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Augmenting Effect of a Nonmutagenic Fraction in Soy Sauce on Mutagenicity of 3-Diazotyramine Produced in the Nitrite-treated Sauce

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When 25 kinds of Japanese soy sauce at a concentration of 5% were incubated with 50m*M* sodium nitrite (pH 2) at 37° for 1 hr, the reaction mixtures induced 34–834 (average 368±228) revertants per microliter of soy sauce equivalent in *Salmonella typhimurium* strain TA100 in the absence of S9 mix. The mutagen(s) formed was very unstable under natural daylight and a fluorescent lamp but quite stable under a yellow lamp as well as in the dark. In addition to the known precursors, i.e., tyramine and 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, 1-methyl-1,2,3,4-tetrahydro-β-carboline, which caused weak mutagenesis, was found in the soy sauce. However, the sum of the activities of the three mutagen-precursors after nitrite treatment accounted for only a part of the mutagenicity of nitrite-treated soy sauce. There was in the soy sauce a factor which increased ninefold the mutagenicity of nitrite-treated tyramine, 3-diazotyramine. Therefore, tyramine was considered the principal precursor of the mutagen produced in the nitrite-treated soy sauce. These three precursors together with the mutagenicity augmentation accounted for all the mutagenicity of nitrite-treated sauce. The mutagenicity-augmenting factor in the soy sauce was nonmutagenic before and after nitrite treatment and was stable to heat and light irradiation.

Key words: Soy sauce — Nitrite — Tyramine — Mutagenicity-augmenting factor — Photosensitivity

Hartman's epidemiological study[1] showed that there is a significant positive correlation between nitrate intake and gastric cancer mortality in 12 countries. In Japan, which had the highest gastric cancer mortality among those countries, about 4.8 mmol of nitrate per day per capita[2] was ingested; this was the highest level in the world. Nitrate itself is chemically stable and has no abnormal action in humans at a low concentration. However, when it is reduced to nitrite in the mouth or intestinal tract of man the nitrite easily reacts with amines and amides in foods and drugs under physiological conditions and produces carcinogenic *N*-nitroso compounds.[3–5] Of these compounds, various kinds of *N*-nitrosamides and *N*-nitrosamines might induce gastric cancer.[6–9]

On the other hand, Wakabayashi *et al.*[10] reported that soy sauce has the highest mutagenicity among Japanese foodstuffs after nitrite treatment and that 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-caboxylic acid (MTCCA)[*4] is a precursor of the mutagen produced in nitrite-treated soy sauce. Subsequently it was found that tyramine in soy sauce becomes a potent mutagen after nitrite treatment.[11] It was reported that 3-diazotyramine, a mutagen produced by nitrite treatment of tyramine, induces squamous cell carcinomas of the oral cavity of rats.[12, 13] Wakabayashi *et al.*[14] found that the mutagenicity of MTCCA and tyramine accounts for most of the mutagenicity of soy sauce after nitrite treatment and they suggested that those direct-acting mutagens might be the causes of human gastric cancer. Later, Tahira *et al.*[15] suggested that some unknown precursor(s) may be present in soy sauce

[*3] To whom correspondence should be addressed.
[*4] Abbreviations: MTCCA, 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid; MTC, 1-methyl-1,2,3,4-tetrahydro-β-carboline; CSS, charcoal-treated soy sauce; MAF, mutagenicity-augmenting factor; HPLC, high-performance liquid chromatography.

because MTCCA and tyramine could not account for the mutagenicity of soy sauce treated with a low concentration of nitrite.

The present paper reports that soy sauce contains tyramine, MTCCA, 1-methyl-1,2,3,4-tetrahydro-β-carboline (MTC)[16] and a factor that augments the mutagenicity of 3-diazotyramine (the product of tyramine treated with nitrite), and that these four components account for the mutagenicity of the nitrite-treated soy sauce.

Materials and Methods

Soy Sauce and Chemicals Twenty-three brands of soy sauce commercially produced in three regions in Japan (Kanto, Kansai and Tokushima) and two kinds of homemade soy sauce in Tokushima were purchased. Tyramine and MTCCA were obtained from Sigma Chemical Co., St Louis, MO. MTC was synthesized from tryptamine and paraldehyde[17,18] as previously reported.[19] 3-Diazotyramine, 4-(2-aminoethyl)-6-diazo-2,4-cyclohexadienone, was given to us by Drs. M. Nagao and K. Wakabayashi, National Cancer Center Research Institute, Tokyo.

