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Taste Enhancements Between Various Amino Acids and IMP

Misako Kawai, Atsushi Okiyama and Yoichi Ueda

Food Research and Development Laboratories, Ajinomoto Co., Inc., 1-1 Suzuki-cho, Kawasaki-ku, Kawasaki 210-8681, Japan

Correspondence to be sent to: Misako Kawai, Institute of Life Sciences, Ajinomoto Co., Inc., 1-1 Suzuki-cho, Kawasaki-ku, Kawasaki 210-8681, Japan. e-mail: misako_kawai@ajinomoto.com

Abstract

It is well known that a strong synergistic interaction of umami occurs between L-α-amino acids with an acidic side chain, such as L-Glu or L-Asp, and 5′-mononucleotides, such as inosine 5′-monophosphate (IMP). We tested taste interactions between various L-α-amino acids and IMP by the psychophysical method and found that taste enhancement occurred when IMP was added to several sweet amino acids, such as L-Ala, L-Ser and Gly. The enhanced quality of taste was recognized as umami, and was not blocked by the sweetness inhibitor ±2-(*p*-methoxyphenoxy)propanoic acid. The total taste intensities of various concentrations of the amino acid and IMP mixtures were measured using magnitude estimation. The results showed that the potentiation ratios were larger than 1 in the cases of L-Ala, L-Ser and Gly. However, the ratio was ~1 in the case of D-Ala, which had an enhanced taste of sweetness. Thus the umami taste enhancement of several sweet L-α-amino acids by IMP was synergistic rather than additive as that of acidic amino acids.

Introduction

Each amino acid contributes, to differing degrees, to the taste of foods. Although individual amino acids do not have a strong taste in themselves, their taste is intensified in the presence of other compounds, such as nucleotides (Kuninaka, 1960) or inorganic salts (Fuke and Konosu, 1991; Ugawa *et al*., 1992). Among various taste interactions, the most prominent phenomenon is a large umami enhancement by mixing of the monosodium salt of L-Glu (MSG) and inosine 5′-monophosphate (IMP) or guanosine 5′-monophosphate (GMP), which has been well investigated. This quality of taste sensation, umami, is the characteristic taste of MSG, IMP or GMP and their mixtures, and constitutes one of the five basic tastes, along with sweetness, saltiness, sourness and bitterness. Yamaguchi measured umami interaction between MSG and IMP using the MSG point of subjective equality method and demonstrated that the enhancement occurs synergistically (Yamaguchi, 1967). Rifkin and Bartoshuk showed that MSG–GMP interaction is a synergistic umami enhancement by the method of magnitude estimation (Rifkin and Bartoshuk, 1980). Only acidic analogues of L-Glu, such as L-Asp, L-homocysteinic acid and ibotenic acid, have been reported to show synergistic umami enhancement by the addition of purine 5′-mononucleotides (Yamaguchi *et al*., 1971; Furukawa, 1991; Kurihara, 1998). Some kinds of acidic peptides, such as L-Glu-Gly-L-Ser, which express weak umami by themselves, have been shown to express enhanced umami by the addition of IMP (Arai *et al*., 1973; Maehashi *et al*., 1999). Furukawa concluded that among protein amino acids, only L-Glu and L-Asp show umami enhancement by the addition of IMP (Furukawa, 1991). However, this result was obtained at a very low concentration, and a relatively small enhancement might have been missed. In addition, several L-α-amino acids with neutral side chains elicit umami at high concentrations (Ninomiya *et al*., 1966; Yoshida and Saito, 1969), therefore they show potential umami enhancement by adding a 5′-mononucleotide.

In this human psychophysical study, taste interactions between various amino acids and IMP were re-examined at various concentrations to investigate whether other non-acidic amino acids have umami synergistic potential.

Experiments and results

General procedure

Twenty- to 40-year-old male or female researchers from the Food Research and Development Laboratories of Ajinomoto Co., Inc., Japan, participated as subjects. All psychophysical tests followed the regulations for sensory evaluation of the Ajinomoto Co., Inc.

Stimulus compounds were as follows. Amino acids and succinic acid of guaranteed reagent grade were from Nacalai Tesque Inc. (Kyoto, Japan); peptides were from Sigma-Aldrich (Tokyo, Japan); food-additive grade IMP·2Na (IMP) was from Ajinomoto Co., Inc. (Tokyo, Japan). In Experiment 3, the sweetness inhibitor ±2-(*p*-methoxy-

phenoxy)propanoic acid (PMP) (Schiffman *et al*., 1999) was purchased from Sigma-Aldrich. Deionized water purified using a Milli-Q water purification system (Millipore, Bedford, MA) was used both for stimuli and as mouth rinse.

