ELSEVIER

PII: S0308-8146(96)00024-6

Food Chemistry, Vol. 56, No. 3, pp. 275–281, 1996

0308-8146/96 $15.00+.00

Molecular mechanisms of sweet taste. Part 6*: the sweet protein, Monellin

Tetsuo Suami,[a] Leslie Hough,[b] Tomoya Machinami[a] & Nobuhiro Watanabe[c]

[a]*Department of Chemistry, College of Science and Engineering, Meisei University, Hino, Tokyo 191, Japan*
[b]*Department of Chemistry, King's College London, University of London, Campden Hill, Kensington, London W8 7AH, UK*
[c]*System Engineering Department, Ajinomoto Co. Inc., Suzuki-cho, Kawasaki 210, Japan*

(Received 29 May 1995; revised version received 27 October 1995; accepted 27 October 1995)

A computer modelling study of the host–guest relationship between Monellin, a sweet protein, and the suggested receptor, an α-helical protein, has revealed an excellent fit, within a cavity of the Monellin conformation. The parameters and stereoselective interactions for sweetness are in accord with our previous data on a range of low molecular weight sweeteners. The $\mathbf{AH_s/B_s}$ is assigned to either Lys-4(A)/Glu-48(B) or Lys-4(A)/Glu-50(B). Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The intensely sweet compound isolated by Inglett & May (1968, 1969) from the berries of the African shrub, *Dioscoreophyllum cumminsii* (family Menispermaceae), the so-called wild red berry, guinea potato or serendipity berry, was named 'Monellin' by Morris & Cagan (1972). This sweet compound is a protein (Morris & Cagan, 1972; Van der Wel, 1972; Cagan, 1973), that is 3000 times sweeter than sucrose on a weight basis (*ca.* 90 000 times sweeter than sucrose on a molar basis). Monellin (MW 11 069) has two separate non-covalently bonded subunits (Bohak & Li, 1976) which are designated A- (MW 5233) and B-chains (MW 5836), containing 44 and 50 amino acid residues, respectively, and their amino acid sequences were established (Frank & Zuber, 1976; Hudson & Biemann, 1976; Higginbotham, 1979). The structure of the B-chains (Frank & Zuber, 1976) is similar to that described by Bohak & Li (1976) with the exception that residues 49 and 50 are transposed (Ariyoshi *et al.*, 1991). Also, some 10% of the A-chains have been found to contain additional phenylalanyl residues on the *N*-terminal arginyl units (Frank & Zuber, 1976).

The tertiary structure of crystalline Monellin (Ogata *et al.*, 1987) has 18 hydrogen bondings, in addition to the usual hydrogen bonds in the α-helical region, in the β-pleated sheet regions, where they serve to hold the conformation in place. This five-stranded, antiparallel β-sheet imposes a severe conformational restriction on the whole molecule of Monellin. The α-helix, 10–25(B), is located in the gently twisted concave side of the β-sheet, and runs almost perpendicular to the β-strands. The α-helix is suspended by two random coils, 6–9(B) and 26–34(B), at both ends of the helix and there appears to be some flexibility in the random coil regions (Ogata *et al.*, 1987). There are 16 basic amino acid residues and 14 acidic residues in Monellin, resulting in a relatively high isoelectric point of 9.03 (Van der Wel, 1972). Monellin is relatively resistant to proteolytic enzymes, such as trypsin and chymotrypsin, without being denatured, in agreement with its compact structure (Higginbotham, 1979).

The native conformation of the Monellin molecule is essential for its sweet taste (Bohak & Li, 1976; Cagan & Morris, 1976; Morris *et al.*, 1978; Kim *et al.*, 1991), since neither isolated subunit is sweet. Using genetic engineering techniques, the two chains (A and B) of Monellin have been fused into a single chain by the formation of a peptide bond between the Arg-1(A) and Glu-50(B) units. This single chain Monellin (named SP1) is just as potent in sweetness as the natural Monellin and their circular dichroism spectra are practically identical at pH 7 (Kim *et al.*, 1989; Kim *et al.*, 1991).

Cleavage of the B-chain of Monellin at the residue adjacent to Met-42 with cyanogen bromide (CNBr) liberates an octapeptide (Lys-Lys-Thr-Ile-Tyr-Glu-Asn-Glu) with concomitant loss of sweetness (Bohak & Li, 1976; Frank & Zuber, 1976). The importance of one or more of the lysine residues in the initiation of sweetness by Monellin was demonstrated by loss of sweetness when they were fully methylated. However, there was no appreciable loss of sweetness (Morris *et al.*, 1978) when 20–40% of the ϵ-amino groups of the lysine residues were methylated. Also, some sweetness was

*For Part 5 see Suami *et al.* (1994).

