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Desmutagenic Effects of Maillard Reaction Products against Mutagenic Heterocyclic Amines

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Each molecular weight (Mw) fraction of melanoidins prepared from a D-glucose and glycine system, i.e., Mw below 1,000, Mw between 1,000 to 5,000 and Mw above 5,000 and nondialyzable and ozone-treated melanoidins were reacted with heat-induced mutagens such as Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2 and IQ at 37°C for 30 min. The inhibitory effects of the melanoidins on the mutagens increased with increasing molecular weight. The reducing ability and antioxidative activity of melanoidins also increased in proportion to the increase in molecular weight, whereas the mutagenic inhibitory effect decreased on reduction of the melanoidins with sodium borohydride. It was also observed that a part of Trp-P-1 was adsorbed to melanoidin molecules. On modification of amino groups of these mutagens with carbonyl compounds derived through the Maillard reaction such as diacetyl and glyceraldehyde, their mutagenic activities were remarkably suppressed. Accordingly, it is speculated that the mutagenic inhibitory action of melanoidins is due to their reducing ability and antioxidative activity, and electrostatic binding and carbonyl groups of the melanoidin molecules.

Introduction

The Maillard reaction is one of the most important reactions occurring during the processing, storage and cooking of foods. On such treatment of foods, several workers reported that various mutagens were formed through the interaction or degradation of food components.

Shibamoto (1983) and Omura et al. (1983) showed the formation of several weak mutagens through the Maillard reaction at 100°C, and also identified some mutagens such as pyrazines, furans and thiazolidines. Sugimura et al. (1977) and coworkers (Yamamoto et al., 1978; Kasai et al., 1980) isolated many strong mutagens from pyrolyzates of amino acids and proteins, and from the charred parts of fishes and meats, such as Trp-P-1(3-amino-1, 4-dimethyl-5H-pyrido{4, 3-b}indole), Trp-P-2 (3-amino-1-methyl-5H-pyrido{4, 3-b}indole), Glu-P-1 (2-amino-6-methyldipyrido{1, 2-a: 3′, 2′-d}imidazole), IQ (2-amino-3-{4, 5-f}quinoline) and so on. Among these mutagens, IQ is easily formed through the Maillard reaction on heating of animal-origin foods containing creatine (Kasai et al., 1980; Jagerstad et al., 1983).

Recently, from the view point of food safety, many investigators have concentrated on the screening of various factors that suppress or modify these mutagens. Naturally occurring substances such as polyphenol components (Fukuhara et al., 1980), porphyrin compounds (Arimoto et al., 1980) and vegetable extracts (Kada et al., 1978) were found to show desmutagenicity. However, there have been few reports on the desmutagenic factors appearing during heat-treatment or storage of foods. Chan et al. (1982) reported the desmutagenic effects of a lysine-fructose reaction mixture and caramelized sucrose, although the effective components were not characterized.

In the present paper, the inhibition of the muta-

genicity of several heat-induced mutagens by melanoidins which are the main products of the Maillard reaction is reported, and an inhibition mechanism is also proposed on the chemically modified melanoidins.

Experimental

1. Chemicals

Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2 and IQ were purchased from Wako Pure Chem. Inc., Ltd. (Tokyo). S-9 was obtained from Oriental Yeast Co., Ltd (Tokyo). Other reagents were of reagent grade and used without further purification.

2. Preparation of melanoidins

Glycine (1 mol), D-glucose (1 mol) and sodium bicarbonate (0.2 mol) were dissolved in 500 *ml* of distilled and deionized water (pH 6.8), and the solution was refluxed at 100 °C for 9 hrs. The reaction solution was fractionated with membrane filters into three fractions; Mw below 1,000, between 1,000 to 5,000, and above 5,000. The reaction solution was also dialyzed against deionized water for 2 weeks at room temperature and then lyophilized to obtain nondialyzable melanoidins. Ozone-treated melanoidins were prepared by ozonolysis of the nondialyzable melanoidins, as previously described (Kim et al., 1985a), but the reduction after ozonolysis was omitted. Reduced melanoidins were prepared by reduced melanoidins were prepared by reduction of the nondialyzable melanoidins with sodium borohydride at pH 8.0 and room temperature for 24 hrs, followed by dialysis against deionized water for 2 days to remove residual salts.

