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Molecular mechanisms of sweet taste 3: aspartame and its non-sweet isomers

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The coupling of the four diastereomers of aspartyl-phenylalanine methyl ester with the L-asparaginyl end of a helical receptor protein has been investigated by molecular modelling. The clockwise, three-point AH/B/X interaction, essential for a sweet taste, is found only in the L,L-isomer, Aspartame. None of the other isomers (L,D-, D,L- and D,D-) could form such a three-point attachment, due to their counterclockwise glucophore, consistent with their lack of sweetness.

INTRODUCTION

In continuation of our studies on sweet amino acids (Suami & Hough, 1991) and sugars (Suami & Hough, 1992), the interaction between the high intensity sweetener, Aspartame (L-aspartyl-L-phenylalanine methyl ester) and a proteinaceous receptor has been investigated by means of CPK molecular models. In order to validate the sweetness mechanism, the situation with the other diastereomers of Aspartame and the receptor model has been examined to explain why they are not sweet.

A tripartite glucophore consisting of AH_s (the subscript 's' denotes the unit of the sweet molecule, to distinguish it from that on the receptor 'r') (proton donor), B_s (proton acceptor) and X_s (lipophilic component), is recognized as an essential factor for all sweet tasting organic compounds (Shallenberger & Acree, 1967; Kier, 1972). Also, the existence of a counterpart $AH_r/B_r/X_r$ system is implicated on the protein receptor, and the sweet taste response arises from a formation of two intermolecular hydrogen bonds between the AH_s/B_r and the B_s/AH_r functions of the sweet compound and the receptor (Shallenberger & Acree, 1967), the intensity of sweetness being determined by the strength of the dispersion bond between the X_s of the stimulus molecule and the X_r of the receptor (Kier, 1972).

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It has been suggested that the receptor is an α-helical protein having L-serinyl, L-threonyl (Suami, 1987) or L-asparaginyl (Suami & Hough, 1991) as the *N*-terminal amino acid residue, where AH_r is the NH_3^+ group and B_r is either the OH or C=O group. On this model, X_r is located on the hydrophobic side chain of the fifth amino acid residue of the receptor, counting from the *N*-terminus. Based on this model, the interactions to elicit a sweet sensation have been interpreted in the two following stages: an initial attraction between the lipophilic sites X_s and X_r by dispersive forces (van der Waals attractive forces), which is accompanied by the formation of an intermolecular hydrogen bond between AH_r and B_s. The second step then involves the formation of the second intermolecular hydrogen bond between AH_s and B_r. The formation of the latter hydrogen bonds is accompanied by the scission of an existing intramolecular hydrogen bond in a recessed state of the receptor, which causes a conformational change in the helical receptor protein around its *N*-terminus. This change can be reversed with the desorption of the sweet compound from the receptor (Suami & Hough, 1991).

Since the receptor protein is assumed to be a right-handed helix, an orientation of the AH_s, B_s and X_s in, for example, the sweet D-amino acids, is a clockwise arrangement, when viewed from the receptor site. On the other hand, the mirror orientations of non-sweet L-amino acids are in the counterclockwise configuration (Suami & Hough, 1991). This generalization is true for sweet and non-sweet chiral compounds, including a wide variety of sucrose derivatives (James *et al.*, 1989).

RESULTS AND DISCUSSION

Based on the above theory of sweetness, we have examined by molecular modelling in conjunction with the Shallenberger–Acree AH/B hypothesis and Kier hydrophobic X site, the three dimensional interaction of the receptor protein and Aspartame (Mazur *et al.*, 1969), a commercial dipeptide ester sweetener (160 times sweeter than sucrose) (Cloninger & Baldwin, 1970) that is the most widely used of the low calorie sweeteners. The two chiral centres in Aspartame, give three other diastereomers and since none are sweet, we have investigated a stereochemical rationale of their behaviour.