High-performance Liquid Chromatography High-performance liquid chromatography (HPLC) was performed with a Shimadzu LC-6A equipped with a UV monitor set usually at 270 nm. The column oven was maintained at 50°. All separations were conducted with columns A (4×250 mm) or B (8×250 mm) packed with Nucleosil 100-$_5C_{18}$ (Macherey-Nagel, D-5160 Duren, West Germany) using one of the following systems: system 1, 10 m*M* sodium phosphate buffer containing 100m*M* $NaClO_4$ (pH 2.6) at 1 ml/min through column A; system 2,[10] 20% methanol at 1 ml/min through column A; system 3, stepwise gradient at 12 min after injection from 10m*M* sodium phosphate buffer containing 100m*M* $NaClO_4$ to methanol containing 100m*M* $NaClO_4$ at 3 ml/min through column B; system 4, 10m*M* sodium phosphate buffer containing 100m*M* $NaClO_4$ (pH 2.6) at 2 ml/min through column B.

Nitrite Treatment Soy sauce was used after filtration through a sterilized membrane filter (pore size, 0.45 μm). The chemicals were dissolved in sterilized water. Samples of 1 ml of tenfold diluted soy sauce were mixed with 1 ml of 0.1*M* sodium nitrite and adjusted to pH 2.0 with 6*N* HCl. The mixtures were incubated in the dark at 37° for 60 min. Nitrosation was stopped by addition of 1 ml of 0.1*M* ammonium sulfamate.[10] Glass-stoppered brown test tubes were used for all nitrite treatments.

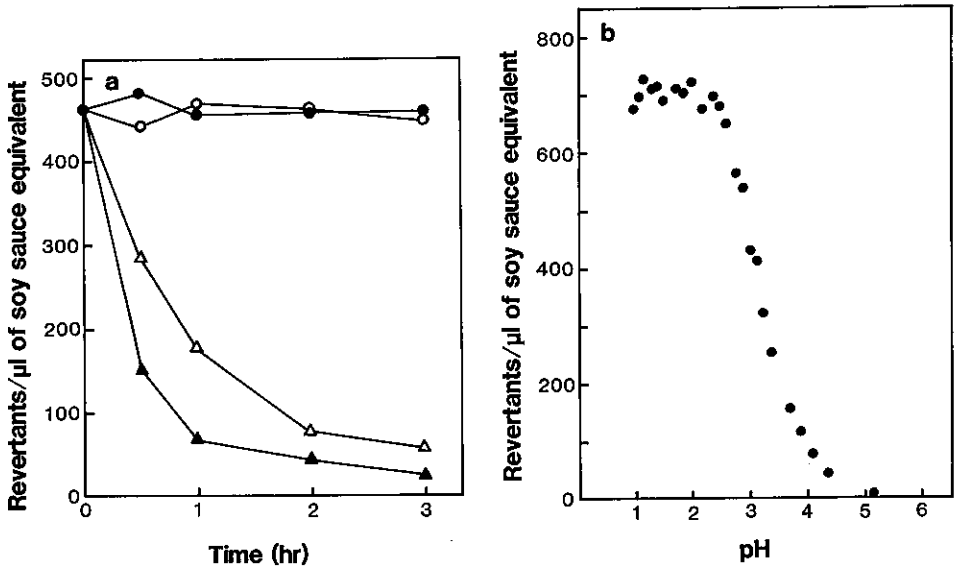

Fig. 1. a. Mutagenicity of nitrite-treated soy sauce after irradiation for 0–3 hr with several light sources. Samples were placed in the dark (●), at 2 m under a 40-W yellow lamp (○), at 0.5 m under a 20-W fluorescent lamp (△) and in daylight (▲) and the mutation assays were performed at 2 m under a 40-W yellow lamp. b. Mutagenicity of soy sauce treated with nitrite in the pH range of 1.01–5.12.

Mutation Test Mutation of *Salmonella typhimurium* strain TA100 was assayed in duplicate by the procedure of Maron and Ames[20] with the modification of preincubation (at 37° for 20 min)[21] in the absence of S9 mix. Mutagens in soy sauce treated with nitrite were found to be stable under a yellow lamp (FL 40S.Y-F, Matsushita Electric Industrial Co., Ltd., Osaka) as well as in the dark, although their mutagenicity decreased rapidly within a few hours in the daylight and under a fluorescent lamp, as shown in Fig. 1a. Therefore all operations involving the reaction mixture were performed under a yellow lamp as the only illumination. Brown test tubes were used for the mutation assays except for the examination for photosensitivity. The number of spontaneous revertants from strain TA100 in the absence of S9 mix was between 107 and 152 (average 130 ± 13) per plate and this number was subtracted in all the data on mutagenicity. As a positive control 2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide (AF-2, 10 ng) was used and induced 429 ± 82 His^+ revertants per plate from strain TA100 in the absence of S9 mix.

Determination of Tyramine, MTCCA and MTC in Soy Sauce Each soy sauce sample was diluted tenfold with methanol, centrifuged for 5 min at 3,000 rpm and passed through a membrane filter (pore size, 0.45 μm). The filtrate was analyzed by HPLC using system 1 for tyramine and system 2 for MTCCA, which may contain (−)-(1*S*, 3*S*)-MTCCA and (−)-(1*R*, 3*S*)-MTCCA. The MTC content was determined by gas chromatography.[19]

Fractionation of Mutagen Precursors in Soy Sauce Soy sauce passed through a membrane filter (pore size, 0.45 μm) was subjected to HPLC and fractionated (system 3). Each fraction was treated with nitrite and assayed for mutagenicity. The compounds in the fraction showing mutagen-precursor activity were further analyzed.