3. Mutation assay

Mutagenicity was assayed by a modification (Yahagi et al., 1977) of the method of Ames et al. (1975), using Salmonella typhimurium TA 98 in the presence of the S-9 mix. Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2 and IQ were allowed to react with melanoidins and carbonyl compounds at 37°C for 30 min prior to preincubation, the initial pH of the reaction mixtures being 7.0. Desmutagenicity was expressed as the ratio of the percentage of the mutagenicity of each heat-induced mutagen before and after the addition of each melanoidin sample and carbonyl compounds.

4. Determination of reducing ability

Two milligrams of each sample was oxidized with potassium ferricyanide and then the color intensity was monitored at 420 *nm* according to the method of Tonomura et al. (1978). The reducing ability was expressed as the equivalent weight of ascorbic acid per weight of sample.

5. Measurement of antioxidative activity

A melanoidin sample (0.5, 2.0 or 5.0 *mg*) was incubated with 1 *g* of linoleic acid at 45°C for 48 hrs. After incubation, the POV (peroxide value) was determined as described by Hayase and Kato (1984). The POV of linoleic acid before and after incubation were 4.0 and 223, respectively.

6. Measurement of the adsorption of Trp-P-1 on melanoidins

Trp-P-1 (30 *mg*/2 *ml*) 200 *μl*, and nondialyzable and ozone-treated melanoidins (500 *mg*/5 *ml*) 20 *μl* were mixed (pH 7.0) and then incubated at 37°C for 30 min. The degree of adsorption of Trp-P-1 on the melanoidins was expressed as the change in peak height at 224 *nm* seen on gel permeation-HPLC before and after the addition of a melanoidin. HPLC analysis was performed with a Hitachi 638-30 Liquid Chromatograph as follows. Column; stainless steel (500×7.6 *mm* i.d.), prepacked with Asahipak GC-320. Detector; a spectrophotometer (Hitachi model 100-50 spectrophotometer). The column was eluted with 0.4 M NaCl at a flow rate of 1.0 *ml*/min.

Results

1. Desmutagenic effect of each fractionated and ozone-treated melanoidins.

Figures 1 and 2 show the dose-response of the

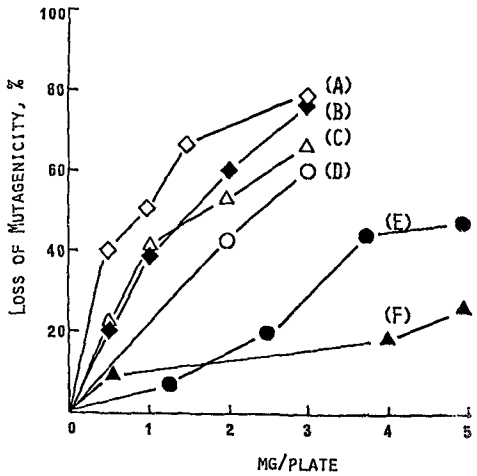

Fig. 1. Effect of Maillard reaction products derived from D-glucose-glycine on the mutagenicity of Trp-P-I.
Trp-P-1(0.18 nmole) was incubated with and without Maillard reaction products at 37°C for 30 min, and the numbers of His^+ revertants of Trp-P-1 per plate were 343.
A, above Mw 5000; B, nondialyzable melanoidin (ML); C, Mw 1000-5000; D, ozone-treated ML; E, unfractionated; F, below Mw 1000