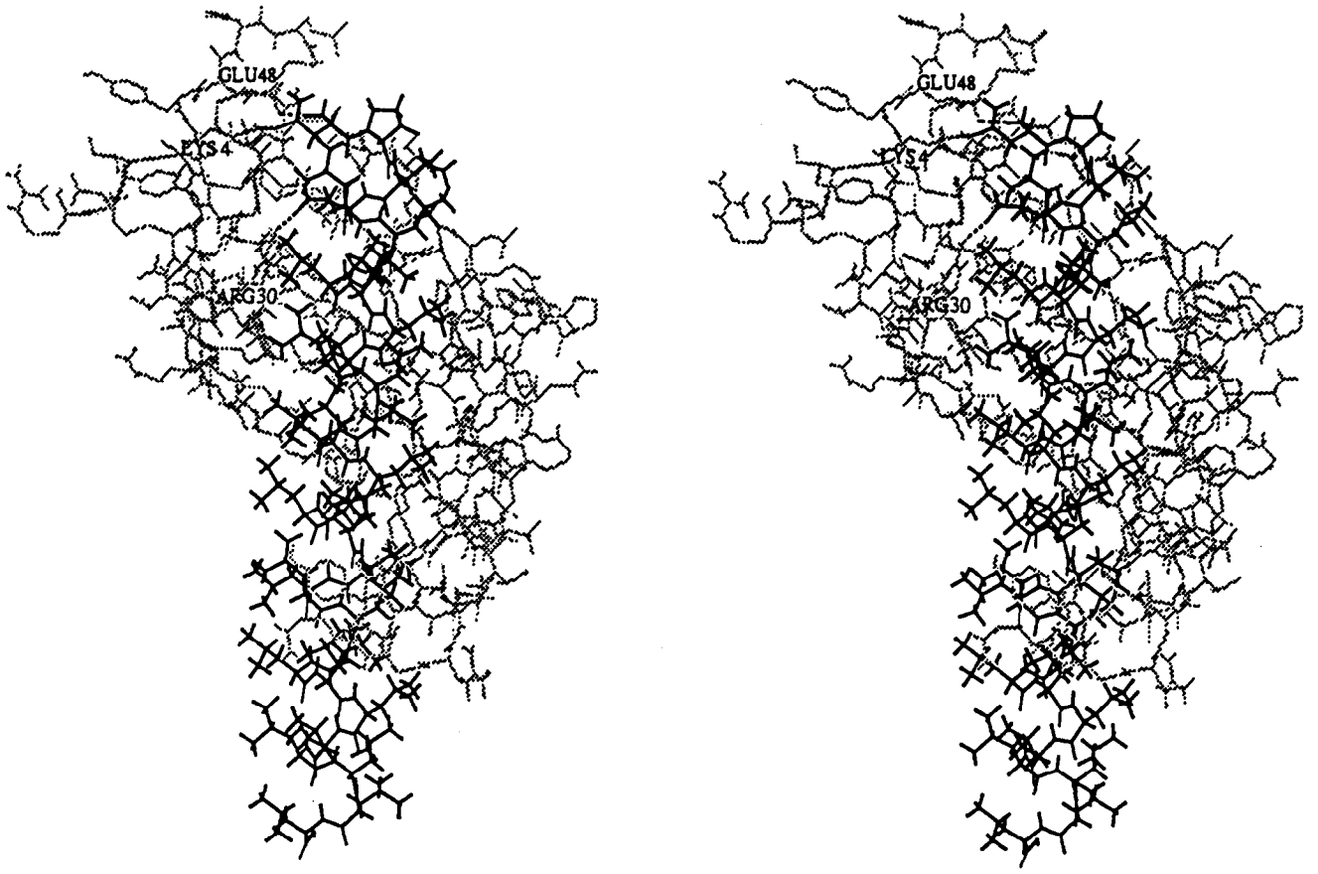

Fig. 1. Stereoviews of the Monellin–α-helical receptor complex, using Lys-4(A)/Glu-48(B) as the AH_s/B_s. The intermolecular H-bonds are shown with dotted lines and the receptor helix is highlighted.

retained after limited hydrolysis of Monellin with a carboxypeptidase (Higginbotham, 1979). The glutamyl *C*-terminus of the B-chain is hydrolysed by a carboxypeptidase whilst that of the A-chain, the prolyl residue, is resistant to this enzyme. Also, its sweetness is pH-dependent, Monellin being tasteless below approximately pH 2 and above pH 9. These factors stimulated us to investigate the molecular interactions between Monellin and our α-helical proteinaceous receptor model (Suami & Hough, 1991) by three dimensional computer techniques. This theoretical receptor has L-asparaginyl ($\mathbf{AH_r}$ and $\mathbf{B_r}$ sites) and L-prolyl residues at the *N*-terminus and adjacent sites, respectively, and L-leucyl residues arranged in a right-handed α-helix (Figs 1 and 2).

MATERIALS AND METHODS

The work was carried out on a Silicon Graphics workstation using the computer programs SYBYL 6.1a (TRIPOS Inc., St Louis, Missouri, USA). Initial coordinates for the Monellin molecule were generated from the Brookhaven protein data bank, and those 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 site, respectively, followed by L-leucyl residues, apart from an L-glutamyl residue at the fourth position, arranged in a right-handed α-helix. Interactive molecular modelling and structure refinements were performed using a Silicon Graphics workstation.

Models for subsequent structure refinements and energy minimizations were performed using the molecular mechanics program MAXIMIN 2 with Professor Kollman's all atom force field and charges (Weiner *et al.*, 1984) in the SYBYL 6.1a system. Initial models for the adducts were generated by docking the receptor model within the main cavity of the Monellin conformation with (1) 1:1 stoichiometry, (2) construction of a hydrogen bond between the NH_3^+ group of the *N*-terminal asparaginyl residue of the receptor and the γ-COO^- group of either Glu-48(B) or Glu-50(B) of the Monellin molecule, (3) construction of a hydrogen bond between the $CONH_2$ group of the *N*-terminal asparaginyl residue of the receptor and the ϵ-NH_3^+ group of Lys-4(A) of the Monellin molecule, and (4) formation of an additional, auxiliary hydrogen bond between the COO^- group of the fourth glutamyl residue of the receptor and the NH_2^+ group of Arg-30(A) of the Monellin molecule. 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 a side-chain of amino acid residues of the receptor model with that of the wall of the main cavity of the Monellin conformation and to remove unfavourable