Aspartame is a flexible molecule whose preferred conformation has been expressed as a combination of the two favourable conformations of the aspartyl and the phenylalanine methyl ester moieties. Lelj and his coworkers (Lelj *et al.*, 1976) investigated the most populated conformations of Aspartame in aqueous solution (over the pH range 3·5–11·7) by means of NMR spectroscopy and potential energy calculations. It was described (van der Heijden *et al.*, 1978) that the conformational isomer of Aspartame which gives the most favourable interaction with the receptor, is a combination of the two staggered rotational isomers of the aspartyl and the phenylalanine methyl ester residues, as shown in Fig. 1. The NH_3^+ and COO^- groups of the aspartyl residue comprise AH_s and B_s, respectively, whilst the hydrophobic function X_s is centred on the aromatic group of the phenylalanine ester unit. In this conformation (Fig. 1) the distances of AH_s—X_s and B_s—X_s are *c.* 5·2 and 7·2 Å, respectively, and that of AH_s—B_s is *c.* 3 Å.

Hatada and his coworkers (Hatada *et al.*, 1985) reported the conformation of Aspartame by X-ray crystal structure analysis and it correlated, apart from AH_s— X_s, to the conformation described by van der Heijden *et al.* (van der Heijden *et al.*, 1978), and the distances for AH_s—B_s, AH_s—X_s and B_s—X_s, as determined by computer analysis, are 3·0, 6·1 and 7·4 Å, respectively.

Molecular models of the conformation of Aspartame

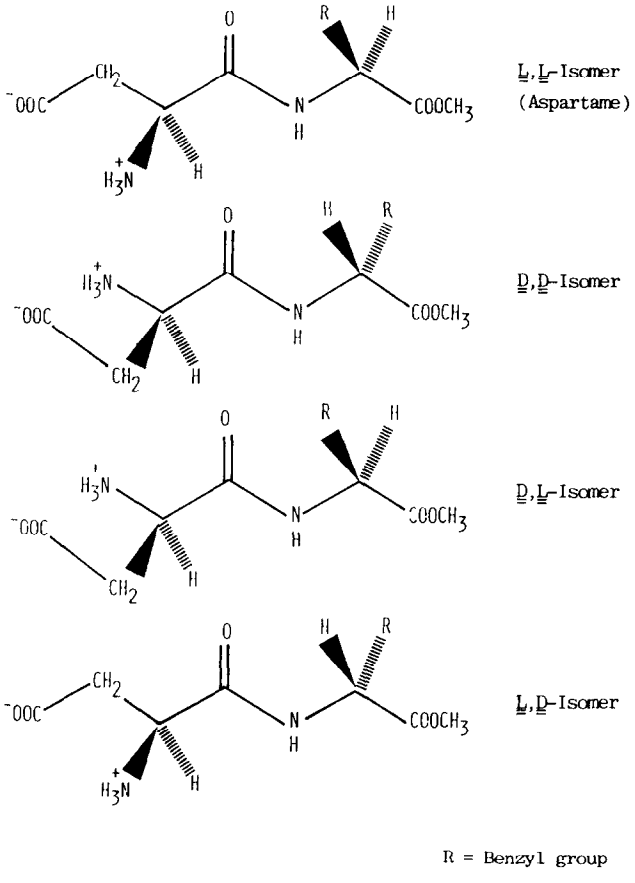

Fig. 1. Perspective representations of Aspartame and its diastereomers.

(Lelj *et al.*, 1976) and the helical receptor revealed that, when the COO group (B_s) is linked with the NH_3^+ group (AH_r) of the *N*-terminal asparaginyl residue, the phenyl group (X_s) of Aspartame is aligned alongside the hydrophobic side chain (X_r) of the fifth amino acid residue of the receptor helix. At this stage, the NH^{3+} group (AH_s) of Aspartame is correctly positioned to form a hydrogen bond with the C=O (CONH$_2$) group (B_r) of the *N*-terminal asparaginyl residue of the receptor (Fig. 2).