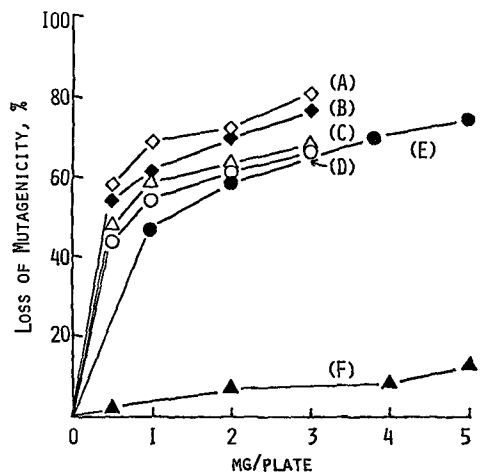

Fig. 2. Effect of Maillard reaction products derived from D-glucose-glycine on the mutagenicity of Trp-P-2.
Trp-P-2(0.08 n mole) was incubated with and without Maillard reaction products at 37°C for 30 min, and the numbers of His^+ revertants of Trp-P-2 per plate were 1745.
A, above Mw 5000; B, nondialyzable melanoidin(ML); C, Mw 1000~5000; D, ozone-treated ML; E, unfractionated; F, below Mw 1000

desmutagenic effect of each melanoidin fraction on the mutagenicity of Trp-P-1 and Trp-P-2. The desmutagenic effect on Trp-P-1 increased with increasing molecular weight and dose of each melanoidin. Although the melanoidins were decolorized and degraded with ozone, still showed a high desmutagenic effect on Trp-P-1, the mutagenicity of Trp-P-1 being reduced by 60% on the addition of 3 *mg* of ozone-treated melanoidin. On Trp-P-2, the extent of desmutagenic effect also showed the similar pattern as Trp-P-1.

The desmutagenic effects of each melanoidin sample on Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2 and IQ are summarized in Table 1. The nondialyzable melanoidins and fractionated melanoidins of above Mw 1000 showed obvious desmutagenic effects on Trp-P-2, Glu-P-1, Glu-P-2 and IQ as in the case of Trp-P-1, whereas the below Mw 1000 fraction did not. The higher molecular weight fraction showed stronger desmutagenicity than the lower molecular weight one. On ozone-treatment of non-

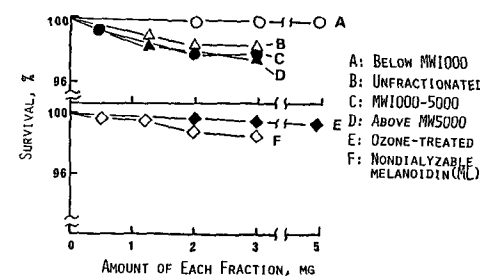

Fig. 3. Effect of heated glucose-glycine reaction products on the growth of S. typhimurium TA 98.
Growth inhibitory action was determined by measurement of the absorbance at 660 *nm* with and without Maillard reaction products.

dialyzable melanoidins, the desmutagenicity still remained, becoming especially stronger against Glu-P-1, Glu-P-2 and IQ.

Each melanoidin at the concentration showing a desmutagenic effect did not show a growth

Table 1. Inhibition of the mutagenicity* of Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2, and IQ by the Maillard reaction products derived from D-glucose-glycine

Maillard reaction product	*mg* Added/ plate	Loss of mutagenicity (%)				
		Trp-P-1	Trp-P-2	Glu-P-1	Glu-P-2	IQ
Unfractionated	2	14.0	59.0	49.4	67.2	63.3
Below Mw 1000	2	11.1	8.3	0.5	11.1	—
Mw 1000~5000	2	52.8	63.9	48.3	72.9	72.4
Above Mw 5000	2	66.2	72.7	67.3	88.9	87.2
Nondialyzable melanoidins	2	62.1	70.8	66.3	75.7	71.9
Ozone-treated melanoidins	2	54.2	61.3	80.3	88.7	88.3

*Trp-P-1 (0.18 n mole), Trp-P-2 (0.08 n mole), Glu-P-1 (0.20 n mole), Glu-P-2 (2.26 n mole) or IQ (0.02 n mole) was incubated with and without each Maillard reaction product at 37°C for 30 min prior to preincubation.

The numbers of His^+ revertants without Maillard reaction products were estimated to 343, 1745, 2400, 992 and 1975 colonies for Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2 and IQ, respectively.