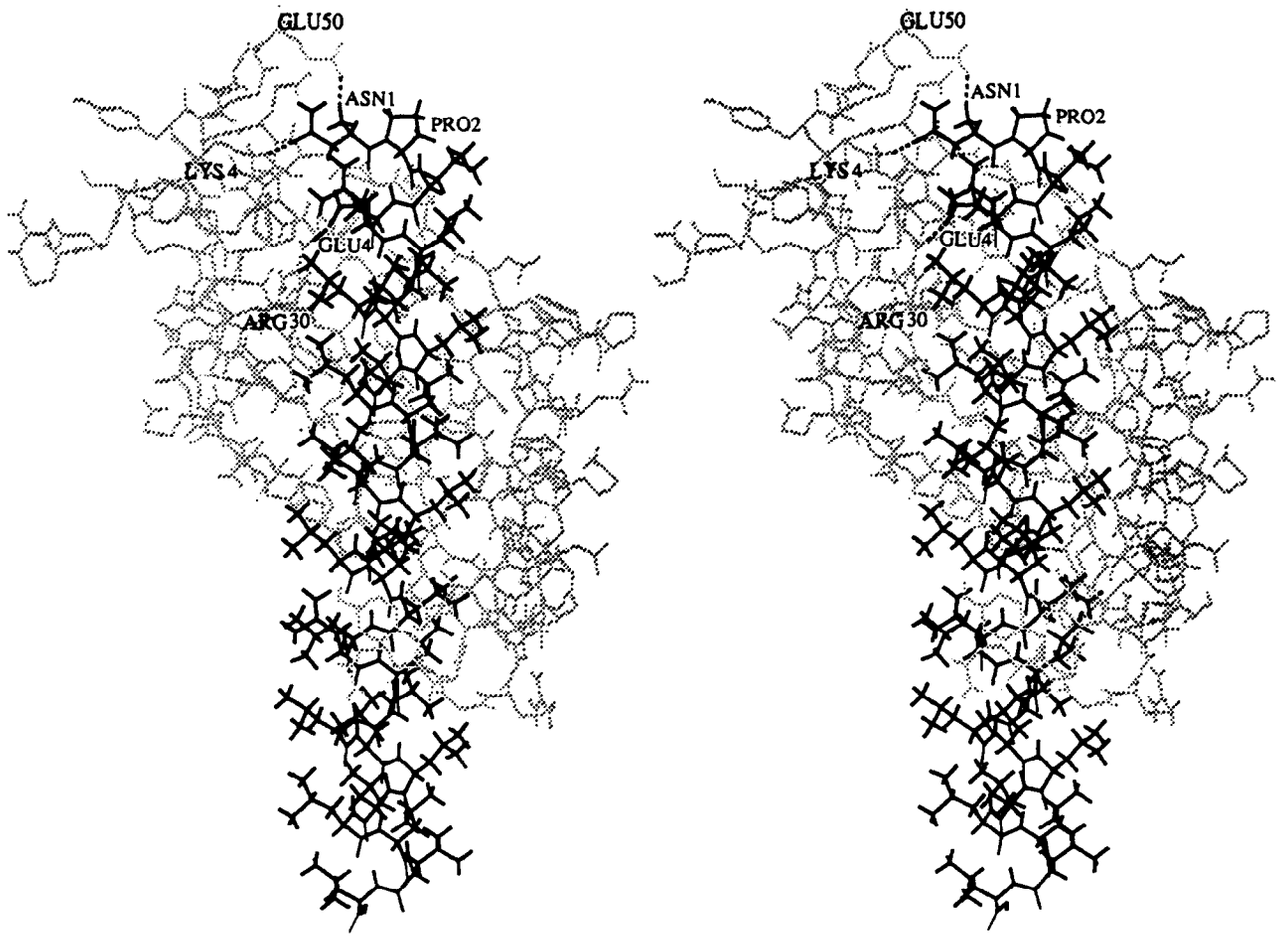

Fig. 2. Stereoviews of the Monellin–α-helical receptor complex, using Lys-4(A)/Glu-50(B) as the AH_s/B_s. The intermolecular H-bonds are shown with dotted lines and the receptor helix is highlighted.

atomic contacts. The stepwise protocol for the structure refinement used 300 steps of conjugate gradient minimization to remove initial bad contacts.

RESULTS AND DISCUSSION

The common molecular feature of all sweet organic compounds is a bifunctional entity composed of $\mathbf{AH_s}$ (a proton donor) and $\mathbf{B_s}$ (a proton acceptor) groups that are 2.5–4.0 Å apart (Shallenberger & Acree, 1967; Shallenberger, 1978) and which operate in conjunction with hydrophobic $\mathbf{X_s}$ components (Kier, 1972; Suami & Hough, 1992). There are 18 proton donating groups in the A-chain (*N*-terminal NH_3^+, Arg-1, Lys-4, Lys-17, Arg-20, Lys-28, Arg-30, Arg-32, Lys-33 and Arg-36) and in the B-chain (*N*-terminal NH_3^+, Lys-17, Lys-25, Arg-31, Lys-36, Arg-39, Lys-43 and Lys-44). This leads to 16 candidates for complementary primary proton accepting groups in the A-chain (Glu-2, Glu-7, Asp-16, Asp-22, Glu-25, Asp-26 and *C*-terminal COO^-) and in the B-chain (Glu-2, Glu-4, Asp-7, Asp-21, Glu-22, Glu-23, Glu-48, Glu-50 and *C*-terminal COO^-).