Stimulus solutions were poured into plastic cups and served at room temperature. Subjects were asked to rinse their mouths thoroughly, then sip the stimulus solution, swirl it around in the mouth for several seconds to taste it, and spit it out. In Experiments 2 and 3, subjects were asked to sip the entire 10 ml aliquot in the cup at once.

Experiment 1

This experiment was conducted to examine how the taste quality changed when IMP at near-threshold concentration was added to each amino acid solution.

Test procedure

In the first test, each amino acid solution was adjusted to a concentration that had an almost 'moderate' total-taste intensity to allow the perception of taste quality (Table 1). These concentrations showed agreement with previous measurements of taste intensity of amino acid solutions by a NaCl standard scale (Ninomiya *et al*., 1966), and with those of taste intensity of NaCl solutions by labelled magnitude scale (Kawai and Okiyama, 1998). Several non-amino acid compounds were also tested under the following concentrations: succinic acid, 2 mM; peptides: Gly–Gly, 60.6 mM and L-Glu–L-Glu, 3.62 mM. Pairs of stimulus solutions with and without 0.5 mM IMP were presented randomly to 10 subjects. The subjects were asked to taste at will in volume of sipping and way of repetition and to describe the difference in intensity between solutions with and without IMP for each quality of taste (sweetness, saltiness, sourness, bitterness, umami, and other tastes) and for total taste using the symbols >, ≥ or =. Based on these responses, numerical values were applied to the solutions with IMP as follows: 'with IMP > without IMP': +2; 'with ≥ without': +1, 'with = without': 0; 'with ≤ without': –1, and 'with < without': –2. Values for the solution without IMP were assumed to be zero. The average value for each taste quality was calculated and a Wilcoxon's signed rank test was performed.

We then recruited 50 subjects for the second sensory test to identify the enhanced quality of taste by adding IMP to each amino acid solution of Gly, L-Ser, L-Ala and D-Ala. Pairs of 50 mM solutions of each amino acid with and without 0.5 mM IMP were presented to subjects. The pair of water and 0.5 mM IMP solution was also presented. The order of presentation of these pairs was randomized and the order within pairs was counter-balanced. The subjects were asked to taste at will as in the previous evaluations and to select the stronger one, and to choose the most different quality of taste in intensity between the two among sweetness, saltiness, sourness, bitterness, umami, and other tastes.

Results

The results from the first test of 26 compounds are presented in Table 1. The taste of many amino acids, except for bitter amino acids with aliphatic side chains, was modified by adding IMP at 0.5 mM, which was its approximate threshold concentration. Several sweet amino acids that do not have acidic side chains showed significant umami enhancement. The dipeptide, L-Glu–L-Glu, also showed umami enhancement.

The results from the latter test of Gly, L-Ser, L-Ala, D-Ala, and water are summarized in Table 2. In the cases of Gly, L-Ser, L-Ala and D-Ala, solutions containing IMP were chosen as the stronger-tasting solutions with significantly greater frequency, while in the case of water the difference in choice was not significant. In the cases of Gly, L-Ser and L-Ala, umami was perceived as the most enhanced quality of taste by addition of IMP. In the case of D-Ala, on the other hand, sweetness was perceived as the most enhanced quality of taste.

Experiment 2

The taste intensities of solution pairs for which the solution with IMP showed significant taste enhancement (Experiment 1) were further examined by modulus-free magnitude estimation.

Test procedure

Eight subjects who had previously participated in magnitude estimation sessions participated in two sessions for each stimulus set. The stimulus set consisted of 19 concentrations of solutions of IMP (0, 0.5, 1.0 and 2.0 mM) and the amino acids Gly, L-Ser, L-Ala or D-Ala (0, 25, 50, 100, 200 mM) except for pure water. Gly, L-Ser and L-Ala were shown to enhance umami, and D-Ala, which is an enantiomer of L-Ala, was shown to enhance sweetness in Experiment 1. Ten millilitre samples of the 19 solutions were served in random order, with the exception that a solution of approximately middle taste intensity was presented as the first sample so that the subjects could make the subsequent ratings easily. Subjects were asked to rate the total-taste intensity of the first sample using any number and to give single magnitude estimates in the form of a ratio to the taste previously sampled. For each of the ratings, subjects were instructed to rinse their mouths more than twice to eliminate the effect of IMP, and sip all of the aliquot in a cup at once to rate total-taste intensity sensed during sample solution in their mouths. Collected data were taken as 16 independent estimates.

