatin and the cleavage of the disulphide linkage distort the

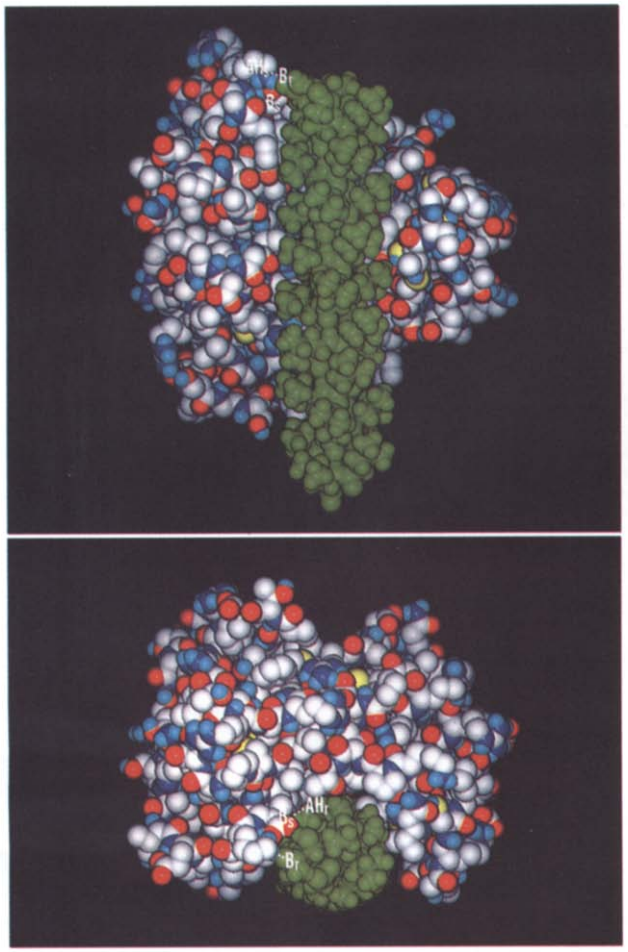

Fig. 4. Side and top views of the intercalation between Thaumatin I and α-helical receptor model bound in the cleft of the stimulus model. The receptor-Thaumatin I complex (with receptor in green) with the key intermolecular H-bonds (shown as dotted lines) is illustrated with CPK molecular models. The complex is viewed from the top of the receptor (bottom panel); the AH_r (top panel) and AH_s (bottom panel) are behind the receptor and the side-chain of Thaumatin I resepctively.

unique conformation of Thaumatin, resulting in loss of sweetness by preventing the highly stereo-specific lock-and-key mode of binding.

3. The fact that alkylation of the Met-112 of Thaumatin I and that of the Met-42(B) of Monellin with iodoacetic acid have no influence on their sweetness can be interpreted as follows. Each methionyl residue is located on the opposite side to the cleft (or cavity) of the protein's conformation and protrudes from the surface of the molecule. Hence, alkylation does not interfere with the interactions between the sweet protein and the proposed receptor model.
4. When Lys-97, the AH_s component of Thaumatin I, is acetylated, it no longer functions and sweetness is lost, whilst *N*-methylation of the Lys-97 does not impede its proton-donating ability, thus sweetness is retained. The AH_s component of Thaumatin I in the present bipartite Lys-97(AH_s)/Asp-98(B_s) glycophore is coincident with one of Van der Wel's proposals based on its homology with Monellin (Edens & Van der Wel, 1985).
5. The arginyl residues of Thaumatin I do not provide the AH_s function; hence, the accessible arginyl residues can be modified by 1,2-cyclohexanedione without loss of sweetness.
6. The proton-accepting power of a carboxyl group is diminished by its esterification, whereas the proton-accepting ability of a carboxyl group is strengthened by its amidation. These facts are clearly reflected in the esterification and amidation of the carboxyl groups of Thaumatin (Van der Wel, 1982; 1994; Higginbotham, 1983).

Hough & Edwardson (1978) noted that Thaumatin can raise antibodies which cross-reacted with a wide variety of non-protein sweet compounds, but also including Monellin. Similarly, antibodies raised against Monellin cross-reacted with Thaumatin, indicating that there is a structural link between these two sweet proteins, despite the limited homology in the amino acid sequences in Monellin and Thaumatin (Iyengar *et al.*, 1979). However, five tripeptide portions in Monellin have their counterparts in Thaumatin I (Gln-94, Tyr-95, Gly-96), (Ile-100, Asp-101, Ile-102), (Asp-101, Ile-102, Ser-103), (Thr-118, Arg-119, Gly-120) and (Ala-128, Asp-129, Ile-130). Van der Wel (1980) suggested that the aforementioned, first tripeptide portion of Thaumatin I, together with the vicinal Lys-97, is located in a β-bend of the tertiary structure on the protein surface and is hence the possible active site responsible for its sweet taste. In fact, our study reveals that the Lys-97 plays an important role as the AH_s component, thus supporting Van der Wel's prediction.

We have shown that all five tripeptide portions of Monellin are involved in the sweetener's interaction with our receptor model (Suami *et al.*, 1996). This is also true of Thaumatin I, where Tyr-95, Gly-96, Ile-100, Asp-101 and Ile-102 are implicated in the interactions with the receptor model. These residues are, therefore, most likely to be responsible for the immunological cross-reactivity, and their positions in the overall conformation probably contribute to the sweetener–receptor attractive forces (De Vos *et al.*, 1985).

There is a suppression of the sweet taste of both Monellin and Thaumatin in the absence of Ca^{2+} (Van der Wel, 1993). The interactive study of Thaumatin I with our receptor model does not provide any plausible explanation of this effect but may well be an intracellular event in the taste cells (Van der Wel, 1993).

CONCLUSIONS

The binding of Thaumatin I to our α-helical receptor model has been studied by a computer-based molecular

modelling program and is based on the accepted theory that all sweet compounds have in common a hydrophilic bipartite AH_s/B_s glycophore to form two hydrogen bonds to reciprocal AH_r and B_r units on the receptor, and that the intensity of sweetness is determined by strategically placed hydrophobic areas of attraction on both the sweetener and the side-chains of the protein receptor. The correlation between the molecular structure of the receptor and the diverse groups of sweet compounds must satisfactorily explain the structure versus activity data, especially the stereospecificity, and account for the varying degrees of intensity of sweetness observed. The bipartite glycophore, AH_s and B_s pair, of Thaumatin I has been assigned to the $\epsilon - NH_3^+$ group of Lys-97 and the β-COO^- group of Asp-98. Our proposed receptor model, right-handed α-helical L-asparaginyl-L-prolyl-poly-L-leucine, provides a plausible explanation for the initiation of sweetness by a widely differing organic sweeteners, including α-amino acids (Suami & Hough, 1991), carbohydrates (Suami & Hough, 1992), chlorodeoxysucroses (Sucralose) (Suami *et al.*, 1994), dipeptide esters (aspartame) (Suami & Hough, 1993*a*), guanidine acetic acids (sucrononic acid) (Suami & Hough, 1993*b*) and, now, the two protein sweeteners, Monellin (Suami *et al.*, 1996) and Thaumatin I. Such unity is convincing, especially with the excellent fit between the molecular dimensions of the cleft of the proven conformations of each of the two intensely sweet proteins, with multiple possible interactions with our α-helical receptor model; hence, it must be very close to the dimensions of the active receptor group on the taste-cell membrane.

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