Charcoal Treatment of Soy Sauce Ten grams of activated charcoal was added to 100 ml of soy sauce, and the sample was shaken slowly for 3 hr and centrifuged at 3,000 rpm for 30 min. Three grams of activated charcoal was added to the supernatant, which was then shaken and centrifuged as noted above. The supernatant was passed through a membrane filter (pore size, 0.45 μm). The filtrate, so-called charcoal-treated soy sauce (CSS), contained neither tyramine, MTCCA, nor MTC, but contained 1.2 times more NaCl than the original soy sauce.

Identification of the Augmentable Mutagen Produced in Nitrite-treated Soy Sauce The nitrite-treated soy sauce (20 μl of original sauce equivalent), nitrite-treated tyramine (20 μg of tyramine equivalent) and 3-diazotyramine were subjected to HPLC and eluted at a flow rate of 2 ml per min (system 4). Each eluate was fractionated and 0.1 ml of each fraction (2 ml) was assayed for mutagenicity with or without 1 μ of CSS per plate. The peak fractions showing mutagenicity were analyzed with a UV-VIS spectrophotometer and their spectra were compared.

Results

Mutagenicity of Nitrite-treated Soy Sauce Preliminary studies on the nitrite treatment of soy sauce indicated that the optimum pH of the reaction was in the range of 1.2–2.4 as shown in Fig. 1b, and that the mutagenicity of the reaction mixture was highest at a soy sauce concentration of 5%, which was in agreement with the findings of Tahira *et al.*[15] The nitrite treatment was therefore carried out at pH 2 and soy sauce was treated at a concentration of 5%.

The 25 kinds of soy sauce were treated with 50m*M* $NaNO_2$ (pH 2) at 37° for 60 min. As shown in Fig. 2 the reaction mixtures induced 34–834 (mean$\pm$SD, 368 ± 228) His^+ revertants per μl of original soy sauce from strain TA100 in the absence of S9 mix. The soy sauce showing the weakest mutagenicity was homemade, while the mutagenicity of the other homemade sample was very high (554 His^+ revertants per μl).

A kind of soy sauce (3.3 μl) treated with 1, 2.5 or 5m*M* $NaNO_2$ (pH 2) at 37° for 60 min

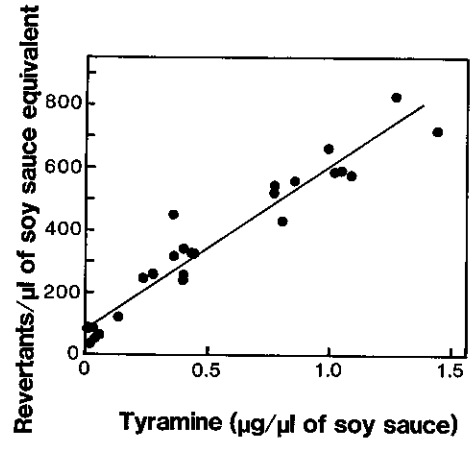

Fig. 2. Correlation of the tyramine content of 25 kinds of soy sauce and the mutagenicity of the nitrite-treated sauce. $Y=513X+86.9$, $r=0.960$, $n=25$.

induced 98, 274 or 495 His^+ revertants per plate, respectively. These mutagenicities were roughly proportional to that induced with 50 m*M* $NaNO_2$.

Amounts of Tyramine, MTCCA and MTC in Soy Sauce and Their Contribution to the Mutagenicity of Nitrite-treated Soy Sauce All the kinds of soy sauce contained tyramine, MTCCA and MTC at concentrations of 10–1,443 (mean±SD, 548±427), 37–774 (487±186) and 0–252 (61.2±71.2) ng per μl, respectively. The amount of tyramine, MTCCA or MTC and the mutagenicity of soy sauce treated with nitrite were statistically examined for correlation. Tyramine content showed a good correlation ($r=0.960$, $P<0.01$) with the mutagenicity of the nitrite-treated soy sauce, as shown in Fig. 2, and the MTC content also had a significant positive correlation ($r=0.767$, $P<0.01$). However, the MTCCA content showed no correlation with the mutagenicity of the nitrite-treated soy sauce ($r=-0.150$).