Data analysis

Prior to analysis, the stimulus was assigned a value of zero in a session, and then this value was replaced with a value

Table 1 Summary of the changes in taste quality of amino acid solutions by the addition of IMP

Amino acid	Conc. (mM)	Sweetness		Saltiness		Sourness		Bitterness		Umami		Other taste		Total taste	
		Ave.	Wilc.	Ave.	Wilc.	Ave.	Wilc.	Ave.	Wilc.	Ave.	Wilc.	Ave.	Wilc.	Ave.	Wilc.
L-Ala	250.0	1.33*	0.010	0.22	0.180	–0.33	0.317	0.00	0.317	1.33**	0.006	0.00	0.317	1.44**	0.006
L-Cys	75.0	1.00*	0.014	0.00	1.000	–1.26	0.206	–0.33	0.380	0.78*	0.038	0.89*	0.038	0.89	0.107
L-Asp	3.0	0.44	0.157	0.11	0.655	–1.00	0.086	–0.22	0.317	1.89**	0.004	–0.11	0.655	–0.22	0.708
D-Ala	250.0	1.33**	0.009	0.00	0.317	–0.56	0.408	0.11	0.408	0.33	0.102	0.00	0.317	1.00*	0.013
L-Glu	1.0	0.00	1.000	0.78	0.059	–1.56*	0.020	–0.11	0.317	1.56*	0.020	–0.11	0.655	0.44	0.420
L-Glu-L-Glu	3.6	–0.11	0.317	0.00	1.000	–0.56	0.386	0.11	0.655	0.89*	0.038	0.22	0.157	–0.56	0.386
L-Phe	25.0	0.00	1.000	0.00	1.000	0.00	1.000	–0.67	0.107	0.11	0.655	0.00	0.317	–0.67	0.084
Gly	250.0	0.11	0.633	0.11	0.180	–0.33	0.888	–0.22	1.000	1.44**	0.007	–0.22	0.564	1.22*	0.029
Gly-Gly	60.6	0.22	0.317	0.00	1.000	–0.78	0.053	–0.33	0.257	–0.11	0.655	0.11	0.317	–1.00*	0.021
L-His	50.0	1.00*	0.024	0.00	1.000	0.00	1.000	0.11	0.773	0.00	1.000	–0.22	0.317	0.89*	0.046
4-OH-L-Pro	100.0	1.22*	0.018	0.00	0.317	–0.11	1.000	–0.11	0.890	0.00	0.317	–0.11	1.000	–0.22	0.715
L-Ile	40.0	–0.22	0.414	0.00	1.000	0.00	1.000	0.22	0.566	–0.11	0.317	0.22	0.317	0.11	0.715
L-Lys-HCl	20.0	–0.11	1.000	0.33*	0.046	0.33	0.102	–0.22	0.655	0.11	0.577	0.00	0.317	0.44	0.248
L-Leu	40.0	0.11	0.317	0.00	1.000	0.22	0.317	–0.78	0.053	0.22	0.317	0.00	1.000	–0.78	0.053
L-Met	50.0	0.33	0.180	–0.11	0.317	–0.56	0.059	–0.22	0.729	1.00*	0.024	0.22	0.131	0.44	0.357
L-Asn	50.0	–0.11	0.317	0.44	0.102	–1.44**	0.009	0.11	0.317	1.78**	0.005	0.11	0.317	0.67	0.196
L-Pro	250.0	0.44	0.417	0.00	0.317	–0.22	0.083	0.22	0.748	0.11	1.000	0.00	1.000	0.56	0.351
DL-*pyro* Glu	3.0	0.22	0.317	–0.11	0.317	–0.22	0.603	–0.11	0.317	0.00	1.000	0.22	0.317	–0.22	0.603
L-Gln	250.0	–0.44	0.201	0.00	0.564	–1.11*	0.014	–0.11	0.396	2.00*	0.011	0.00	1.000	1.22	0.080
L-Arg	10.0	1.11*	0.013	0.00	0.317	0.00	0.317	–0.44	0.584	0.00	0.317	0.00	0.317	0.11	0.720
L-Ser	250.0	0.67	0.272	0.44	0.276	–1.11	0.050	0.11	1.000	2.00**	0.003	0.00	0.655	1.78**	0.006
Succinic acid	2.0	0.11	0.317	0.11	0.317	–1.11*	0.025	–0.22	0.414	0.00	1.000	–0.22	0.334	–0.78	0.149
L-Thr	250.0	0.56	0.270	0.00	0.317	–1.22*	0.020	–0.11	1.000	1.56**	0.006	0.00	0.157	1.22*	0.036
L-Val	50.0	0.44	0.157	0.00	1.000	–0.22	0.157	–0.44	0.465	0.00	1.000	0.22	0.317	–0.22	0.603
L-Trp	10.0	0.22	0.157	0.00	1.000	–0.33	0.180	0.11	0.773	0.00	1.000	0.11	0.317	–0.11	0.855
w/o amino acid		0.28*	0.025	0.06	0.317	–0.22*	0.046	–0.06	0.655	0.17	0.317	0.00	1.000	0.11	0.564