An $\mathbf{AH_s/B_s}$ pair in one and the same subunit (A- or B-chain), can be rejected by the foregoing non-sweet character of the separated subunits. The fused, single chain Monellin (SP1; Kim *et al.*, 1989) is similar in sweetness to the native bimolecular Monellin, suggesting that the *N*-terminal NH_3^+ group of the A-chain and the *C*-terminal COO^- group of the B-chain are not candidates. However, the loss of sweetness caused by the cyanogen bromide cleavage of the B-chain in native Monellin is consistent with either the $\mathbf{AH_s}$ or the $\mathbf{B_s}$ component residing in the liberated octapeptide (Lys-Lys-Thr-Ile-Tyr-Glu-Asn-Glu). The ϵ-NH_3^+ groups of Lys-43 (B-chain) and Lys-44(B) can function as proton donating components ($\mathbf{AH_s}$) and the γ-COO^- groups of Glu-48(B) and Glu-50(B) could act as proton accepting components ($\mathbf{B_s}$). As a result, there are 32 different combinations of potential $\mathbf{AH_s/B_s}$ pairs, and the distance between the two components of each pair in the native conformation of Monellin (Ogata *et al.*, 1987; Brookhaven protein data bank) was determined by computer calculations (Tables 1 and 2). Then, taking the restricted distance of 2.5–4.0 Å between the $\mathbf{AH_s}$ and $\mathbf{B_s}$ pair (Shallenberger & Acree, 1967) into consideration, the Lys-4(A)/Glu-48(B) and Lys-4(A)/Glu-50(B) pairs (distance of $\mathbf{AH_s}$–$\mathbf{B_s}$ = 3.27 and 2.38 Å, respectively) were the most obvious $\mathbf{AH_s/B_s}$ pairs for further investigation; furthermore each pair has the favourable clockwise configurations of the $\mathbf{AH_s/B_s/X_s}$ units (James *et al.*, 1989).

Table 1. Distance (Å) between the proton donor (AH_s candidate) and proton acceptor (B_s candidate) in Monellin

Proton acceptor	Proton donor	
	Lys-43(B)	Lys-44(B)
	AH_s–B_s[a]	AH_s–B_s[a]
Glu-2(A)	12.25	15.38
Glu-7(A)	12.74	5.99
Asp-16(A)	28.60	32.01
Asp-22(A)	15.41	9.63
Glu-25(A)	6.58	13.85
Asp-26(A)	14.02	14.35
C-terminal COO^-(A)	20.88	21.66

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

Table 2. Distance (Å) between the proton donor (AH_s candidate) and proton acceptor (B_s candidate) in Monellin

Proton donor	Proton acceptor	
	Glu-48(B)	Glu-50(B)
	AH_s–B_s[a]	AH_s–B_s[a]
Arg-1(A)	7.07	7.05
Lys-4(A)	3.27	2.38
Lys-17(A)	42.07	42.31
Arg-20(A)	26.38	26.50
Lys-28(A)	6.78	6.29
Arg-30(A)	18.84	19.55
Arg-32(A)	22.64	22.79
Lys-33(A)	20.47	18.86
Arg-36(A)	21.24	20.68

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

Molecular modelling by Corey–Pauling–Koltun precision molecular models (CPK molecular models) revealed that an intimate contact between the conformation of the Monellin molecule and the helical receptor model was obtained using the ϵ-NH_3^+ group of Lys-4(A) as the $\mathbf{AH_s}$ component and the γ-COO^- group of Glu-48(B) or that of Glu-50(B) as the $\mathbf{B_s}$ function. These results were confirmed in all respects, by the following computer modelling study.

Crystalline Monellin (Wlodawer & Hodgson, 1975) tastes intensely sweet, suggesting that the two chains of A and B do not dissociate on crystallization (Higginbotham, 1979). The conformation of crystalline Monellin has been established (Ogata *et al.*, 1987), and whilst that in a water solution is unknown, its compact structure (Ogata *et al.*, 1987; Kim *et al.*, 1989), and hence, its conformation, probably does not deviate significantly in water from that in the crystalline state. Therefore, we have used this conformation for our solution related studies.

Using the three-dimensional structure of Monellin available from the Brookhaven protein data bank, we constructed the two primary intermolecular hydrogen bonds, with the proper distances and correct bond angles, between the *N*-terminal asparaginyl residue of the receptor model (Suami & Hough, 1991) and the Monellin molecule, namely $\mathbf{AH_r}(NH_3^+)\cdots\mathbf{B_s}[\gamma\text{-}COO^-$ of Glu-48(B)] (1.84 Å and 163.6°, Taylor *et al.*, 1984; Jeffrey & Saenger, 1991) and $\mathbf{B_r}(CONH_2)\cdots\mathbf{AH_s}[-NH_3^+$ of Lys-4(A)] (1.89 Å and 160.0°, Taylor *et al.*, 1984; Jeffrey & Saenger, 1991). An additional, auxiliary intermolecular hydrogen bond was then introduced between the fourth glutamyl residue of the receptor (Suami *et al.*, 1994) and Monellin: $\mathbf{B_r^4}(COO^-)\cdots\mathbf{AH_s^4}[NH_2^+$ group of Arg-30(A)] (1.77 Å and 157.5°; Taylor *et al.*, 1984). The receptor model could then be accommodated comfortably in an optimum position within the cavity of the Monellin conformation for a variety of favourable van der Waals and hydrogen-bonding attractions to be observed, after energy minimizations were performed by the molecular mechanics program MAXIMIN 2 with Professor Kollman's all atom force field and charges (Weiner *et al.*, 1984) in the SYBYL system (Tripos Associates, Inc., St Louis, Missouri, USA, SYBYL 6.1a, 1995; Fig. 1)

