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Influence of Maillard Reaction Products on DNA Damage in Human Lymphocytes

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The effect of Maillard reaction products (MRPs) on induced DNA damage in human lymphocytes was investigated using single-cell gel electrophoresis (comet assay). Three MRPs, Xyl-Lys MRP, Glu-Lys MRP, and Fru-Lys MRP, were prepared by heating lysine with xylose, glucose, and fructose, respectively, at pH 9.0 and 100 °C for 3 h and called undialyzed MRPs. The prepared MRPs were further dialyzed, and three undialyzable MRPs were obtained. The undialyzed MRPs caused significant ($p < 0.05$) DNA damage in human lymphocytes at a concentration of 0.05−0.1 mg/mL by the comet assay. Compared with the control, the undialyzable Xyl-Lys MRP and Glu-Lys MRP caused significant DNA damage in human lymphocytes at a concentration >0.1 mg/mL, whereas Fru-Lys MRP did so at a concentration >0.2 mg/mL. Moreover, undialyzed MRPs caused less DNA damage than did undialyzable MRPs. The undialyzable MRPs did not affect the activity of glutathione peroxidase or lipid peroxidation in human lymphocytes at a concentration of 0.05−0.8 mg/mL. However, these three undialyzable MRPs decreased the glutathione (GSH) contents and the activities of GSH reductase and catalase in human lymphocytes. On the basis of the results of the formation of 8-hydroxy-2′-deoxyguanosine, radicals, and hydrogen peroxide, the radicals might play an important role in the DNA damage in human lymphocytes induced by these MRPs in this reaction system.

KEYWORDS: Maillard reaction products; lysine; free radical; DNA damage; lymphocytes

INTRODUCTION

The Maillard reaction, between carbonyl and amino compounds, is an important reaction that occurs in foods during processing and storage. The reaction can occur under severe or mild heating conditions and through many complex chemical intermediates, which ultimately lead to the production of brown polymeric compounds, known as melanoidins. The formation of Maillard reaction products (MRPs) is greatly influenced by both the reaction conditions and the sources of the reaction sugars and amino acids. The Maillard reaction has been extensively studied from various chemical, technological, physiological, and toxicological points of view in foods and medicines (*1*).

Many studies have reported that MRPs exhibit antioxidant activity (*2*), antimutagenicity (*3*), and mutagenicity (*4*) in in vitro systems. Furthermore, some studies also have indicated that MRPs may potentially induce DNA damage. Wijewickreme and Kitts (*5*) found that Glu-Lys and Fru-Lys MRPs induced PM2 bacteriophase DNA damage. Hiramoto et al. (*6*) found that hydroxyquinone isolated from MRPs in coffee extract also exhibited the ability to break DNA strands. Some dihydropyrazines were also reported to induce DNA strand breakage (*7*). Pischetsrieder et al. (*8*) found that aminoreductones have antioxidant activity and that they cause oxidative damage of protein and lipoprotein in the presence of reducing agents and metal ions. In addition, flavonoid quercetin can behave as both an antioxidant and a prooxidant, depending on its concentration. It was also clearly demonstrated that quercetin could protect cutaneous tissue-associated cell types from injury induced by oxidative stress (*9*). Furthermore, the *o*-quinone produced by the metabolic activation of quercetin can induce cell damage (*10*). Although the antioxidant properties of MRPs have been studied in various investigations, it would be interesting to know whether MRPs play a protective or damaging role in human cell systems. In our previous study (*11*), Xyl-Lys MRP, Glu-Lys MRP, and Fru-Lys MRP showed higher brown intensity. Therefore, the purpose of this study was to investigate the effect of MRPs (Xyl-Lys MRP, Glu-Lys MRP, and Fru-Lys MRP) on DNA damage in human lymphocytes and to elucidate their possible mechanisms.