Authentic tyramine, MTCCA and MTC treated with nitrite induced 101, 17.6 and 6.9 His^+ revertants per μg, respectively, from strain TA100 without S9 mix. The contributions of tyramine, MTCCA and MTC in each soy sauce sample to the mutagenicity of nitrite-treated soy sauce were in the ranges of 1.2–20.4 (mean±SD, 12.9±4.8), 0.1–24.4 (5.3±6.8) and 0–0.29% (0.10±0.07), respectively, and the sum of the three was 10.8–31.7% (18.4±5.0). When the nitrite-treated tyramine, MTCCA and MTC were allowed to stand under a fluorescent lamp, the mutagenicity of nitrite-treated tyramine decreased markedly, as shown in Fig. 3, similarly to that of the nitrite-treated soy sauce in Fig. 1a, whereas that of the nitrite-treated MTCCA and MTC decreased only a little. Therefore the tyramine seemed to have a closer relationship to the mutagenicity of nitrite-treated soy sauce than the other two precursors, although the contribution of the nitrite-treated tyramine to the mutagenicity was not enough to account for all the mutagenicity.

Fractionation of Mutagen-precursors in Soy Sauce A small amount of soy sauce was fractionated by HPLC using system 3. The eluate with retention time of 3 to 20 min was divided into six portions, A–F, as shown in Fig. 4a. Each fraction was evaporated to reduce the volume, treated with nitrite and assayed for mutagenic activity. The muta-

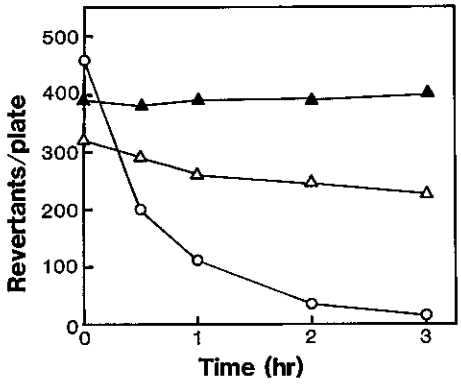

Fig. 3. Mutagenicity of nitrite-treated tyramine (5 μg, ○), MTCCA (30 μg, △) and MTC (75 μg, ▲) after irradiation for 0–3 hr with a 20-W fluorescent lamp from a distance of 0.5 m.

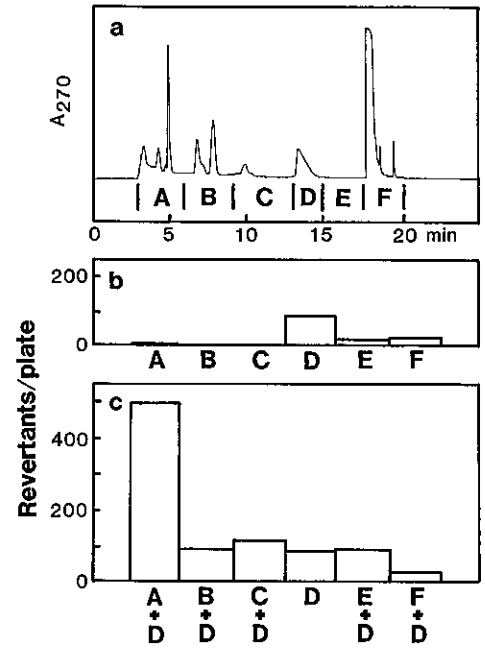

Fig. 4. HPLC profile of soy sauce and mutagenicity of nitrite-treated eluates. System 3 was used for HPLC analysis (a). Eluates from 3 to 20 min were divided into six fractions and each fraction was evaporated. Single (b) or mixed (c) samples were treated with nitrite and their mutagenicity was assayed with strain TA100 without S9 mix.

Table I. Mutagenicity of Nitrite-treated Samples of Soy Sauce, Charcoal-treated Soy Sauce and Charcoal-treated Soy Sauce Containing Tyramine

Soy sauce	Revertants/plate/μl of soy sauce equivalent[a]		
	Soy sauce + $NaNO_2$	Charcoal-treated soy sauce + $NaNO_2$	Charcoal-treated soy sauce + tyramine (1.27 μg)[b] + $NaNO_2$
A	1060	16	1210
B	864	0	1130
C	616	17	1100
D	433	15	1250
E	377	0	948
F	275	2	1070
G	75	0	1320
H	60	0	1450
I	49	6	1120
J	28	0	496
Mean $\pm$ SD	384 $\pm$ 364	5.6 $\pm$ 7.4	1110 $\pm$ 257

a) Mutagenicity was assayed with strain TA100 without S9 mix.
b) Soy sauce A contained 1.27 μg of tyramine per μl.

Table II. Effect of Charcoal-treated Soy Sauce on the Mutagenicity of Nitrite-treated Tyramine

Treatment	Revertants/plate/μl of soy sauce equivalent[a]	%
Soy sauce + $NaNO_2$	827	100
Tyramine (1.27 μg)[b] + $NaNO_2$	85	10
Charcoal-treated soy sauce + $NaNO_2$	25	3
Tyramine + Charcoal-treated soy sauce + $NaNO_2$	1060	128
(Tyramine + $NaNO_2$) + (Charcoal-treated soy sauce + $NaNO_2$)	1060	128
Tyramine + (Charcoal-treated soy sauce + $NaNO_2$)	8	1
(Tyramine + $NaNO_2$) + Charcoal-treated soy sauce	994	120

a) Mutagenicity was assayed with strain TA100 without S9 mix.
b) Soy sauce used in this experiment was soy sauce A in Table I and it contained 1.27 μg of tyramine per μl.

