as found for calcium pectate[14] (fig. 1, straight line 2). The $\gamma_{Ca^{2+}}$ values determined in solutions of calcium pectinates prepared by the action of pectinesterases of different origins are compared with those corresponding to pectinates with a random distribution of free carboxyl groups achieved by alkaline deesterification[14] (curve 1) and with the $\gamma_{Ca^{2+}}$ value assigned to block-wise arrangement (straight line 2).

The activity coefficients $\gamma_{Ca^{2+}}$ of calcium pectinates, prepared by the action of *Trichoderma reesei* pectinesterase (table; fig., curve 3) are close to those of pectin samples partially deesterified by tomato[3], orange[7] and alfalfa[3] pectinesterases (fig.). The results demonstrate the formation of blocks of free carboxyl groups in the linear macromolecule of pectin. Somewhat higher $\gamma_{Ca^{2+}}$ values, corresponding to pectin samples deesterified only slightly (d.e. 55–65%) by these enzymes are caused by formation of shorter blocks of free carboxyl groups, where $\gamma_{Ca^{2+}}$ is already a function of the block length. On the other hand, $\gamma_{Ca^{2+}}$ values corresponding to pectin samples deesterified by *Aspergillus foetidus*[3] and *A. niger*[7] pectinesterases are very close to curve 1 and represent therefore a random distribution of free carboxyl groups in the pectin molecule.

Pectinesterases of tomato, orange, alfalfa and *Trichoderma reesei* are basic proteins with isoelectric points in the range 8.3–11.0 and pH-optima also in alkaline region. On the contrary, pectinesterases of *Aspergillus* species, catalyzing a random deesterification of pectin in the same way as alkali, have isoelectric points and pH-optima in the acidic region. From these findings it is possible to conclude that the mode of deesterification of pectin by pectinesterases is independent of their origin (fungal or higher plant), but is determined by the character of the enzyme proteins reflected e.g. in isoelectric points and pH-optima.

1 Speiser, R., and Eddy, C.R., J. Am. chem. Soc. *68* (1946) 287.
2 Kohn, R., Furda, I., and Kopec, Z., Colln Czech. chem. Commun. *33* (1968) 264.
3 Kohn, R., Markovič, O., and Machová, E., Colln Czech. chem. Commun. *48* (1983) 790.
4 Solms, J., and Deuel, H., Helv. chim. Acta *38* (1955) 321.
5 Heri, W., Neukom, H., and Deuel, H., Helv. chim. Acta *44* (1961) 1945.
6 Dongowski, G., and Bock, W., Nahrung *25* (1981) (3) K5.
7 Kohn, R., Dongowski, G., and Bock, W., Nahrung, in press.
8 Ishii, S., Kiho, K., Sugiyama, S., and Sugimoto, H., J. Food Sci. *44* (1979) 611.
9 Baron, A., Rombouts, F., Drilleau, J.F., and Pilnik, W., Lebensm Wiss. Technol. *13* (1980) 330.
10 Markovič, O., Slezárik, A., and Labudová, I., prepared for publication.
11 Tibenský, V., Rosík, J., and Zitko, V., Nahrung *7* (1963) 321.
12 Kohn, R., and Tibenský, V., Chem. Zvesti *19* (1965) 98.
13 Kohn, R., and Furda, I., Colln Czech. chem. Commun. *32* (1967) 1925.
14 Kohn, R., and Luknár, O., Colln Czech. chem. Commun. *40* (1975) 959.

0014-4754/84/080842-02$1.50 + 0.20/0

Peptide interactions with taste receptors: Overlap in taste receptor specificity

R.H. Cagan[1]

Veterans Administration Medical Center and Monell Chemical Senses Center and University of Pennsylvania, Philadelphia (Pennsylvania 19104, USA), 21 September 1983

Summary. Two hitherto unrelated areas of taste appear to provide an important insight into a class of taste receptor sites. A region of the sweet protein monellin is similar to a savory tasting beef octapeptide, and this peptide region could represent an overlap in the specificity of binding to a common peptide taste receptor site. Such an overlap in binding specificity explains the low but significant level of sweetness observed with savory tasting stimuli.

The biochemical basis of taste receptor specificity appears to depend upon recognition of stimuli in a weak binding interaction with receptors located in the cell plasma membrane[2]. An experimental model emerged from the discovery a decade ago[3,4] of the sweet-tasting protein monellin, which consists of 2 dissimilar polypeptide subunits of known sequence[5,6]. Native monellin tastes intensely sweet, being around 10^5 times more effective in eliciting a sweet taste (at threshold levels) than the common sugars[7]. The structural features of monellin required for sweetness are not elucidated in detail, but the evidence[8–12] demonstrates the importance of the tertiary and quaternary structures, and supports the hypothesis[7] that its conformation is critical in its ability to evoke a sweet sensation.

