Model System Study for the Mutagenicity of Sugar-Glycine Systems

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Abstract The mutagenicity after heating of different sugars (glucose, fructose, galactose, and tagatose) on the non-enzymatic browning reaction in different sugars and glycine model system was investigated. The model system containing 0.2 M glycine and 0.2 M of different sugars in 10 mL water was heated at $150\pm5^{\circ}C$ for 30 min. After heating, degree of non-browning reaction intensity and mutagenicity using *Salmonella typhimurium* TA 98 were examined. Heated glycine model systems containing different sugars increased their mutgenicity ranged from 30 to 372 revertant colonies. After heating for 40 min, mutagenicity was achieved with glycine model systems containing 4 different sugars with by 145, 356, 206, and 369 revertants per plate, respectively. The glycine model systems containing fructose or tagatose were significantly ($p<0.05$) higher mutagenic activity than glycine model systems containing glucose or galactose after 40 min of heating. The linear regression between Maillard reaction intensity and mutagenic activities (slope=32.38, R^2=0.93) indicates that mutagenicity could be fully ascribed to Maillard reaction products.

Keywords: model system, sugar, glycine, Maillard reaction, mutagenicity

Introduction

When food is cooked, carbonyl and amino compounds react via the Maillard reaction to produce several hundreds of reaction products. Some of these contribute to the color and flavor of the cooked food. The Maillard reaction may also impact the nutritional value of the food (1). Furthermore, in some cases, the Maillard reaction can lead to the formation of food mutagen or carcinogens (2-5). Epidemiological studies have shown that diet and life style are closely related to human cancer. Many mutagens and carcinogens have been identified in foods. Recently, several foods and constituents of foods have been investigated for their inhibitory or promotional effects on carcinogenesis (6,7).

The Maillard reaction takes place in food through a series of reactions between reducing sugars such as glucose and fructose, and compounds possessing available amino groups such as amino acids, peptides, and proteins. The combining of an available amino group and a reducing sugar produces a glycosylamine which undergoes an Amadori rearrangement to produce a 1-amino-2-keto sugar. This intermediate may then be broken down into 2- and 3-carbon fragments by two pathways (3-deoxyhexosone and methyl α-dicarbonyl routes), leading to the formation of a variety of compounds such as aldehydes, ketones, and melanoidin pigments. Pyrazine and pyridines can be produced from the interaction of the α-dicarbonyls from the Maillard reaction with amino acids, the so-called Strecker degradation (8). Aldehydes, furanones, sulfur-containing heterocyclic compounds, pyridines, pyrazines, and pyrroles are volatile compounds produced via the Maillard reaction which contribute to the flavor and aroma of cooked foods. Melanoidines are color compounds formed during the Maillard reaction which contribute to brown color formation in breads and cooked meats (8).

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The Ames *Salmonella typhimurium* mutagenicity assay has been used to test the mutagenicity of Maillard reaction products. *S. typhimurium* strain TA 98, a detector of frameshift mutations, and strain TA 100, a detector of basepair change mutations are the most common strains used in the assay. It has been reported that mutagenicity of Maillard reaction products can be produced with glucose as the source of carbonyl group and amino acids as the source of amino group (5). However, the relationship of reaction mixtures consisting of different sugars to mutagenicity of Maillard reaction products is less known. Therefore, the objectives of this study were to investigate the effects of different sugars on the non-enzymatic browning reaction and mutagenicity in different sugars and glycine model system.

Materials and Methods

Materials All chemicals were analytical grade. Glucose, fructose, glycine, and galactose were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) and tagatose were supplied by Fluka Chemical Co. (Buchs, Switzerland).

The heating module was an Isotemp, model DW110, made by Daeil Technol. Co. (Seoul, Korea). Stainless steel test tubes, 2.3 mL capacity, with threaded, self-sealing stainless steel caps were manufactured by the Dongwha Mechanic & Machine Shop (Seoul, Korea). A new set of stainless steel test tubes was used for each amino acid to avoid carryover from one experiment to another.

Model systems The model system contained 0.2 M glycine and 0.2 M of different sugars in 10 mL water. The reactants were added directly to the stainless test tubes and sealed with threaded caps wrapped with teflon tape. The reaction was carried out in a closed hood. The heating module was preheated for a minimum of 1.5 hr before heating the samples. The heating temperature was $150\pm5^{\circ}C$ and silicon oil (0.5 mL) was placed in each cavity in the heating block to facilitate heat transfer from the block to the test tubes. The stainless test tubes were heated for 30

min and then immediately cooled in an ice-bath. The contents of each test tube were quantitatively transferred to microvials (1.5 mL capacity) and stored at $5\pm1°C$ until required.

