Short Communication

Desmutagenicity of Melanoidins against Mutagenic Pyrolysates

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The Maillard reaction is considered to be one of the most predominant reactions occurring in the processing, storage and cooking of foods. Many mutagens are known to be formed through the interaction of specific food components or pyrolysis of them. Omura *et al.*[1] showed the formation of several weak mutagens through the Maillard reaction at 100°C. Sugimura and coworkers[2,3] isolated many strong mutagens from pyrolysates of amino acids and proteins. Some of these mutagens are formed in foods cooked with oil or a naked flame.[3] On the other hand, from the viewpoint of food safety improvement, some investigations involving screening of factors suppressing the mutagenicity have been performed. Naturally occurring substances such as polyphenol components,[4] porphyrin compounds[5] and the extracts of vegetables[6,7] were found to show desmutagenicity. However, there have been few reports on desmutagenic factors formed during heat-treatment or storage of foods. Chan *et al.*[8] described the desmutagenic effects of a heated lysine-fructose mixture and caramelized sucrose, but the effective components remain unknown.

In the present communication, the inhibition of the mutagenicity of several pyrolysates by melanoidins, the main products of the Maillard reaction, is described for the first time, and the inhibition mechanism is discussed, with reference to the properties of fractionated or chemically modified melanoidins.

Melanoidin samples were prepared as follows. Glycine (1 mol), D-glucose (1 mol) and sodium bicarbonate (0.2 mol) were dissolved in 500 ml of deionized water (pH 6.8) and then refluxed at 100°C for 9 hr. Each reaction solution was dialyzed against distilled and deionized water for 2 weeks at room temperature and then lyophilized to obtain the nondialyzable melanoidin. The reaction solution was also fractionated with membrane filters into melanoidins with molecular weights (MW) below 1000, MW of 1000 to 5000, and MW above 5000. An ozone-treated melanoidin was prepared by ozonolysis of the nondialyzable melanoidin, as previously described.[9] A reduced melanoidin was prepared by reduction of the nondialyzable melanoidin with sodium borohydride at pH 8.0 and room temperature for 24 hr. Trp-P-1 (3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole, Trp-P-2 (3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole), Glu-P-1 (2-amino-6-methyldipyrido[1,2-*a*: 3′, 2′-*d*]imidazole), Glu-P-2 (2-aminodipyrido-[1,2-*a*: 3′,2′-*d*]imidazole) and IQ (2-amino-3-methylimidazo[4,5-*f*]quinoline) were purchased from Wako Pure Chem. Ind., Ltd., Tokyo. S-9 was purchased from Oriental Yeast Co., Ltd., Tokyo.

Mutagenicity was assayed by a modification[10] of the method of Ames *et al.*,[11] using *Salmonella typhimurium* TA98 in the presence of the S-9 mixture. The desmutagenic effect was expressed as the percent loss of the mutagenicity. Antioxidative activity was estimated by the peroxide value test according to Hayase and Kato.[12] Reducing ability was measured by the method of Tonomura *et al.*[13]

The experimental results are summarized in Table I. The nondialyzable melanoidin and the fractionated melanoidins of above MW 1000 showed obvious desmutagenic effects against Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2 and IQ, whereas the fraction below MW 1000 did not. The higher MW fraction showed stronger desmutagenicity than the lower MW fraction. It

TABLE I. COLOR INTENSITY, REDUCING ABILITY, ANTIOXIDATIVE ACTIVITY AND DESMUTAGENIC EFFECT OF MAILLARD REACTION PRODUCTS PREPARED FROM GLYCINE AND GLUCOSE

Maillard reaction products	Color intensity[a]	Reducing ability[b]	Antioxidative activity (POV)[c]		Loss of mutagenicity (%)[d]				
			0.5 mg	2.0 mg	Trp-P-1	Trp-P-2	Glu-P-1	Glu-P-2	IQ
Unfractionated	2.00	0.22	147.0	24.0	14.0	59.0	49.4	67.2	63.3
Below MW 1000	0.14	0.19	265.0	37.0	11.1	8.3	0.5	11.1	—
MW 1000 to 5000	5.67	0.62	41.5	21.0	52.8	63.9	48.3	72.9	72.4
Above MW 5000	7.09	0.68	31.1	21.3	66.2	72.7	67.3	88.9	87.2
Nondialyzable melanoidin	6.89	0.71	31.6	21.7	62.1	70.8	66.3	75.7	71.9
Ozone-treated melanoidin	0.52	0.17	36.5	28.5	54.2	61.3	80.3	88.7	88.3
Reduced melanoidin	2.31	0.37	37.0	20.0	46.6	—	—	—	56.4

[a] Expressed as optical density at 470 nm; 2 mg of each Maillard reaction product was dissolved in 1 ml of deionized water.

[b] 2 mg of a sample was oxidized with potassium ferricyanide,[13] and the reducing ability was expressed as the equivalent weight of ascorbic acid per weight of sample.

[c] 0.5 or 2.0 mg of a sample was incubated with 1 g of linoleic acid at 45°C for 48 hr.[12] The POV (peroxide value, millieq./kg) of linoleic acid before and after incubation was 4.0 and 223, respectively.

[d] Trp-P-1 (0.18 nmol), Trp-P-2 (0.08 nmol), Glu-P-1 (0.20 nmol), Glu-P-2 (2.26 nmol) or IQ (0.02 nmol) was incubated with or without each Maillard reaction product (2 mg) at 37°C for 30 min prior to preincubation. At the concentration of each sample (2 mg/plate) the growth of *Salmonella typhimurium* TA 98 was not affected. The numbers of His^+ revertants without Maillard reaction products were 343, 1745, 2400, 992 and 1975 colonies for Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2 and IQ, respectively.

seems that there are correlationships among the color intensity, reducing ability, antioxidative activity and desmutagenicity of the fractionated melanoidins and the reduced melanoidin. It has been established that the antioxidative activity of Maillard reaction products is partly due to their reducing ability or reductone structure.[14] Furthermore, a considerable amount of data has been presented on the inhibition of cancer by antioxidative substances.[15] Subsequently, the reductone structure or antioxidative activity of melanoidins possibly participate in their desmutagenicity.

When the nondialyzable melanoidin was reduced with $NaBH_4$, the desmutagenicity decreased with the decreases in color intensity and reducing ability, although the antioxidative activity did not decrease so much. The carbonyl groups of the melanoidin, which would react with the basic groups of the mutagens, are reduced with $NaBH_4$. We have also observed that carbonyl compounds such as diacetyl and dihydroxyacetone show desmutagenic effects against basic mutagens.[16] Accordingly, the reactivity of carbonyl groups of melanoidins should not be overlooked.

On ozone-treatment of the nondialyzable melanoidin, most of the color intensity and reducing ability disappeared, whereas the desmutagenicity still remained, that against Glu-P-1, Glu-P-2 and IQ becoming stronger. In the previous paper,[9] we described that, on ozone-treatment, carboxylic groups of melanoidins increased and their isoelectric points decreased. In this case, electrostatic interaction between the ozone-treated, acidic melanoidins and basic mutagens is considered, in addition to participation of the carbonyl groups that increased on ozonolysis.

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