Table 2. Mutagenicity of Maillard reaction products derived from D-glucose-glycine system

Maillard reaction product	Amount of sample added (*mg*/plate)	Revertants per plate
Unfractionated	2	80
Below Mw 1000	2	124
Mw 1000~5000	2	87
Above Mw 5000	2	95
Nondialyzable melanoidins	2	59
Reduced melanoidins	2	68
Ozone-treated melanoidins	2	106

*Mutagenicity was assayed with the preincubation method using S. typhimurium TA 100 in the absense of S-9 mix.

inhibitory effect or mutagenicity against Salmonella typhimurium TA 98 and TA 100 (Figure 3 and Table 2).

2. Effects of reducing ability and antioxidative activity on the desmutagenicity.

Table 3 shows the desmutagenic effects of reduced melanoidin and butylated hydroxytoluene(BHT). When melanoidins were reduced with sodium borohydride, the desmutagenic effects on Trp-P-1 and IQ decreased to 40% and 20% compared with those before the reduction, respectively. The mutagenicity of Trp-P-1 and IQ also decreased with the addition of an antioxidant such as BHT.

Table 3. Effect of nondialyzable melanoidins, reduced melanoidins and BHT on the loss of mutagenicity of Trp-P-1 and IQ

Sample	Amount of sample added (*mg*/plate)	Loss of mutagenicity %	
		Trp-P-1	IQ
Nondialyzable melanoidins	2	62.1	71.9
Reduced melanoidins	2	33.2	56.4
Butylated hydroxytoluene (BHT)	0.2	46.6	52.3

*Trp-P-1 (0.39 n mole) or IQ (0.02 n mole was incubated with and without each sample at 37°C for 30 min prior to preincubation. The numbers of His^+ revertants derived from Trp-P-1 and IQ were 763 and 1975, respectively.

Subsequently, we examined the reducing ability and antioxidative activity of each melanoidin. The results are summarized in Table 4. The reducing ability and antioxidative of each melanoidin increased with increasing molecular weight and color intensity. Melanoidins of above Mw 1000 showed obvious reducing ability and antioxidative activity, whereas the below Mw 1000 fraction showed only weak activities. When nondialyzable melanoidins were reduced with sodium borohydride, their color intensity, reducing ability and desmutagenicity remarkably decreased, but their antioxidative activity did not decrease so much,

Table 4. Antioxidative activity of Maillard reaction products derived from D-glucose-glycine

Maillard reaction product	Color intensity*	Peroxide value (*meq./kg*) per the weight of sample incubated		
		0.5 *mg*	2.0 *mg*	5.0 *mg*
Unfractionated	2.00	147.0	24.0	18.0
Below Mw 1000	0.14	265.0	37.0	31.1
Mw 1000~5000	5.67	41.5	21.0	16.0
Above Mw 5000	7.09	31.1	21.3	16.9
Nondialyzable melanoidins	6.89	31.6	21.7	14.5
Ozone-treated melanoidins	0.52	36.5	28.5	26.2
Reduced melanoidins	2.31	37.0	20.0	17.5
Butylated hydroxyanisole	—	17.7	12.0	10.0
Butylated hydroxytoluene	—	9.0	9.0	9.0

*Indicated as optical density at 470 *nm* (2 *mg* of each Maillard reaction product was dissolved into 1 *ml* of deionized water).

**Peroxide value of linoleic acid alone was 223.0 (Peroxide value of linoleic acid before incubation was 4.0).

3. The adsorption of mutagens on melanoidins.

Table 5 shows the adsorption of Trp-P-1 on the nondialyzable and ozone-treated melanoidins. The ozone-treated melanoidins showed approximately three times more adsorption of Trp-P-1 than the nondialyzable melanoidins.