Degree of browning reaction intensity The browning reaction intensity of the heated model system reaction mixtures was determined by measuring the absorbance at 420 nm with a ultra violet (UV)/vis spectrophotometer (Mecasys Co., Seoul, Korea). The samples were diluted 4 times with 16%(w/w) sodium dodecyl sulfate (SDS) to reduce scattering due to protein aggregation. Distilled water was used as a blank.

***Salmonella* mutagenicity assay** The mutagenic activity of the sample extracts was determined using the standard plate incorporation assay described by Ames *et al.* (9) using *Salmonella typhimurium* TA98 (Molecular Toxicology, Inc., Boone, NC, USA). Aroclor-induced rat liver S-9 mixture (Molecular Toxicology, Inc.) was used for metabolic activation. Dimethyl sulfoxide (DMSO) was used as a negative control (spontaneous revertant colonies), while 2-aminoanthracene was used as a positive control for *S. typhimurium* TA98. Negative control (spontaneous revertant colonies) and positive control gave an average of 26 ± 3 and 859 ± 37 revertants/μg, respectively. The mutagenic activity was corrected for spontaneous mutagens by subtracting the number of revertants in the negative control. The result of the assay was considered mutagenic if the total number of revertants per plate was at least twice as high as the number of spontaneous revertants per plate. Mutagenic activity was calculated from the linear portion of the dose-response curve. A minimum of 4 dose points from duplicate platings were used, and the linear portion of the curves was used to calculate the revertants/plate.

Statistical analysis The results were analyzed by Sigma Stat 2.0 (Jandel Corp., San Rafael, CA, USA). One-way analysis of variance (ANOVA) was performed. Appropriate comparisons were made using the Student-Newman-Keuls test for one-way ANOVA analysis. Calculation of mutagenic activity was made by linear regression analysis of the dose response curves of revertants/plate.

Results and Discussion

Heated glycine model systems containing different sugars (glucose, fructose, galactose, and tagatose) were tested for their mutagenicity. A dose response of mutagenic activity was observed in model systems heated at 150°C for 40 min (Fig. 1). When sample extracts were added at the 0.1, 0.2, 0.4, and 0.6 mL levels per plate, heated model systems increased the mutagenicity ranged from 30 to 372 revertant colonies. Analysis of variance revealed that the addition of heated different sugars and glycine model systems at levels from 0.1 to 0.4 mL samples per plate significantly increased the mutagenicity ($p<0.05$). However, statistical analysis revealed no significant difference between the mutagenicity achived with 0.4-0.6 mL samples per plate ($p>0.05$) (Fig. 1).

Based on the results of this study, 0.4 mL of samples per plate of different sugars (glucose, fructose, galactose, and tagatose) and glycine model systems were chosen for further
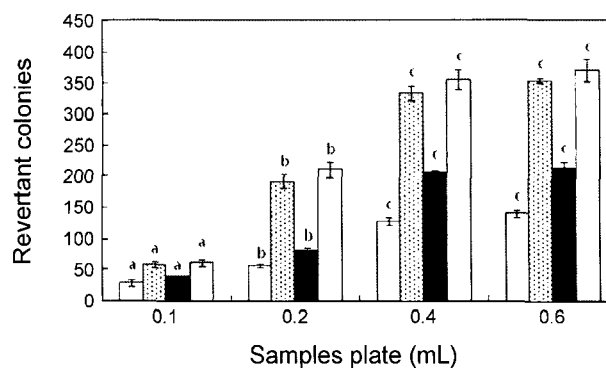
Fig. 1. Dose response of mutagenicity from glucose-glycine (blank), fructose-glycine (black dotted), galactose-glycine (black), and tagatose-glycine (gray) heated for 40 min. Means with different letters are not significantly different ($p<0.05$); comparisons are made only within the same bar. Means±SD; $n=3$ for all treatments.

investigation of heating time response of mutagenicity in model systems.

Heating time response on the mutagenicity of different sugars (glucose, fructose, galactose, and tagatose) and glycine model systems were evaluated by the Ames *S. typhimurium* assay using the tester strain TA98 (Table 1). The mutagenicity of the model systems varied with the different sugars. Different sugars and glycine model systems showed an increase of revertant colonies with heating time. After heating for 40 min, mutagenicity was achieved with 4 different sugars (glucose, fructose, galactose, and tagatose) with glycine model systems by 145, 356, 206, and 369 revertants per plate, respectively. The glycine model system containing fructose or tagatose showed a significantly higher mutagenic activity than glycine model systems containing glucose or galactose after 40 min of heating ($p<0.05$). Results of this study showed that mutagenicity could be related to Maillard reaction products. Brands *et al.* (10) demonstrated that sugars with higher reactive in the Maillard reaction form higher amounts of mutagenic compounds. The mutagenic activity of galactose-glycine system was higher than that of the glucose-glycine system. The increased mutagenic activity of galactose-glycine system could be explained by its higher amount present in the acyclic form of sugars (11). The ketose (fructose and tagatose)-glycine system showed the higher mutagenicity as compared to aldose (glucose and galactose)-glycine system. The difference in mutagenicity