genicity of nitrite-treated fraction D was 16.7% of the mutagenicity of nitrite-treated soy sauce, and those of fractions E and F were 3.6 and 4.8%, respectively (Fig. 4b). There was no mutagenicity in fractions A, B and C after nitrite treatment. Therefore, the total recovery of mutagenicity was 25.1%. In contrast, when these six fractions were mixed and treated with nitrite, the mutagenicity of the mixture was 61.5% of that of the nitrite-treated soy sauce, suggesting that the soy sauce contained some mutagenicity-augmenting factor(s) (MAF). To find the fraction containing the MAF, pairs of fractions were mixed, treated with nitrite and assayed for mutagenicity. It was then found that the nitrite-treated mixture of fractions A and D had 96.2% of the mutagenicity of nitrite-treated soy sauce (Fig. 4c). Authentic tyramine was eluted in fraction D and authentic MTCCA and MTC in fraction F. These results suggested that the mutagenicity of nitrite-treated tyramine or other nitrite-treated components in fraction D was augmented by components or nonmutagenic nitrite-treated components in fraction A.

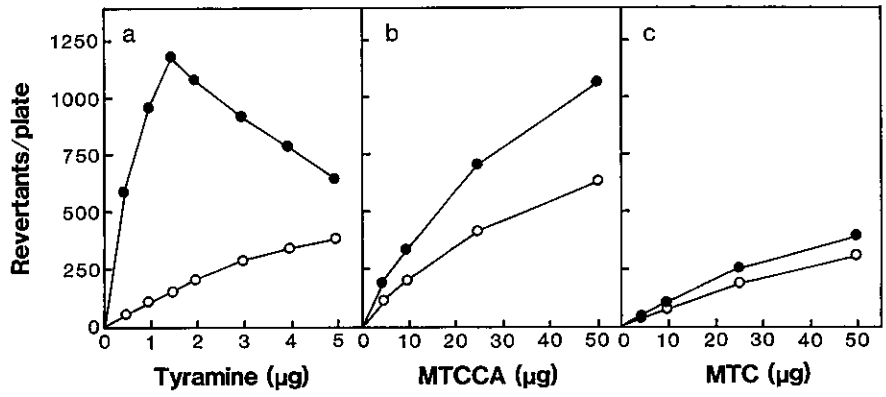

Fig. 5. Mutagenicity of nitrite-treated tyramine (a), MTCCA (b) and MTC (c) with (●) and without (○) charcoal-treated soy sauce A (1 μl/plate).

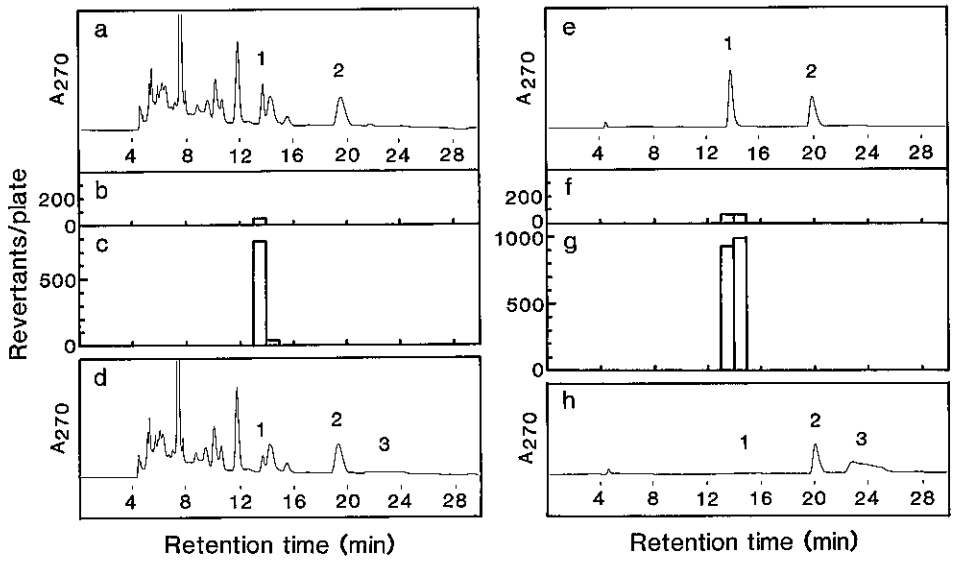

Fig. 6. HPLC profiles of nitrite-treated soy sauce (a–d) and nitrite-treated tyramine (e–h) before (a and e) and after (d and h) light irradiation and mutagenicity of eluates (shown in panels a and e) in the absence (b and f) and presence (c and g) of charcoal-treated soy sauce A (1 μl per plate). System 4 was used for HPLC analysis. Eluates from 0 to 30 min were fractionated every minute and their mutagenicity was assayed with strain TA100 without S9 mix. Nitrite-treated tyramine (inducing 138 His^+ revertants per plate from strain TA100) was subjected to HPLC (e and f) and the peak at 13.7 min showed 149 His^+ revertants per plate (recovery 108%). Samples (d and h) were irradiated with a 20-W fluorescent lamp from a distance of 20 cm for one hour. Peak 1, 3-diazotyramine; peak 2, tyramine; peak 3, unknown product induced by light irradiation.