Analogously, we constructed the two primary intermolecular hydrogen bonds: $\mathbf{AH_r}(NH_3^+)\cdots\mathbf{B_s}[\gamma\text{-}COO^-$ of Glu-50(B)] (1.83 Å and 169.3°) and $\mathbf{B_r}(CONH_2)\cdots\mathbf{AH_r}(\epsilon\text{-}NH_3^+$ of Lys-4(A)] (1.84 Å and 160.1°). An additional intermolecular hydrogen bond was introduced between the 4th glutamyl residue of the receptor and Arg-30(A) of Monellin: $\mathbf{B_r^4}(COO^-)\cdots\mathbf{AH_s^4}[NH_2^+$ of Arg-30(A)] (1.66 Å and 163.2°). While these distances and angles of the hydrogen bonds were slightly different from the correct values as described above, the deviations were within allowable limits (Jeffrey & Saenger, 1991). The refinement of the complex was performed analogously as described above, and once these three hydrogen bonds were in place, the receptor model was also accepted, without any hindrance, within the cavity of the Monellin conformation, when energy minimizations were carried out by the same system as described above (Fig. 2).

The main-chain structures of the Monellin molecule before and after (highlighted) the intercalation with the receptor model are shown in Figs 3 and 4. The major portions of each pair of skeletal structures were practically superimposable, except for the flexible portions of the structures: 1–4(A), 6–9, 26–34 and 47–50(B), on which the α-helix, 10–25(B), is hanging.

In each of these two sweetener–receptor combinations, the Monellin molecule was able to wind itself around the helical receptor, with the receptor intercalated within the wide cavity (or cleft) on the surface of the Monellin molecule. The receptor was surrounded by the five-stranded, anti-parallel β-sheet, made of two strands from the B-chain and three from the A-chain, and the B-chain α-helix, located in the gently twisted concave side of the β-sheet (Ogata *et al.*, 1987). In this conformational interaction, a number of hydrophobic amino acids, such as Ile-23(A), 6, 8(B), Phe-37(A), 11, 18, 34(B), Leu-10(A), 15, 32(B), Val-12(A), 20, 37(B), Ala-14, 21(A) and 19(B) resided within the wide cavity. Also, the cavity is lined with several hydrophilic amino acids, such as Lys-4(A), 43(B), Arg-30, 32(A), Glu-25(A), 2, 4, 23, 48(B), Ser-15(A), Thr-12, 45(B), Tyr-6,

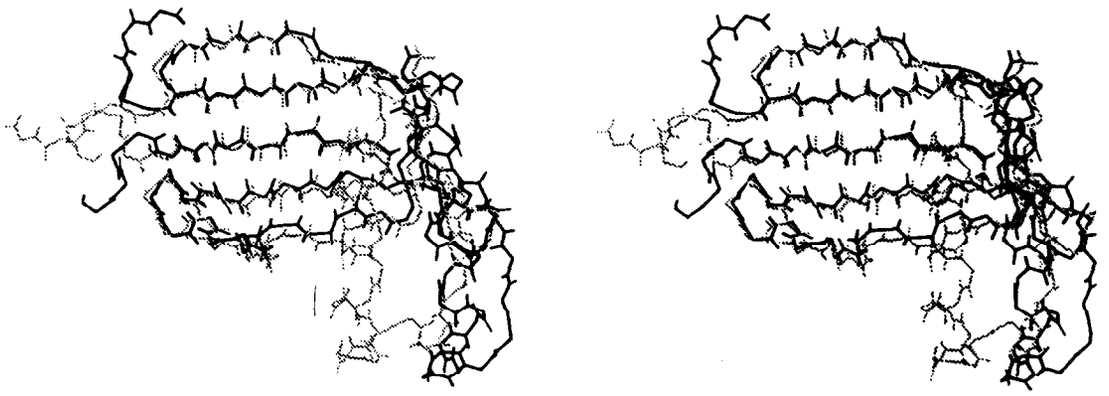

Fig. 3. Stereoviews of main-chain structures of Monellin before and after (highlighted) the intercalation with the receptor model, using Lys-4(A)/Glu-48(B) as the AH_s/B_s.

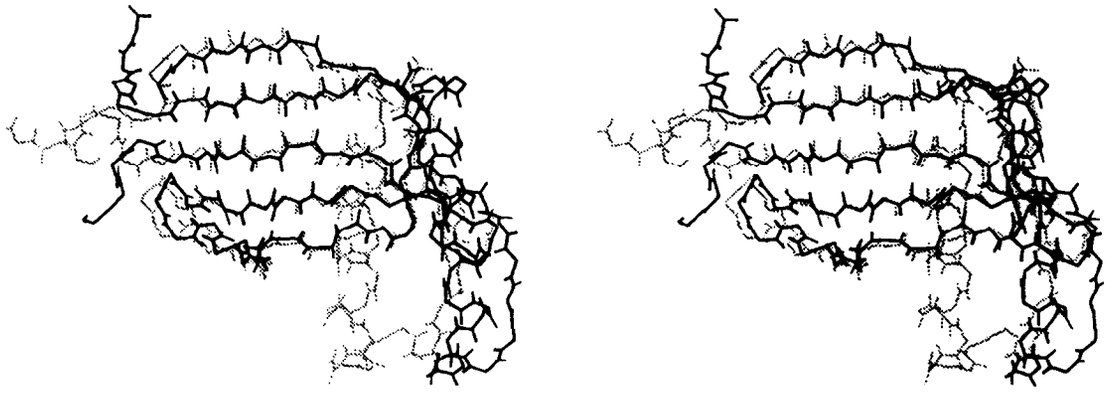

Fig. 4. Stereoviews of main-chain structures of Monellin before and after (highlighted) the intercalation with the receptor model, using Lys-4(A)/Glu-50(B) as the AH_s/B_s.