Ave, average; Wilc., Wilcoxon.

*$P < 0.05$; **$P < 0.01$—statistically significant differences based on Wilcoxon's signed rank test.

Table 2 The change in taste quality of 50mM of Gly, L-Ser, L-Ala and D-Ala solutions and pure water by the addition of 0.5mM IMP

Amino acid	No. of samples w/IMP chosen		Enhanced quality of taste by adding IMP[c]		
	Number[a]	P[b]	Umami	Sweetness	Bitterness
Gly	43***	0.000	29	4	3
L-Ser	46***	0.000	35	6	3
L-Ala	47***	0.000	34	9	2
D-Ala	36**	0.002	5	31	3
w/o amino acid	28	0.240	8	5	11

[a]Number of subjects who chose the solution with IMP as the stronger.

[b]Significance level by one-tailed binomial test: **$P < 0.01$; ***$P < 0.001$.

[c]Number of subjects responding.

equal to 1% of the second smallest number of the session. To eliminate arbitrary numbers between sessions, standardization was performed by multiplication as if the sum of the numbers of each session was 100. The geometric mean of standardized values for each stimulus, i.e. the taste intensity, was calculated by taking the arithmetic mean of the logarithmic values. Then, each geometric mean value was converted to a relative value by division with the value for the 2.0 mM IMP unmixed solution. The potentiation ratio was calculated by dividing the taste intensity of the mixture by the sum of taste intensities of the individual components in the mixture.

Results

In all tested amino acids at low concentrations, the potentiation ratio exceeded 1. At higher concentrations, the

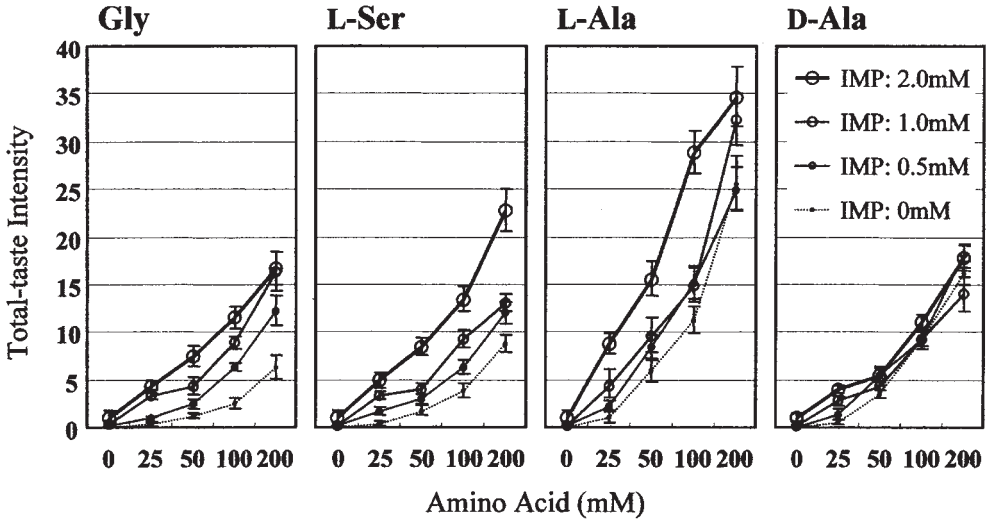

Figure 1 The mean taste intensity of each amino acid and/or IMP solution measured by magnitude estimation with an SE bar when the intensity of 2.0 mM IMP solution is 1. Symbols are represented in the D-Ala graph.