The specific regions of the protein that bind to taste receptor sites are not known. Partial methylation of the lysines of monellin does not markedly affect its taste, while extensive methylation leads to loss of sweetness[13]. The necessity for the sulfhydryl group of the single cysteine to remain in the reduced form for the protein to retain its sweetness was demonstrated[5,14], although the cysteine is buried in the interior and is relatively inaccessible to reaction. We demonstrated that ^{3}H-labeled methylated monellin[13] binds to taste tissue preparations from bovine and human circumvallate papillae[15].

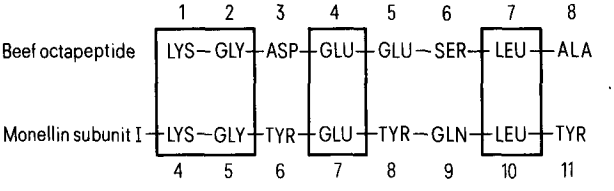
A seemingly unrelated area is that of monosodium L-glutamate (MSG), the taste of which is called *umami* in Japanese[16,17], meaning 'delicious' or 'savory' taste[18]. Possible mechanisms of action of L-glutamate in eliciting this unique taste sensation were discussed in an earlier review[19], which stressed that the well known taste synergism of certain 5′-ribonucleotides in combination with MSG[17,20,21] could be involved in the mechanistic basis for the unique 'flavor enhancing' action of MSG. We demonstrated direct binding of L-[^{3}H]glutamate to bovine taste tissue preparations and enhancement of this binding by certain 5′-ribonucleotides[22]. Yamasaki and Maekawa[23,24] have isolated an octapeptide from beef gravy which elicits a 'delicious' or 'savory' taste. They established its amino acid sequence[23] and synthesized the octapeptide[24].

In this communication an interesting structural similarity is pointed out between the octapeptide of beef gravy and a portion of the primary structure of the sweet protein monellin. The octapeptide shows a similarity to the amino acid region 4–10 of subunit I of monellin (fig.). This region of monellin has amino acids 4, 5, 7 and 10 identical with those of the octapeptide. It is postulated that this similarity results in the octapeptide being able to interact at monellin binding sites on the taste receptor cells. This interaction could explain the low level of sweetness of the octapeptide. The hypothesis necessitates that the region of monellin shown in the figure contain at least 1 moiety that binds to the receptors. The presence of a common receptor site does not, however, imply exclusivity for these 2 ligands; this site need not be the only receptor site to which these stimulus compounds can bind. Rather, it is postulated that a site type exists to which both of these peptides can bind and therefore overlap in the taste quality of sweetness; the converse hypothesis, that monellin is able to bind to other octapeptide (glutamate) binding sites is not postulated, because a savory quality of monellin has not been observed.

Synthesis of the octapeptide and of related smaller peptides was reported by Yamasaki and Maekawa[24]. They carried out sensory evaluations of these peptides, not only for savory taste, but also for sweet, sour, bitter and astringent tastes. The findings are extremely interesting. Peptides lacking GLU showed no savory taste, which is not surprising in view of the specific taste quality of L-glutamate itself[16–20]. An unexpected result was the concomitant appearance of a low level of sweetness along with the more pronounced savory taste. The hexapeptide (amino acids 3–8) lacking the N-terminal LYS-GLY tasted mainly sour, but showed low levels of both savory (10%) and sweet (5%) tastes. The octapeptide itself was principally savory (60%), but also had sour (30%) and sweet tastes (10%). The pentapeptide lacking ASP-GLU-GLU was bitter and astringent, with neither savory nor sweet qualities. In an earlier study of taste responses to various amino acids using multidimensional scaling, Yoshida and Saito found[16] that the major portion of the taste of MSG was accounted for by *umami* (71%), but that low levels of salty (13%) and sweet (10%) were present. The data taken together therefore support the hypothesis of a relation between sweet and savory (glutamate) taste reception. Further, they point to an essential role of GLU in position 4 of the octapeptide and position 7 of monellin.

Electrophysiological recordings from the chorda tympani nerve of the rat in response to stimulation of the tongue with L-glutamate and other compounds were reported several years ago. From these recordings[25,26], a correlation was shown between responsiveness of single nerve fibers to sucrose and to mixtures of MSG + 5′-ribonucleotides. Fibers sensitive to NaCl tended to be sensitive to MSG alone, perhaps reflecting stimulation by Na^+ in both cases. Fibers sensitive to sucrose, however, tended to be sensitive to the MSG-ribonucleotide mixture; this suggests a relationship between stimulation by sucrose and by glutamate-nucleotide in the rat. These findings could also support the hypothesis proposed here of an overlap in binding specificity.