8(A) and 29(B). Outside this cavity, a considerable number of hydrophilic amino acids [Lys-17, 28, 33(A), 17, 25, 36, 44(B), Arg-1, 20, 36(A), 31, 39(B), Glu-2, 7(A), 22, 50(B), Gln-9(A), 13, 28(B), Asp-16, 22, 26(A), 7, 21(B), Asn-38(A), 14, 24, 35, 49(B), Pro-40, 42, 44(A), 10, 40(B), Ser-24(A), Thr-29(A), 33(B), Tyr-11, 13, 27(A) and 47(B)] were located on the periphery of this conformation and hence exposed to any surrounding water molecules. The excellent fit between the receptor and Monellin offers the opportunity for multiple hydrogen bonds and van der Waals interactions between the two extended proteins.

A van der Waals attraction between two hydrophobic amino acid side-chains requires that they should be separated by *ca.* 5 Å (Shallenberger, 1993). In our case, the receptor lodged in the hydrophobic cavity of Monellin accounted for a considerable number of such van der Waals forces with the hydrophobic amino acid side-chains of Monellin. Two hydrophilic amino acid side-chains are presumed to interact by hydrogen bonding, if they are within *ca.* 3 Å (Shallenberger, 1993) and several hydrophilic amino acid side-chains of Monellin were observed to have such contacts with the receptor protein model. At the present time, it is premature to comment in any detail on these contacts, apart from a few probabilities, since the primary structure of the receptor protein is unknown.

The proposed receptor model closely matched the topography of the binding sites of Monellin without any major conformational distortions of the constituents, and the structure of the Monellin–receptor complex conformed to the traditional shape of a lock-and-key model of binding, suggesting that it is close to the dimensions of the native receptor (Fig. 5). Therefore, the isolation, identification and characterization of the natural receptor are urgently awaited for further development of our proposed mechanism.

The contribution of the lysyl residues to the sweetness of Monellin was demonstrated by methylation studies (Morris *et al.*, 1978). Of the nine lysyl residues, four residues: Lys-33(A), 17, 25 and 36(B), are situated on the periphery of the conformation of the Monellin molecule, and selective methylation of these accessible units did not influence the tertiary structure of Monellin significantly. Hence the interaction with the receptor was not impeded, with no loss of sweetness. Further methylation, however, resulted in significant modification of the tertiary structure of Monellin, with consequential loss of sweetness.

The interactions described above provide an explanation of several previous observations on the structure–sweetness relationships noted for Monellin. (1) The intense sweetness and a prolonged period of sweet taste perception (Morris & Cagan, 1972) of Monellin can be

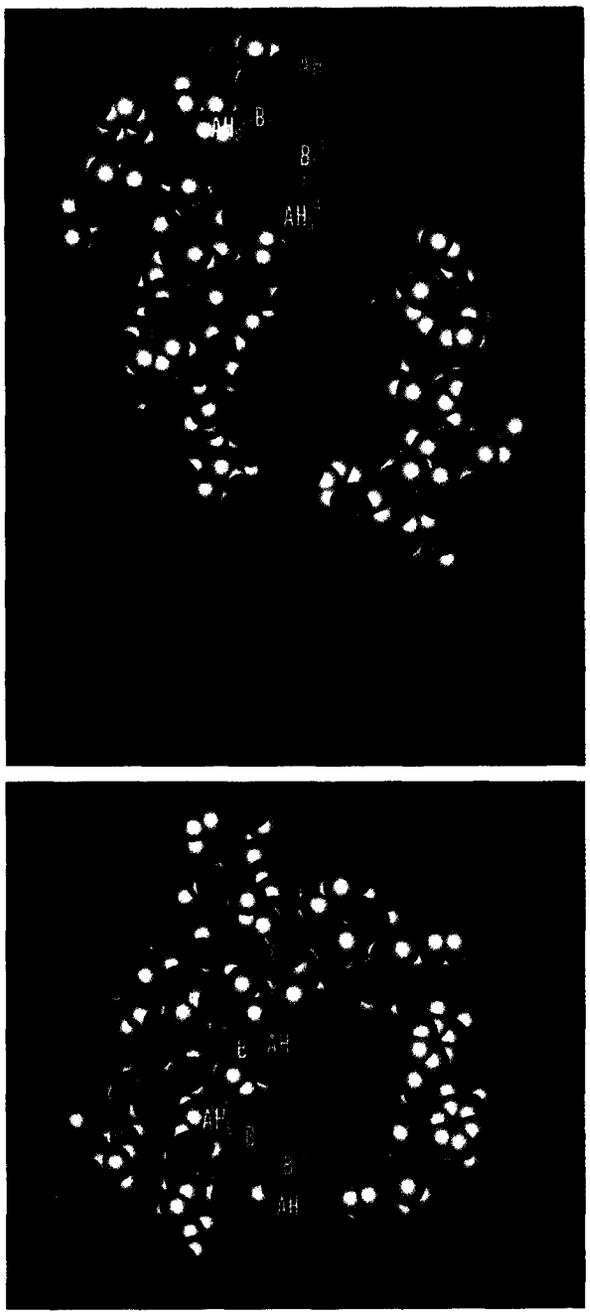

Fig. 5. Side- and top-views of the interactions between Monellin and α-helical receptor model bound in the cavity of Monellin molecule, using Lys-4(A)/Glu-48(B) as the $\mathbf{AH_s/B_s}$. The receptor protein (coloured green)–Monellin complex with the key intermolecular H-bonds (shown in dotted lines) is illustrated above. The $\mathbf{B_s}$ is behind, and within the cavity, as in the side view. The complex is viewed from the top of the receptor helix (bottom).