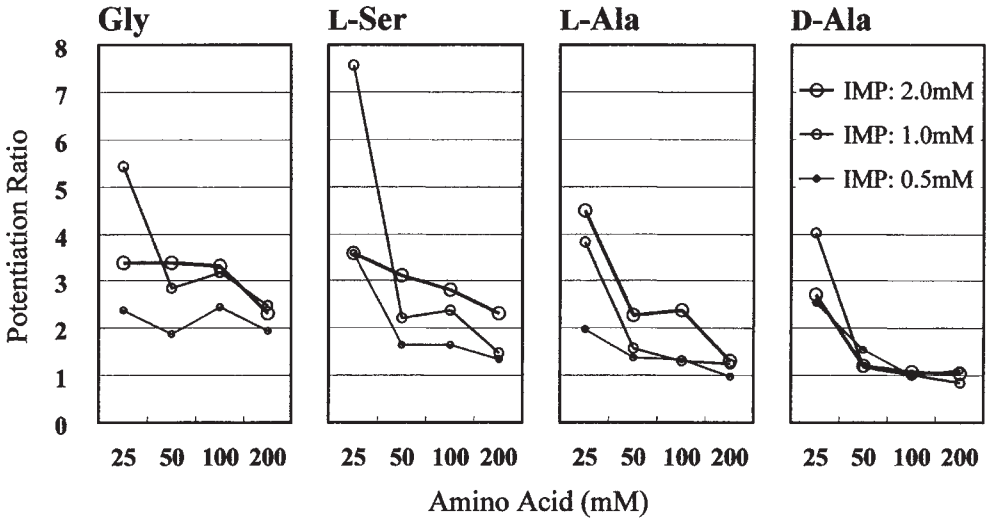

Figure 2 The potentiation ratio of amino acid–IMP solutions derived from mean taste intensities. Symbols are represented in the D-Ala graph.

potentiation ratios of Gly, L-Ser and L-Ala exceeded 1, but that of D-Ala was ~1 (Figures 1 and 2).

Experiment 3

The results from Experiment 1 show that umami was the perceived quality of the enhanced taste when several sweet L-amino acids were mixed with IMP. In this experiment, we attempted to qualify the quality of the enhanced taste, whether umami or sweetness.

Test procedure

Four male subjects performed 80 sessions of triangle tests. Two cups of 100 mM Gly and one cup of 100 mM Gly + 0.5 mM IMP or 200 mM Gly were presented in the presence of 0.5 mM PMP. The latter two stimuli showed almost the same total taste intensity as they did in Experiment 2 (Figure 1). Sets of three 10 ml solutions were presented randomly, and for each set the subjects were asked to choose the odd one, and then to label the discriminability of this choice as 'Very easy', 'Easy' or 'Difficult'. Based on signal detection measures, d' was estimated according to the correct ratio (Ennis, 1993; Bi *et al*., 1997).

Results

The ratio of each response is listed in Table 3. In the presence of PMP, although 100 mM Gly + 0.5 mM IMP was easily discriminated from 100 mM Gly, the discrimination of 200 mM Gly from 100 mM Gly was not easy. The

Table 3 Discriminability of 200 mM Gly or 100 mM Gly + 0.5 mM IMP from 100 mM Gly in the presence of PMP

	Answer						Correct ratio %[a]	d' [b]
	Correct			Error				
	Very easy	Easy	Difficult	Difficult	Easy	Very easy		
200 mM Gly	1	8	26	32	12	1	43.75*	1.12 ± 0.54
100 mM Gly + 0.5 mM IMP	36	31	6	2	4	1	91.25***	4.18 ± 0.69

[a]Asterisks represent statistically significant differences based on the results of a one-tailed binomial test: *$P < 0.05$; ***$P < 0.001$.

[b]d' value and 90% confidence limit.

difference between the two d' values was highly significant ($Z = 5.74$) (Bi *et al*., 1997). Though dispersion was observed in the correct ratio and discriminability among the answers of the four subjects, the above tendency showed consistency among subjects.