Mutagenicity-augmenting Action of Ten Kinds of Soy Sauce Ten kinds of soy sauce, which induced 28–1,060 His^+ revertants per μl from strain TA100 without S9 mix after nitrite treatment, were treated with activated charcoal. The CSS contained neither tyramine, MTCCA, nor MTC and showed little mutagenicity after nitrite treatment (Table I).

However, when 1.27 μg of tyramine, which was present in 1 μl of soy sauce A, was added to each CSS and the mixture was treated with nitrite, the mutagenicity of almost all samples showed the same high levels as that of nitrite-treated soy sauce A (Table I), indicating that all the kinds of soy sauce tested contained high levels of mutagenicity-augmenting compound(s) after nitrite treatment.

The mutagenicity of a mixture of CSS and nitrite-treated tyramine was as high as that of a mixture of nitrite-treated CSS and nitrite-treated tyramine (Table II). This finding indicates that the MAF in CSS was able without nitrite treatment to augment the mutagenicity of nitrite-treated tyramine.

The effect of the MAF in charcoal-treated soy sauce A on the mutagen-precursors in soy sauce was determined as shown in Fig. 5. Addition of 1 μl of the CSS increased the mutagenicity of nitrite-treated MTCCA and MTC 1.7-fold and 1.4-fold, respectively, at a concentration of 25 μg. On the other hand, addition of the same amount of CSS increased the mutagenicity of 1 μg of tyramine after nitrite treatment 9.1-fold. The decrease in the mutagenicity of nitrite-treated tyramine at concentrations of more than 2 μg in the presence of CSS was probably due to killing of the test bacteria which was observed with a light microscope.

The mutagenicity-augmenting activity of the MAF was stable to light irradiation for a long time and also did not decrease after heating of MAF at 100° for 10 min (data not shown).

Identification of the Augmentable Mutagen Produced in Nitrite-treated Soy Sauce

Nitrite-treated soy sauce (inducing 830 His^+ revertants per plate per μl of original soy sauce equivalent from strain TA100 in the absence of S9 mix) was subjected to HPLC and fractionated by using system 4 as described in "Materials and Methods" (Fig. 6a). Each one-minute fraction was assayed for mutagenicity. Only the fraction with a peak appearing at 13.7 min showed weak mutagenicity (44 His^+ revertants per plate, recovery 5.3%) (Fig. 6b). However, the mutagenicity of the fraction was markedly augmented by addition of the CSS (808 His^+ revertants per plate, recovery 97%) (Fig. 6c). No other mutagenic fraction with or without CSS was detected in this system. When nitrite-treated soy sauce was irradiated with a 20-W fluorescent lamp at a distance of 20 cm for 60 min, the sample showed almost no mutagenicity (data not shown). At the same time, the area of the peak at 13.7 min (peak 1 in Fig. 6a and d) was reduced significantly and a new broad peak (peak 3 in Fig. 6d) appeared. The peak that appeared at 13.7 min in the HPLC chromatogram of nitrite-treated soy sauce (peak 1 in Fig. 6a) was identified as 3-diazotyramine because the retention time and the UV spectrum (data not shown) were the same as those of nitrite-treated tyramine (Fig. 6e) and those of authentic 3-diazotyramine (data not shown). In addition the mutagenicity of the peak of nitrite-treated tyramine (Fig. 6f and g) and authentic 3-diazotyramine was increased more than tenfold by addition of CSS. Moreover, the peak of nitrite-treated tyramine disappeared after light irradiation (Fig. 6h).

Discussion

The mutagenicity of nitrite-treated soy sauce presented in this paper (Fig. 2 and Table I) was considerably higher than that reported in earlier papers,[10, 11, 15] presumably not only because the conditions of nitrite treatment were modified but also because the mutagenicity was assayed under a yellow light to prevent photoinactivation of the mutagens produced. For the same reasons, the mutagenicity of nitrite-treated tyramine was higher than that reproted by Ochiai *et al.*[11] On the other hand, the mutagenicity of nitrite-treated MTCCA or MTC was not very different from that reported in the past[10, 22] because it seemed to be not particularly affected by light (Fig. 3).

Nonmutagenic MTC present in soy sauce[16] is considered to be a mutagen-precursor in soy sauce because it became mutagenic after nitrite treatment as did tyramine and MTCCA. However, the mutagenicity of nitrite-treated MTC was not as strong as that of nitrite-treated tyramine or MTCCA (Fig. 5), partly because the reaction of MTC with nitrite may be slow.