interpreted by the unique and intimate interactions between the peptides within the cavity of the stimulant molecule and those in the proposed receptor. Intensity of sweetness depends upon the strength of binding to the receptor, which is roughly correlated with the multiplicity of these interactions. (2) Limited methylation (20–40%) of the four accessible lysyl residues, Lys-33(A), 17, 25 and 36(B), resulted in only a slight conformational change, restricted to the periphery of the Monellin molecule, but insufficient to prevent the cleft interaction with the receptor; therefore its sweetness was maintained. (3) Cyanogen bromide cleavage of the B-chain in Monellin destroys the crucial $\mathbf{AH_s/B_s}$ pair by removing the $\mathbf{B_s}$ components (Glu-48 and Glu-50) with loss of sweetness. (4) Limited hydrolysis of Monellin by carboxypeptidase within Glu-50(B) and Asn-49(B) does not remove the $\mathbf{B_s}$ component in Glu-48(B) and it remains sweet. However, further hydrolysis of the B-chain, with removal of the Glu-48(B), results in loss of sweetness, owing to a lack of the $\mathbf{B_s}$ component. (5) Both A- and B-chains participate in the sweetness mechanism by providing the $\mathbf{AH_s}$ and $\mathbf{B_s}$ components, respectively, hence accounting for the observation that neither of the individual A- or B-chain is sweet. (6) The formation of a peptide bond between Arg-1(A) and Glu-50(B) provides the single-chain protein (SP1), which exhibits almost equal sweetness to natural Monellin, apparently because the unification of the A- and B-chains does not disturb the proposed Lys-4(A)/Glu-48(B) and Lys-4(A)/Glu-50(B) pair systems.

Hough & Edwardson (1978) and Van der Wel & Bel (1978) first found that antibodies raised against the sweet protein, Thaumatin, cross-react with Monellin. This observation suggests that some structural feature present in Thaumatin, and which interacts with the sweet receptor, is the major antigenic determinant. The occurrence of five identical tripeptides in Thaumatin and in Monellin has been described (Iyengar *et al.*, 1979). The present study on the sweetness of Monellin reveals that the following five homologous tripeptides of Monellin [**1**: Gln-28· Tyr-29· Gly-30(B), **2**: Ile-6· Asp-7· Ile-8(B), **3**: Asp-22· Ile-23· Ser-24(A), **4**: Thr-29· Arg-30· Gly-31(A) and **5**: Ala-21· Asp-22· Ile-23(A)] interact with the receptor model. That is, Ile-6, Ile-8, Tyr-29 of the B-chain and Ile-23, Arg-30 of the A-chain of Monellin are most likely to be involved in the interactions with the helical receptor and may well be related to the antibody interactions.

We are now investigating the host–guest relationship between the sweet protein Thaumatin and our receptor model, and our initial observations suggest a similar interaction to that found with Monellin, namely the receptor helix binding to a large cleft in the conformation of the sweet protein.

CONCLUSIONS

The $\mathbf{AH_s/B_s}$ pair of Monellin has been assigned to the ϵ-NH_3^+ of Lys-4(A)/ the γ-COO^- of Glu-48(B) with the Lys-4(A)/Glu-50(B) pair as an alternative. An additional hydrogen bond between the Arg-30(A) ($\mathbf{AH_s^4}$) of the Monellin molecule to the fourth glutamyl residue ($\mathbf{B_r^4}$) of the receptor helix is suggested. When these groups are hydrogen-bonded to the proposed receptor model, the two macromolecules dovetail together in a perfect fit, with the receptor helix lying within a cavity of the conformation of the Monellin molecule. The intense, lingering sweetness of Monellin is then accounted for by

the multiple interactions, both hydrophobic forces and intermolecular hydrogen bonds, that can arise from intimate association of the two proteins.

The present study reveals that the receptor protein occupies most of the space available in the main binding cavity of the sweet protein Monellin. It suggests that the host–guest interaction between the helical receptor protein and the cavity of the sweet protein is of the lock-and-key model of enzyme binding. This is a widely accepted concept that is stereospecific for an enzyme and its substrate, but in the case of Monellin the ligand (or substrate) is the receptor protein itself. Hence, the molecular recognition of the receptor protein for the macromolecular sweet protein is a reversal of the role of enzyme for its substrate.

ACKNOWLEDGEMENTS

The authors wish to express their thanks to Dr Kunisuke Izawa for his helpful suggestions, Miss Wakana Takaha and Mr Ryoichiro Nakamura for their invaluable technical assistance. They are grateful for partial support of this study by a grant from the Ajinomoto Co., Inc.

REFERENCES

Ariyoshi, Y., Kohmura, M., Hasegawa, Y., Ota, M. & Nio, N. (1991). Sweet Peptides and Proteins. In *Sweeteners, Discovery, Molecular Design, and Chemoreception*, eds D. E. Walters, F. T. Orthoefer & G. E. DuBois. ACS Symp. Ser., **450**, pp. 41–56.

Bohak, Z. & Li, S.-L. (1976). The structure of monellin and its relation to the sweetness of the protein. *Biochem. Biophys. Acta*, **427**, 153–70.