Discussion

Umami was the enhanced quality of taste when IMP was added to some sweet L-α-amino acids

In prior human sensory evaluation studies, only acidic amino acids have been reported to have enhanced umami by addition of purine 5′-monophosphates (Yamaguchi *et al*., 1971; Furukawa, 1991). In Experiment 1 of the present study, however, addition of IMP changed the taste of various amino acids as follows: (1) umami was enhanced for L-Ser, L-Gln, L-Asp, L-Asn, L-Thr, L-Glu, Gly, L-Ala, L-Met, L-Cys and peptide L-Glu–L-Glu; (2) sweetness was enhanced for L-Ala, D-Ala, 4-hydroxy-L-Pro, L-Arg, L-His and L-Cys; and (3) Sourness was reduced for L-Glu, L-Asn, L-Thr, L-Gln, and succinic acid. With regards to (1), although many of the amino acids had a dominant sweet taste in themselves, umami was perceived as the basic quality of the enhanced taste. Next, we used 50 subjects to investigate the perceived quality of enhanced taste by adding IMP at near-threshold concentration to L-Ala, D-Ala, Gly and L-Ser, the total-taste intensities of which were found to be significantly enhanced by IMP addition in the first test. Subjects did not agree as to quality of enhanced taste by addition of 0.5 mM IMP to pure water, while umami was clearly perceived as the quality of the enhancement for L-Ala, Gly and L-Ser, and sweetness was perceived as that for D-Ala.

Although umami and sweetness are independent basic tastes, people sometimes integrate the two and cannot accurately discriminate between them in mixed-taste solutions, such as in mixtures of sweet amino acids and IMP. For this reason, the quality of the enhanced taste should be identified not only by a subjective test but also by a more objective method. In Experiment 3, we further investigated whether the quality of the enhanced taste was umami or sweetness using Gly–IMP solutions with a sweetness inhibitor, PMP. This compound is an analogue of a sweetener, dulcin, and reduces sweet taste as a competitive antagonist having a broad inhibitory spectrum (Schiffman *et al*., 1999). One hundred mM Gly + 0.5 mM IMP was determined to be equivalent to 200 mM Gly in total-taste intensity in Experiment 2, and both solutions were easily distinguished from 100 mM Gly. In the presence of PMP, only the discrimination between 200 mM Gly and 100 mM Gly became difficult. PMP inhibited the sweetness that was perceived as the intrinsic dominant taste of Gly, and did not inhibit the taste enhanced by addition of IMP. Similar phenomena were observed in the cases of L-Ser and L-Ala (data not shown). Thus, the quality of the enhanced taste was perceived as umami and was not inhibited by the sweetness inhibitor, PMP.

Umami enhancement is synergistic

We measured the taste intensity of amino acid and amino acid–IMP solutions by magnitude estimation. We designed our study to give estimates not of the individual basic taste qualities, but of the total-taste intensity of amino acid and amino acid–IMP solutions in order to eliminate idiosyncrasies in taste discrimination of sweetness–umami mixed solutions. The slopes of the psychometric amino acid concentration–taste intensity curve got steeper as the concentration of IMP was higher in the cases of Gly, L-Ser and L-Ala, while in the case of D-Ala the slope did not change (Figure 1).

Based on the fact that magnitude estimates have shown a high correlation with neural responses (Borg *et al*., 1967), we applied the function of potentiation ratio (PR) to our results, as has been done in nerve response animal studies (Yamamoto *et al*., 1991). The PR value patterns of Experiment 2 (Figure 2) can be classified into two cases: Case 1: L-Ala, Gly and L-Ser; PR > 1; and Case 2: D-Ala; PR = 1 (PR > 1 at 25 mM, approximate threshold concentration). Thus, the umami enhancement in Case 1 was synergistic rather than additive, while the sweetness enhancement in Case 2 was additive. The larger PRs at low stimulus con-