Although no other new potent mutagen-precursor was detected, we found a compound augmenting the mutagenicity of mutagens produced from precursors in soy sauce

by nitrite treatment. The MAF and three known precursors accounted for all the mutagenicity of nitrite-treated soy sauce. The mutagenicity-augmenting activity was found in all soy sauce samples tested. If the MAF is produced by fermentation, it may also be present in soybean paste and other foods.

Three mutagen-precursors in soy sauce, i.e., tyramine, MTCCA and MTC, induced 101, 17.6 and 6.9 His^+ revertants, respectively, from strain TA100 in the absence of S9 mix when 1 μg of each chemical was treated with nitrite in our system. Their average quantities in the 25 kinds of soy sauce were 548, 487 and 61.2 ng/μl, respectively. Since 1 μl of the CSS increased the mutagenicity of nitrite-treated tyramine, MTCCA and MTC by 9.1-, 1.7- and 1.4-fold, respectively (Fig. 5), the average contribution of the three precursors to the mutagenicity of the 25 kinds of nitrite-treated soy sauce was calculated to be 100:3.0:0.13, respectively. Moreover, nine of ten kinds of CSS seemed to have about tenfold mutagenicity-augmenting activity for nitrite-treated tyramine, and the mutagenicity-augmenting activity of CSS needed no nitrite (Tables I and II). In addition, mutagenicity of nitrite-treated soy sauce was correlated strongly with the tyramine content of the soy sauce (Fig. 2). Therefore, these results suggest that tyramine is the most important precursor in regard to the mutagenicity of nitrite-treated soy sauce.

Although the mutagenic activity of nitrite-treated tyramine is highly photosensitive (Fig. 3), the decrease in the mutagenicity must be almost negligible in the human gastrointestinal tract because of the darkness. Tyramine occurs not only in soy sauce but also in some soybean products, cheese and other fermentation products.[23-25] Although it has been said that the pH of the stomach contents does not drop below 3 until about an hour after a meal[26] and that the nitrite level does not rise above 5mM even in extremely abnormal cases,[27] the mutagenicity of a limited amount of direct-acting mutagens generated by nitrite in the acidic human stomach may be augmented by the MAF when foods containing it are eaten.

The structure and the mechanism of action of MAF and the physiological action on animal cells are currently under study.

Acknowledgments

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References

1) Hartman, P. E. Putative mutagens and carcinogens in foods. I. Nitrate/nitrite ingestion and gastric cancer mortality. *Environ. Mutag.*, **5**, 111–121 (1983).
2) Kawabata, T., Ohshima, H., Uibu, J., Nakamura, M., Matsui, M. and Hamano, M. Occurrence, formation, and precursors of *N*-nitroso compounds in Japanese diet. *In* "Naturally Occurring Carcinogens-Mutagens and Modulators of Carcinogenesis," ed. E. C. Miller, J. A. Miller, I. Hirono, T. Sugimura, and S. Takayama, pp. 195–209 (1979). Japan Scientific Societies Press, Tokyo/Univ. Park Press, Baltimore.
3) Hartman, P. E. Nitrates and nitrites: ingestion, pharmacodynamics, ad toxicology. *In* "Chemical Mutagens," Vol. 7, ed. F. J. de Serres and A. Hollaender, pp. 211–294 (1982). Plenum Publishing Corp., New York and London.
4) Magee, P. N. and Barnes, J. M. Carcinogenic nitroso compounds. *Adv. Cancer Res.*, **10**, 163–246 (1967).
5) Lijinsky, W. and Taylor, H. W. Nitrosamines and their precursors in food. *In* "Origins of Human Cancer," Book C, ed. H. H. Hiatt, J. D. Watson and J. A. Winsten, pp. 1579–1590 (1977). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
6) Mirvish, S. S. The etiology of gastric cancer: intragastric nitrosamide formation and other theories. *J. Natl. Cancer Inst.*, **71**, 629–647 (1983).
7) Shephard, S. E., Schlatter, C. and Lutz, W. K. Assessment of the risk of formation of carcinogenic *N*-nitroso compounds from dietary precursors in the stomach. *Food Chem. Toxicol.*, **25**, 91–108 (1987).

8) Endo, H., Ishizawa, M., Endo, T., Takahashi, K., Utsunomiya, T., Kinoshita, N., Hidaka, K. and Baba, T. A possible process of conversion of food components to gastric carcinogens. *In* "Origins of Human Cancer," Book C, ed. H. H. Hiatt, J. D. Watson and J. A. Winsten, pp. 1591–1607 (1977). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

9) Tannenbaum, S. R., Archer, M. C., Wishnok, J. S., Correa, P., Cuello, C. and Haenszel, W. Nitrate and the etiology of gastric cancer. *In* "Origins of Human Cancer," Book C, ed. H. H. Hiatt, J. D. Watson and J. A. Winsten, pp. 1609–1625 (1977). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

10) Wakabayashi, K., Ochiai, M., Saito, H., Tsuda, M., Suwa, Y., Nagao, M. and Sugimura, T. Presence of 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, a precursor of a mutagenic nitroso compound, in soy sauce. *Proc. Natl. Acad. Sci. USA*, **80**, 2912–2916 (1983).