Table 5. Adsorption of Trp-P-1 on the nondialyzable and ozone-treated melanoidins

Melanoidin	Amount of sample added, (*mg*)	Trp-P-1 adsorbed, (%)
Nondialyzable melanoidins	0.4	3.8
Ozone-treated melanoidins	0.4	10.6

4. The effect of carbonyl groups on the desmutagenicity.

Heat-induced mutagens such as Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2 and IQ have free amino groups in their molecules. Therefore, these amino groups may be modified with carbonyl groups present in melanoidins. We used glyceraldehyde and diacetyl as model carbonyl compounds. The desmutagenic effects of glyceraldehyde and diacetyl on heat-induced mutagens are shown in Figures 4 and 5. The desmutagenicity of glyceraldehyde and diacetyl against each mutagen increased with the dose of the carbonyl compounds. Diacetyl showed much stronger desmutagenicity than glyceraldehyde.

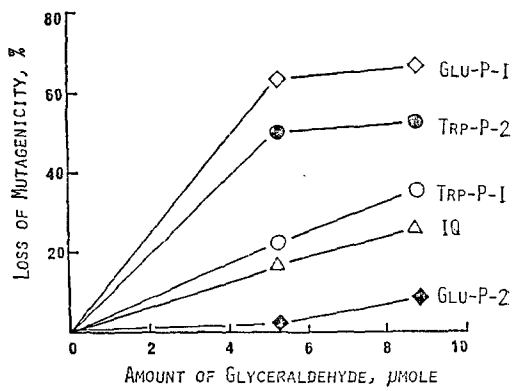

Fig. 4. Inhibition of the mutagenicity of amino acid pyrolysis products by glyceraldehyde, Trp-P-1(0.18 n mole), Trp-P-2(0.08 n mole), Glu-P-1 (0.20 n mole), Glu-P-2 (2.26 n mole) or IQ (0.02 n mole) was incubated with and without glyceraldehyde at 37°C for 30 min prior to preincubation.

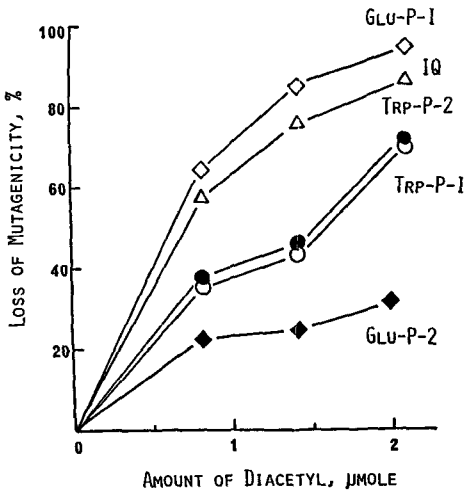

Fig. 5. Inhibition of the mutagenicity of amino acid pyrolysis products by diacetyl. Trp-P-1(0.18 n mole), Trp-P-2(0.08 n mole), Glu-P-1(0.20 n mole), Glu-P-2(2.26 n mole) or IQ (0.02 n mole) was incubated with and without diacetyl at 37°C for 30 min prior to preincubation.

Discussion

Heat-induced heterocyclic mutagens such as Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2 and IQ etc. were shown to be the main mutagenic heterocyclic amines in pyrolyzates of amino acids and proteins, and in cooked foods (Nagao et al., 1983), and were also shown to be carcinogenic in animal experiments (Matsukura et al., 1981; Ohgaki et al., 1984). However, the formation of anti- or desmutagens as well as mutagens on heat processing of foods might be a reasonable assumption. With this in mind, the authors investigated the desmutagenic effect of melanoidins on mutagenic heterocyclic amines, and as a result, a desmutagenic effect of melanoidins was found.

The fractionated melanoidins of above Mw 1000 and nondialyzable melanoidins showed strong desmutagenic effects, whereas the below Mw 1000 fraction did not. The color intensity, reducing ability and antioxidative activity as well as the desmutagenic effect of melanoidins increased with increasing molecular weight.