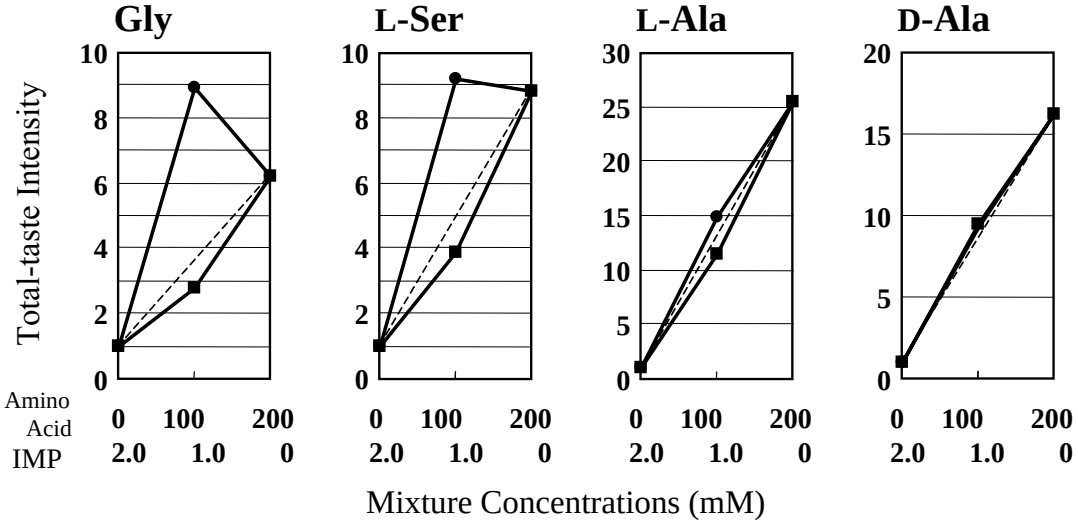

Figure 3 Results from stimulus addition analysis. Mean taste intensities are shown as filled circles connected by a solid line, taste intensities predicted by the sum of perceived intensities of single-ingredient solutions are shown as filled squares connected by a solid line, and taste intensities predicted by stimulus addition analysis are shown as dashed lines.

centrations might have been due to quasi-zero ratings for both amino acids and IMP unmixed solutions.

We further tested whether our result met Rifkin and Bartoshuk's criteria of synergism (Rifkin and Bartoshuk, 1980). Figure 3 compares three values: the observed taste intensity of mixture solutions, the predicted value made by the sum of perceived intensities of single-ingredient solutions, and the value by stimulus addition analysis calculated in molar concentration. In the cases of Gly, L-Ala and L-Ser, observed intensity of mixtures exceeded not only the sum of perceived intensities but also the value by stimulus addition analysis. Therefore, the enhancement patterns agreed with Rifkin and Bartoshuk's criteria of synergism. On the other hand, in the case of D-Ala, these values were almost the same.

The essential structure for synergistic umami is L-α-amino acid

Amino acids that showed umami enhancement by IMP in our study have umami in addition to their dominant sweet taste at high concentrations (Yoshida and Saito, 1969). Noguchi *et al.* reported that some peptides, such as L-Glu–L-Glu, also have umami at neutral pH; L-Glu–L-Glu also showed umami enhancement in our study (Noguchi *et al.*, 1975). These findings indicate that many amino acids other than L-Glu and L-Asp and some peptides might have weak interactions with umami receptor(s) in a mode similar to that of MSG. Our findings that umami enhancement occurs synergistically verify the action of this mechanism. On the other hand, the sweetness enhancement of D-Ala, an enantiomer of L-Ala, by IMP occurred only in an additive manner. Furthermore, succinic acid, which was isolated as an umami compound from clams, did not show umami enhancement by the addition of IMP. Our results suggest that the basic structure necessary for synergistic umami might be L-α-amino acid. Umami has been said to be the guide taste for protein, and our result that among protein amino acids not only acidic but also various neutral amino acids showed umami enhancement might support this idea.

Chaudhari *et al.* cloned cDNA of a truncated analogue of the brain metabotropic glutamate receptor, taste mGluR4, from rat taste cells (Chaudhari *et al.*, 2000). More recently, the hetero-dimeric G-protein-coupled receptors, T1R1–T1R3, from human taste cells was found to respond to L-Glu synergistically with IMP (Li *et al.*, 2002; Nelson *et al.*, 2002). It is shown that human T1R1 influences sensitivity to L-Ala and L-Ser as well as L-Glu. In other words, L-Ala and L-Ser activate robustly receptors containing human T1R1, and IMP potentiates the response synergistically. These latest reports support our psychophysical findings in a molecular manner. Though the subunit T1R3 is shared with the sweet taste receptor T1R2–T1R3 (Nelson *et al.*, 2001), the umami elicited by mixing various sweet L-α-amino acids and IMP was barely reduced by PMP in Experiment 3, and the umami of MSG and MSG–IMP mixture was also barely inhibited (data not shown). If we can find and use an umami-specific taste inhibitor, our findings from the above psychophysical experiments to divide total taste into each quality would be strongly supported.

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References

Arai, S., Yamashita, M. Noguchi, M. and **Fujimaki, M.** (1973) *Tastes of L-glutamyl oligopeptides in relation to their chromatographic properties*. Agric. Biol. Chem., 37, 151–156.