11) Ochiai, M., Wakabayashi, K., Nagao, M. and Sugimura, T. Tyramine is a major mutagen precursor in soy sauce, being convertible to a mutagen by nitrite. *Gann*, **75**, 1–3 (1984).

12) Nagao, M., Wakabayashi, K., Fujita, Y., Tahira, T., Ochiai, M., Takayama, S. and Sugimura, T. Nitrosatable precursors of mutagens in vegetables and soy sauce. *In* "Diet, Nutrition and Cancer," ed. Y. Hayashi, M. Nagao, T. Sugimura, S. Takayama, L. Tomatis, L. W. Wattenberg and G. N. Wogan, pp. 77–86 (1986). Japan Scientific Societies Press, Tokyo and VNU Scientific Press, Utrecht.

13) Fujita, Y., Wakabayashi, K., Takayama, S., Nagao, M. and Sugimura, T. Induction of oral cavity cancer by 3-diazotyramine, a nitrosated product of tyramine present in foods. *Carcinogenesis*, **8**, 527–529 (1987).

14) Wakabayashi, K., Nagao, M., Ochiai, M., Tsuda, M., Yamaizumi, Z., Saito, H. and Sugimura, T. Presence of 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acids and tyramine as precursors of mutagens in soya sauce after nitrite treatment. *In* "*N*-Nitroso Compounds: Occurrence, Biological Effects and Relevance to Human Cancer," IARC Scientific Publications No. 57, ed. I. K. O'Neill, R. C. von Borstel, C. T. Miller, J. Long and H. Bartsch, pp. 17–24 (1984). IARC, Lyon.

15) Tahira, T., Fujita, Y., Ochiai, M., Wakabayashi, K., Nagao, M. and Sugimura, T. Mutagenicity of soy sauce treated with a physiologically feasible concentration of nitrite. *Mutat. Res.*, **174**, 255–258 (1986).

16) Higashimoto, M., Handa, Y. and Matano, K. 1-Methyl-1,2,3,4-tetrahydro-β-carboline (1-MTHBC) in fermented seasonings. *J. Jpn. Soc. Nutr. Food Sci.*, **40**, 43–46 (1987) (in Japanese).

17) Tatsui, G. Synthesis of carboline derivatives. *J. Pharm. Soc. Jpn.*, **48**, 453–459 (1928) (in Japanese).

18) Akabori, S. and Saito, K. Synthetische Versuche in der Indol-Gruppe, VIII. Synthese von Harman und Harmin. *Ber. Dtsch. Chem. Ges.*, **63**, 2245–2248 (1930).

19) Higashimoto, M. and Matano, K. Gas chromatographic determination of 1-methyl-1,2,3,4-tetrahydro-β-carboline (1-MTHBC) in alcoholic beverages. *J. Jpn. Soc. Nutr. Food Sci.*, **38**, 317–322 (1985) (in Japanese).

20) Maron, D. M. and Ames, B. N. Revised methods for the *Salmonella* mutagenicity test. *Mutat. Res.*, **113**, 173–215 (1983).

21) Yahagi, T., Nagao, M., Seino, Y., Matsushima, T., Sugimura, T. and Okada, M. Mutagenicities of *N*-nitrosamines on *Salmonella*. *Mutat. Res.*, **48**, 121–129 (1977).

22) Ochiai, M., Wakabayashi, K., Sugimura, T. and Nagao, M. Mutagenicities of indole and 30 derivatives after nitrite treatment. *Mutat. Res.*, **172**, 189–197 (1986).

23) Sung, S. K., Lee, C. M., Young, J. D. and Chen, C. N. High levels of tyramine in some Chinese foodstuffs. *Hum. Psychopharmacol.*, **1**, 103–107 (1986).

24) Wakabayashi, K., Nagao, M., Chung, T. H., Yin, M., Karai, I., Ochiai, M., Tahira, T. and Sugimura, T. Appearance of direct-acting mutagenicity of various foodstuffs produced in Japan and Southeast Asia on nitrite treatment. *Mutat. Res.*, **158**, 119–124 (1984).

25) Yamamoto, S., Wakabayashi, S. and Makita, M. Gas-liquid chromatographic determination of tyramine in fermented food products. *J. Agric. Food Chem.*, **28**, 790–793 (1980).

26) James, A. H. and Pickering, G. W. The role of gastric acidity in the pathogenesis of peptic ulcer. *Clin. Sci.*, **8**, 181–210 (1949).

27) Correa, P., Cuello, C., Gordillo, G., Zarama, G., Lopez, J., Haenszel, W. and Tannenbaum, S. The gastric microenvironment in populations at high risk to stomach cancer. *Natl. Cancer Inst., Monogr.*, **53**, 167–170 (1979).