When nondialyzable melanoidins were reduced with sodium borohydride, the desmutagenicity decreased with the decrease in color intensity and reducing ability, although the antioxidative activity did not decrease so much. All carbonyl groups in melanoidin molecules which would react with amino groups of mutagens and a part of the reductone structure are reduced with sodium borohydride. It has been established that the antioxidative activity of Maillard reaction products is partly due to their reducing ability and reductone structure (Kato, 1973). Furthermore, considerable data showing the inhibition of cancer by antioxidative substances or reducing compounds have been reported (Ames et al., 1981; Ames, 1983).

These facts may suggest that the reducing ability, reductone structure and antioxidative activity of melanoidins play major roles in the desmutagenic effect.

On ozone-treatment of nondialyzable melanoidins, the color intensity and reducing ability decreased, whereas the desmutagenicity remained. In the previous paper (Kim et al., 1985a), we reported that, on ozone-treatment, the carboxylic groups of melanoidins increased and the isoelctric point decreased. In the present study, ozone-treated melanoidins showed three times more adsorption of Trp-P-1 than nondialyzable melanoidins. In this case, the electrostatic interaction between the ozone-treated acidic melanoidins and basic mutagens is considered.

Mutagens induced in pyrolysis of amino acids and proteins have free amino groups in their molecules. These amino groups play an important role in the expression of the mutagenic activity (Hashimoto et al., 1980; Okamoto et al., 1981). If a free amino group of these mutagens was blocked or modified, their strong mutagenic activity will be inhibited. Melanoidins have carbonyl groups in their molecules, and these carbonyl groups increase with ozone-treatment (Kim et al., 1985a). Accordingly, the reactivity of carbonyl groups of melanoidins should not be overlooked. The mutagenicity of these mutagens

was remarkedly suppressed by carbonyl compounds such as glyceraldehyde and diacetyl. The authors identified diacetyl as the most abundant compound in the headspace volatiles formed from a glucose-glycine system (Hayase et al., 1985; Kim and Park, 1986). We have also observed that various carbonyl compounds known to be formed through the Maillard reaction, show desmutagenic effects on mutagenic heterocyclic amines (Kim et al., 1985b; Kim et al., 1986).

In conclusion, we propose that the desmutagenicity of melanoidins against heat-induced mutagens is closely related to their reducing ability and antioxidative activity, due to the reductone structure, and also due to the reactivity of carbonyl groups and further to the electrostatic binding through polyanion groups of melanoidin molecules.

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Desmutagenic Effects of Maillard Reaction Products against Mutagenic Heterocyclic Amines

變異原性 Heterocyclic Amine에 대한 Maillard 反應生成物의 變異原性 抑制效果

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D-glucose-glycine系로 부터 調製한 Maillard 反應生成物을 限外여과로 各 分子量別로 분획(分子量 1,000이하, 1,000~5,000, 5,000이상)하고, 투석에 의하여 非透析性 melanoidin을, 오존처리에 의하여 오존처리 melanoidin을 각각 얻었다. 이들 각 시료를 아미노산 및 단백질의 加熱分解 由來의 變異原性物質인 Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2 및 IQ에 각각 作用(37°C, 30分) 시켜서, 變異原性抑制效果를 檢討하였다. 그 結果, Maillard反應生成物의 變異原性抑制效果는 反應生成物의 分子量의 크기에 비례하여 높게 나타났다. Maillard反應生成物의 還元力 및 抗酸化力 또한 分子量이 큰 획분일수록 크게 나타났다. 그러나, Sodium borohydride로 melanoidin을 還元시켰을 때, melanoidin의 變異原性抑制效果 및 還元力이 減少하였다. 또한, Trp-P-1의 一部가 melanoidin分子中에 吸着되는 것이 밝혀졌고, 카르보닐化合物(diacetyl 및 glyceraldehyde)로 이들 變異原性物質의 아미노기를 修飾함으로써 變異原性物質의 變異原活性이 크게 低下하였다. 따라서, Maillard反應生成物 즉 melanoidin의 變異原性抑制效果는 melanoidin의 還元力 및 抗酸化能을 비롯하여 靜電氣的인 吸着 및 melanoidin分子中의 카르보닐기에 기인한다고 推察된다.