Bi, J., Ennis, D.M. and **O'Mahony, M.** (1997) *How to estimate and use the variance of d' from difference test*. J. Sensory Stud., 12, 87–104.

Borg, G., Diamant, H., Strom, L. and **Zotterman, Y.** (1967) *The relation between neural and perceptual intensity*. J. Physiol., 192, 13–20.

Chaudhari, N., Landin, A.M. and **Roper, S.D.** (2000) *A novel metabotropic glutamate receptor function as a taste receptor*. Nat. Neurosci., 3, 113–119.

Ennis, D.M. (1993) *The power of sensory discrimination methods*. J. Sensory Stud., 8, 353–370.

Fuke, S. and **Konosu, S.** (1991) *Taste active components in some foods: a review of Japanese research*. Physiol. Behav., 49, 863–868.

Furukawa, H. (1991) *Synergistic effects in the taste of α-aminodicarboxylic acids and L-homocysteinate with 5'-inosinate*. J. Agric. Chem. Soc. Jpn., 65, 163–169.

Kawai, M. and **Okiyama, A.** (1998) *Labeled magnitude scale in Japanese*. Jpn. J. Taste Smell Res., 5, 399–402.

Kuninaka, A. (1960) *Studies on taste of ribonucleic acid derivatives*. J. Agric. Chem. Soc. Jpn., 34, 489–492.

Kurihara, K. (1998) *Physiological studies on taste*. Kagaku to Seibutsu, 36, 801–805.

Li, X., Staszewski, L., Xu, H., Durick, K., Zoller, M. and **Adler, E.** (2002) *Human receptors for sweet and umami taste*. Proc. Natl Acad. Sci. USA, 99, 4692–4696.

Maehashi, K., Matsuzaki, M., Yamamoto, Y. and **Udaka, S.** (1999) *Isolation of peptides from an enzymic hydrolyzate of food proteins and characterization of their taste properties*. Biosci. Biotechnol. Biochem., 63, 555–559.

Nelson, G., Hoon, M.A., Chandrashekar, J., Zhang, Y., Ryba, N.J.P. and **Zuker, C.S.** (2001) *Mammalian sweet taste receptors*. Cell, 106, 381–390.

Nelson, G., Chandrashekar, J., Hoon, M.A., Feng, L., Zhao, G., Ryba, N.J.P. and **Zuker, C.S.** (2002) *An amino-acid taste receptor*. Nature, 416, 199–202.

Ninomiya, T., Ikeda, S., Yamaguchi, S. and **Yoshikawa, T.** (1966) *Studies on the taste of various amino acids*. Report of the 7th Sensory Evaluation Symposium, JUSE, pp. 109–123.

Noguchi, M., Arai, S., Yamashita, M., Kato, H. and **Fujimaki, M.** (1975) *Isolation and identification of acidic oligopeptides occurring in a flavor potentiating fraction from fish protein hydrolysate*. J. Agric. Food Chem., 23, 49–53.

Rifkin, B. and **Bartoshuk, L.M.** (1980) *Taste synergism between monosodium glutamate and disodium 5'-guanylate*. Physiol. Behav., 24, 1169–1172.

Schiffman, S.S., Booth, B.J., Sattely-Miller, E.A., Graham, B.G. and **Gibes, K.M.** (1999) *Selective inhibition of sweetness by the salt of ±2-(4-methoxyphenoxy) propanoic acid*. Chem. Senses, 24, 439–447.

Ugawa, T., Konosu, S. and **Kurihara, K.** (1992) *Enhancing effect of NaCl and Na phosphate on human gustatory responses to amino acids*. Chem. Senses, 17, 811–815.

Yamaguchi, S. (1967) *The synergistic taste effect of monosodium glutamate and disodium 5'-inosinate*. J. Food Sci., 32, 473–478.

Yamaguchi, S., Yoshikawa, T., Ikeda, S. and **Ninomiya, T.** (1971) *Measurement of the relative taste intensity of some L-α-amino acids and 5'-nucleotides*. J. Food Sci., 36, 846–849.

Yamamoto, T., Matsuo, R., Fujimoto, Y., Fukunaga, I., Miyasaka, A. and **Imoto, T.** (1991) *Electrophysiological and behavioral studies on the taste of umami substances in the rat*. Physiol. Behav., 49, 919–925.

Yoshida, M. and **Saito, S.** (1969) *Multidimensional scaling of the taste of amino acids*. Jpn. Psychol. Res., 11, 149–166.

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