This three-point coupling between Aspartame and

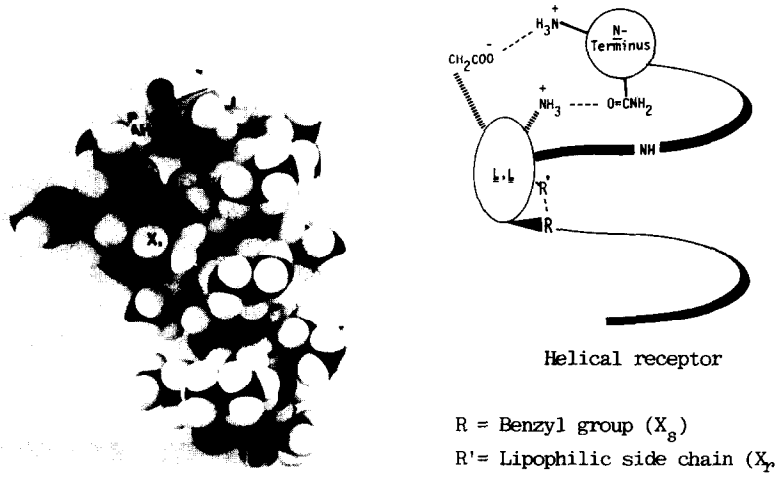

Fig. 2. Interaction between Aspartame and the receptor with CPK molecular models.

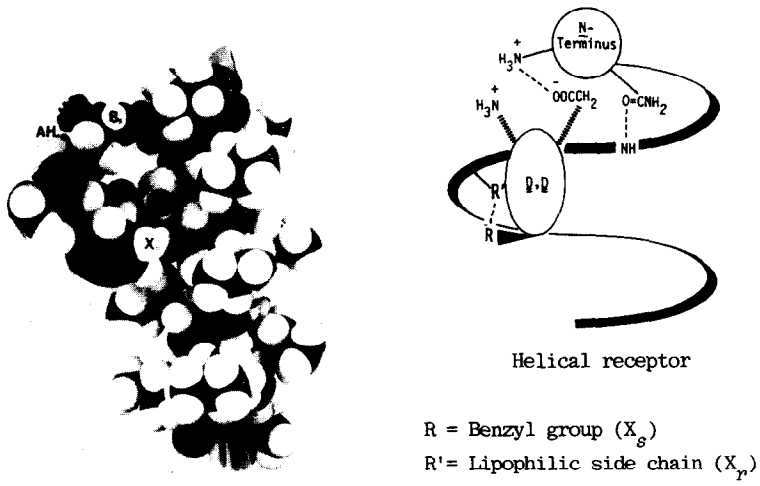

Fig. 3. Interaction between the D,D-isomer and the receptor with CPK molecular models.

the receptor requires the AH_s, B_s and X_s of Aspartame to be a clockwise arrangement, when viewed from the AH_r, B_r and X_r site. The distance between the AH_s and B_s is now approximately 3·0 Å and the X_s site is at distances of *c.* 5·4 and 7·4 Å from the AH_s and B_s, respectively. These values are coincident with those described by van der Heijden *et al.* (1978) for the favoured conformation of Aspartame. Thus, the molecular conformation that gives the best fit with the receptor model, is similar to that shown by X-ray crystallographic analysis. It should be noted that the phenyl ring is perpendicular to the peptide backbone and not coplanar with the zwitterionic ring of aspartic acid (Hatada *et al.*, 1985).

Of the four diastereomers (L,L; D,D; D,L and L,D) of the aspartyl–phenylalanine methyl ester, only the L,L-isomer, Aspartame, is sweet. In the case of D-aspartyl-D-phenylalanine methyl ester, the NH_3^+ group (AH_s) is unable for steric reasons to form the second hydrogen bond with the B_r function in the second stage of the interaction, after the COO^- group (B_s) and the lipophilic phenyl group (X_s) bind to the AH_r and X_r. Furthermore, the AH_s, B_s and X_s of the D,D-isomer are in a counterclockwise arrangement; thus, the required three-point coupling with the receptor is impossible and, therefore, it cannot initiate the sweet sensation on a stereochemical basis (Fig. 3).

Whilst the preferred conformation of the D,L-isomer has never been described, the absolute configuration of the two asymmetric carbon atoms are (R) and (S). Therefore, it is assumed from the preferred conformation of the (S)L,(S)L-isomer, Aspartame, that the (R)D,(S)L-isomer is closely related to the conformation shown in Fig. 1. Thus, the COO^- group (B_s) and the phenyl group (X_s) of this isomer can bind to the AH_r and X_r, but the NH_3^+ group (AH_s) cannot approach the intramolecularly hydrogen bound B_r component of the recessed state of the receptor (Fig. 4). Again, the AH_s, B_s and X_s groups are anticlockwise, thus accounting for the lack of sweetness in the D,L-isomer.