It is of further interest that the region noted (fig.) is not present in the primary structure of thaumatin[27], a different sweet-tasting protein. Certain other dipeptide and tripeptide regions of monellin and thaumatin were noted[27] to be the same. A very interesting finding by Hough and Edwardson[28] was that antibody prepared to thaumatin cross-reacts with monellin and with several other sweet compounds. In an independent study the cross-reactivity of anti-thaumatin antibody with monellin was confirmed[29]. The hypothesis proposed here would suggest that an anti-monellin antibody could cross-react with the octapeptide.



Structurally similar regions of monellin and beef octapeptide. The structure of monellin is from Bohak and Li[5] and Hudson and Biemann[6]; that for the octapeptide was determined by Yamasaki and Maekawa[23,24]

1 The work from this laboratory was supported by NIH Research Grant No. NS-15740 from NINCDS and by the Veterans Administration.

2 Cagan, R.H., in: Biochemistry of Taste and Olfaction, p. 175. Eds R.H. Cagan and M.R. Kare. Academic Press, New York 1981.
3 Morris, J.A., and Cagan, R.H., Biochim. biophys. Acta *261* (1972) 114.
4 Morris, J.A., Martenson, R., Deibler, G., and Cagan, R.H., J. biol. Chem. *248* (1973) 534.
5 Bohak, Z., and Li, S.-L., Biochim. biophys. Acta *427* (1976) 153.
6 Hudson, G., and Biemann, K., Biochem. biophys. Res. Commun. *71* (1976) 212.
7 Cagan, R.H., Science *181* (1973) 32.
8 Morris, J.A., and Cagan, R.H., Proc. Soc. exp. Biol. Med. *150* (1975) 265.
9 Jirgensons, B., Biochim. biophys. Acta *446* (1976) 255.
10 Van der Wel, H., and Loeve, K., FEBS Lett. *29* (1973) 181.
11 Brand, J.G., and Cagan, R.H., Biochim. biophys. Acta *493* (1977) 178.
12 Cagan, R.H., Brand, J.G., Morris, J.A., and Morris, R.W., in: Sweeteners and Dental Caries, p. 311. Eds J.H. Shaw and G.G. Roussos. Information Retrieval, Inc., Washington, D.C. 1978.
13 Morris, R.W., Cagan, R.H., Martenson, R.E., and Deibler, G., Proc. Soc. exp. Biol. Med. *157* (1978) 194.
14 Cagan, R.H., and Morris, J.A., Proc. Soc. exp. Biol. Med. *152* (1976) 635.
15 Cagan, R.H., and Morris, R.W., Proc. natl Acad. Sci. USA *76* (1979) 1692.
16 Yoshida, M., and Saito, S., Jap. psychol. Res. *11* (1969) 149.
17 Yamaguchi, S., Yoshikawa, T., Ikeda, S., and Ninomiya, T., J. Food Sci. *36* (1971) 846.
18 Komata, Y., in: Microbial Production of Nucleic Acid-Related Substances, p. 299. Eds K. Ogata, S. Kinoshita, T. Tsunoda and K. Aida. John Wiley and Sons, New York 1976.
19 Cagan, R.H., in: The Chemical Senses and Nutrition, p. 343. Eds M.R. Kare and O. Maller. Academic Press, New York 1977.
20 Yamaguchi, S., J. Food Sci. *32* (1967) 473.
21 Rifkin, B., and Bartoshuk, L.M., Physiol. Behav. *24* (1980) 1169.
22 Torii, K., and Cagan, R.H., Biochim. biophys. Acta *627* (1980) 313.
23 Yamasaki, Y., and Maekawa, K., Agric. biol. Chem. *42* (1978) 1761.
24 Yamasaki, Y., and Maekawa, K., Agric. biol. Chem. *44* (1980) 93.
25 Sato, M., Yamashita, S., and Ogawa, H., Jap. J. Physiol. *20* (1970) 444.
26 Yamashita, S., Ogawa, H., and Sato, M., Jap. J. Physiol. *23* (1973) 59.
27 Iyengar, R.B., Smits, P., Van der Ouderaa, F., Van der Wel, H., Van Brouwershaven, J., Ravenstein, P., Richters, G., and Van Wassenaar, P.D., Eur. J. Biochem. *96* (1979) 193.
28 Hough, C.A.M., and Edwardson, J.A., Nature, Lond. *271* (1978) 381.
29 Van der Wel, H., and Bel, W.J., Chem. Senses Flav. *3* (1978) 99.

0014-4754/84/080843-02$1.50 + 0.20/0