Cagan, R. H. (1973). Chemostimulatory protein: a new type of taste stimulus. *Science*, **181**, 32–35.

Cagan, R. H. & Morris, J. A. (1976). The sulfhydryl group of monellin: its chemical reactivity and importance to the sweet taste. *Proc. Soc. Exp. Biol. Med.*, **152**, 635–40.

Frank, G. & Zuber, H. (1976). The complete amino acid sequences of both subunits of the sweet protein monellin. *Hoppe-Seyler's Z. Physiol. Chem.*, **357**, 585–92.

Higginbotham, J. D. (1979). Protein sweeteners. In *Developments in Sweeteners-1*, eds C. A. M. Hough, K. J. Parker & A. J. Vlitos. Applied Science Publishers Ltd, London, UK, pp. 93–103.

Hough, C. A. M. & Edwardson, J. A. (1978). Antibodies to thaumatin as a model of the sweet taste receptor. *Nature*, **271**, 381–3.

Hudson, G. & Biemann, K. (1976). Mass spectrometric sequencing of proteins. The structure of subunit I of monellin. *Biochem. Biophys. Res. Commun.*, **71**, 212–20.

Inglett, G. E. & May, J. F. (1968). Tropical plants with unusual taste properties. *Econ. Bot.*, **22**, 326–31.

Inglett, G. E. & May, J. F. (1969). Serendipity berries—Source of a new intense sweetener. *J. Food Chem.*, **34**, 408–11.

Iyengar, R. B., Smits, P., van der Ouderaa, F., van der Wel, H., van Brouwershaven, J., Ravestein, P., Richters, G. & van Wassenaar, P. D. (1979). The complete amino-acid sequence of the sweet protein Thaumatin I. *Eur. J. Biochem.*, **96**, 193–204.

James, C. E., Hough, L. & Khan, R. (1989). Sucrose and its derivatives. In *Progress in the Chemistry of Natural Products*, eds W. Herz, H. Griesenbach, G. W. Kirby & Ch. Tamm. Springer-Verlag, Vienna, **55**, pp. 117–84.

Jeffrey, G. A. & Saenger, W. (1991). *Hydrogen Bonding in Biological Structures*. Springer-Verlag, Berlin, Heidelberg, New York, pp. 128–131.

Kier, L. B. (1972). A molecular theory of sweet taste. *J. Pharm. Sci.*, **61**, 1394–7.

Kim, S.-H., Kang, C. H., Kim, R., Cho, J. M., Lee, Y. B. & Lee, T. K. (1989). Redesigning a sweet protein: increased stability and renaturability. *Protein Engineering*, **2**, 571–5.

Kim, S.-H., Kang, C. H. & Cho, J. M. (1991). Sweet proteins, biochemical studies and genetic engineering. In *Sweeteners, Discovery, Molecular Design, and Chemoreception*, ACS Symp. Ser., **450**, pp. 28–40.

Morris, J. A. & Cagan, R. H. (1972). Purification of monellin, the sweet principle of *Dioscoreophyllum Cumminsii*. *Biochem. Biophys. Acta*, **261**, 114–22.

Morris, R. W., Cagan, R. H., Martenson, R. E. & Diebler, G. (1978). Methylation of the lysine residues of monellin. *Proc. Soc. Exp. Biol. Med.*, **157**, 194–9.

Ogata, C., Hatada, M., Tomlinson, G., Shin, W. C. & Kim, S.-H.. Crystal structure of the intensely sweet protein monellin. *Nature*, **328**, 739–42.

Shallenberger, R. S. & Acree, T. E. (1967). Molecular theory of sweet taste. *Nature*, **216**, 480.

Shallenberger, R. S. (1978). Intrinsic chemistry of fructose. *Pure Appl. Chem.*, **50**, 1409–20.

Shallenberger, R. S. (1993). *Taste Chemistry*. Blackie Academic & Professional, pp. 64–73.

Suami, T. & Hough, L. (1991). Molecular mechanisms of sweet taste. 1. Sweet and non-sweet tasting amino acids. *J. Carbohydr. Chem.*, **10**, 851–60.

Suami, T. & Hough, L. (1992). Molecular mechanisms of sweet taste. 2. Glucopyranose, fructopyranose and sucrose. *J. Carbohydr. Chem.*, **11**, 953-67.

Suami, T., Hough, L., Tsuboi, M., Machinami, T. & Watanabe, N. (1994). Molecular mechanisms of sweet taste. 5. Sucralose and its derivatives. *J. Carbohydr. Chem.*, **13**, 1079–92.

Taylor, R., Kennard, O. & Versichel, W. (1984). The geometry of the N-H···O=C hydrogen bond. 3. Hydrogen-bond distances and angles. *Acta Cryst.*, **B40**, 280–8.

Van der Wel, H. (1972). Isolation and characterization of the sweet principle from *Dioscoreophyllum cumminsii* (Stapf) Diels. *FEBS Lett.*, **22**, 88–90.

Van der Wel, H. & Bel, W. J. (1978). Structural investigation of the sweet-tasting proteins thaumatin and monellin by immunological studies. *Chem. Senses Flavour*, **3**, 99–104.

Weiner, S. J., Kollman, P. A., Case, D. A., Singh, U. C., Ghio, C., Alagona, G., Profeta, S. & Weiner, P. (1984). A new force field for molecular mechanical simulation of nucleic acids and proteins. *J. Am. Chem. Soc.*, **106**, 765–84.

Wlodawer, A. & Hodgson, K. O. (1975). Crystallization and crystal data of monellin. *Proc. Nat. Acad. Sci. USA*, **72**, 398–9.