The L,D-isomer, the mirror image of the D,L-isomer, is not sweet but bitter. When the molecular model of the L,D-isomer was attached to the receptor model by two linkages between the B_s and AH_r, and between the X_s and X_r sites, the AH_s component was remote from the intramolecularly hydrogen bound B_r function and could not form an intermolecular hydrogen bond (Fig. 5). The AH_s, B_s and X_s order is ambiguous, but

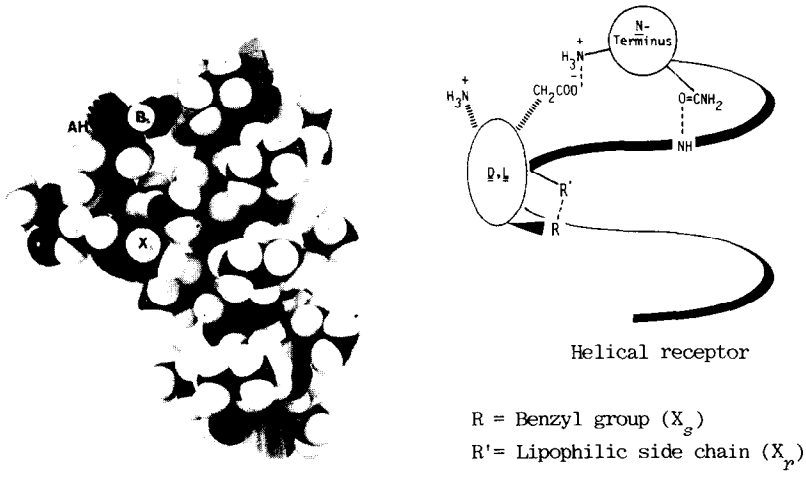

Fig. 4. Interaction between the D,L-isomer and the receptor with CPK molecular models.

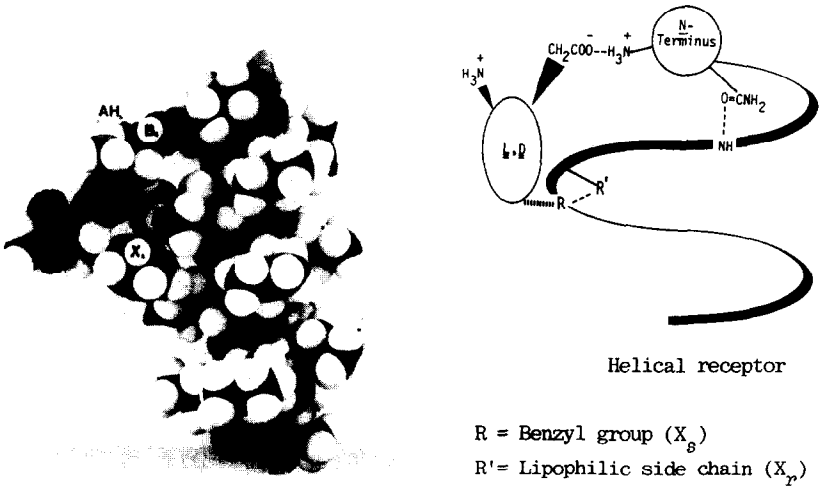

Fig. 5. Interaction between the L,D-isomer and the receptor with CPK molecular models.

the dimensions do not conform with those required by the Shallenberger–Kier theory for sweetness.

CONCLUSION

The appearance of a sweet taste in only the L,L-isomer of aspartyl–phenylalanine methyl ester is correlated with the preferred conformation of the molecule. The clockwise arrangement of the AH_s, B_s and X_s groups is recognized as an essential requirement for the perception of sweet taste by permitting the initial step of the stereospecific interaction between the stimulus molecule and the receptor. On the other hand, the counter-clockwise arrangements of the AH_s, B_s and X_s in the D,D-, D,L- and L,D-isomers do not permit the necessary three-point coupling with the receptor, and none are sweet.

ACKNOWLEDGEMENTS

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