**Table 1. Effect of different sugars on mutagenicity from sugar-glycine model systems[1]**

Sugar	Heating time (min)		
	10	20	40
Glucose	31.7 ± 5.2^a	69.8 ± 6.5^a	145.6 ± 10.5^a
Fructose	101.3 ± 12.6^{bc}	269.5 ± 14.5^{bc}	355.7 ± 27.1^c
Galactose	87.4 ± 10.6^b	129.9 ± 13.1^b	206.0 ± 16.5^b
Tagatose	112.8 ± 18.5^{bc}	305.3 ± 23.6^{bc}	368.8 ± 31.2^c

[1]Means in the same columns bearing different superscripts are significantly different ($p<0.05$); data represent the triplicate mean±SD of per treatment.

Table 2. Nonenzymatic browning (absorbance 420 nm) of different sugars and glycine model systems[1]

Sugar	Heating time (min)		
	10	20	40
Glucose	1.8 ± 0.4^a	4.1 ± 1.2^a	6.3 ± 1.5^a
Fructose	4.1 ± 1.5^a	7.3 ± 2.5^{ab}	11.6 ± 2.8^a
Galactose	3.2 ± 1.2^a	5.8 ± 2.0^a	8.1 ± 2.5^a
Tagatose	5.3 ± 1.8^b	9.4 ± 2.6^b	13.4 ± 2.2^b

[1]Means in the same columns bearing different superscripts are significantly different ($p<0.05$); data represent the triplicate mean±SD of per treatment.
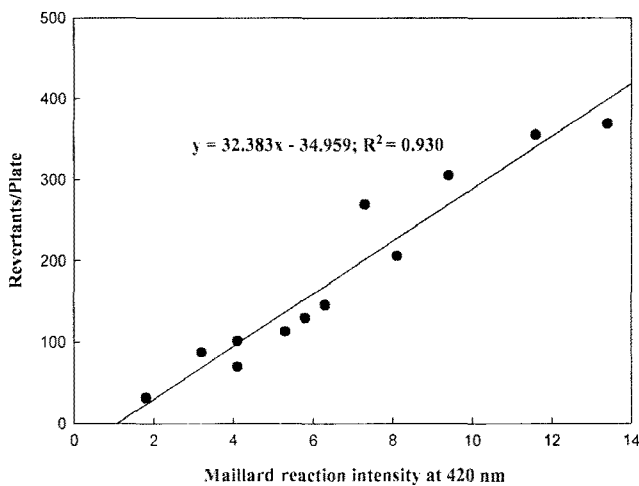
Fig. 2. Plot of mutagenic activity measured in *S. typhimurium* TA98 and Maillard reaction intensity at 420 nm in different sugars and glycine model systems.

between the ketose and aldose-glycine systems was caused by a difference in Maillard reactivity and difference in reaction mechanism.

Table 2 shows the changes of optical density in different sugars and glycine model systems with respect to different heating time. The optical density of different sugars and glycine model systems increased with heating time. The color development of non-enzymatic browning with different sugars and glycine model systems was in the following descending order: galactose > glucose. The ketose sugars browned more quickly than their aldose isomers.

The plot of Maillard reaction intensity and mutagenic activity is shown in Fig. 2. The linear regression between Maillard reaction intensity and mutagenic activities (slope=32.38, R^2=0.93) indicates that mutagenicity coud be fully ascribed to Maillard reaction products. These observations agree with those of Brands *et al.* (10) who reported that different sugars reacted with casein model systems showed a higher mutagenic activity corresponding with a higher Maillard reactivity. Also, the ketose-glycine system showed the higher mutagenic activity compared with the aldose-glycine system. The results presented here indicate that the higher Maillard reactivity of ketose-glycine system, as measured by spectrophotometer analyses, is accompanied by a concomitant increase in the mutagenicity of the model system. These results agree with the data of Powrie *et al.* (12), who demonstrated that mutagenicity

could be fully ascribed to Maillard reaction products. The difference in mutagenic activity was caused by a difference in chemical reaction mechanism between ketose and aldose. Ketose contributes to browning through caramelization reaction, whereas caramelization may not be as a significant browning mechanism in aldose system (8). The scatter in the data is probably due to a combination of measurement errors in model system procedures and the accumulation of errors in each analytical method. The Maillard reaction produces numerous mutagenic compounds, where as some may be antimutagenic compounds (13,14). Brands *et al.* (10) demonstrated that the amount of antimutagenic compounds formed in the ketose-casein system was much lower than in the aldose-casein system, where as the higher mutagenic activity formed in the ketose-casein system compared with the aldose-casein system. This study demonstrated that differences in mutagenic activity due to the Maillard reaction between ketoses and aldoses. More research is required to provide a better understanding with mutagenicity due to the Maillard reaction for food industry.

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