MATERIALS AND METHODS

Materials. D-Fructose, D-glucose, D-xylose, L-lysine monohydrochloride, 1-chloro-2,4-dinitrobenzene (CDNB), glutathione (GSH), glutathione disulfide (GSSG), glutathione reductase, NADPH, Triton X-100, trypan blue, ethidium bromide, peroxidase (from horseradish),

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proteinase K, nuclease P1, 8-OH-dG, and 2′-dG were obtained from the Sigma Chemical Co. (St. Louis, MO). Agarose, low-melting-point agarose, RPMI 1640 mediun, Dulbecco's modified Eagle medium, fetal bovine serum (FBS), trypsin EDTA (T/E), penicillin−streptomycin, L-glutamine, MEM sodium pyruvate solution, and MEM nonessential amino acid (NEAA) were purchased from Gibco BRL Co. (Grand Island, NY). Snakeskin pleated dialysis tubing (MWCO = 7000) was obtained from the Pierce Chemical Co. (Rockford, IL). All other reagents were of analytical grade.

Preparation of Maillard Reaction Products. MRPs were prepared under the reaction conditions described by Yen and Hsieh (*3*). Three MRPs, Xyl-Lys MRP, Glu-Lys MRP, and Fru-Lys MRP, were prepared by heating lysine (0.1 mol) with xylose, glucose, and fructose (0.1 mol), respectively, at pH 9.0 and 100 °C for 3 h and were called undialyzed MRPs. The pH values of the above three reaction mixtures after heating were 6.5, 5.06, and 5.99, respectively. The prepared MRPs were further dialyzed with dialysis tubing (MWCO = 7000) against deionized distilled water at 4 °C overnight, and three undialyzable MRPs were obtained. The undialyzed MRPs and undialyzable MRPs were freeze-dried and stored at −20°C for further use.

Lymphocyte Isolation. Blood samples were obtained from healthy volunteers (female and male) not exceeding the age of 35 years. Blood was drawn by venipuncture and heparinized with a calparine. Red blood cells and granulocytes were removed from undiluted whole blood using a cell separation tube. Lymphocytes were separated using a separation medium with a density of 1.077 g/mL, composed of an aqueous solution of Ficoll (57 g/L). The purity of the prepared lymphocytes was >96% as determined by Giemsa staining.

Cytotoxicity Analysis. To measure the acute cytotoxicity, 0.49 mL of each original cell suspension was mixed with 10 μL of a 0.4% trypan blue solution and checked for viability 5 min later. The cells were analyzed through microscopic observation, and the percentage of viable cells was determined (*12*).

Analysis of DNA Damage (Comet Assay). The effect of MRPs on DNA damage in human cells was investigated using the comet assay or single-cell gel electrophoresis under alkaline conditions according to the method of Singh et al. (*13*) with slight modification. The MRPs were adjusted to different concentrations by dissolving them in deionized−distilled water and then added into cell suspension treated at 37 °C for 30 min. After treatment with MRPs, the cells were centrifuged and resuspended in preheated 1% low-melting-point agarose. The cell suspension was then added to Dakin fully frosted microscope slides (Menzel-Glaser, Germany), precoated with 1% normal melting agarose. After application of a third layer of 1% low-melting-point agarose, the slides were immersed in cold lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% sodium lauryl sarcosineaate, 1% Triton X-100, 10% DMSO, pH 10) for at least 1 h at 4 °C. The microscope slides were then placed in an electrophoresis tank, and the DNA was allowed to unwind for 20 min in freshly prepared alkaline electrophoresis buffer (1 mM EDTA, 0.3 N NaOH, pH 13). Electrophoresis was conducted at 4°C for 20 min at 25 V and 300 mA. The slides were then neutralized with Tris buffer (0.4 M, pH 7.5) and stained with ethidium bromide. All of the steps were performed under yellow light to prevent additional damage. The slides were observed using a fluorescent microscope attached to a CCD camera connected to a personal computer based image analysis system (Komet 3.0; Kinetic Imaging Ltd.). For each analysis, 50 individual cells were calculated, and in most cases, three separate experiments were conducted for each series. Single cells were analyzed under the fluorescent microscope as desired. The DNA damage was expressed as percent tail DNA, where percent tail DNA = tail DNA/(head DNA + tail DNA) × 100. A higher percent tail DNA meant a higher level of DNA damage.

Determination of Glutathione Content. The content of glutathione in cells was measured according to the method of Hu et al. (*14*). The cell lysate (0.2 mL) was mixed with 5% TCA (0.8 mL) and centrifuged after cooling on ice for 5 min. The supernatant (0.3 mL) was mixed with 0.7 mL Tris/EDTA (pH 8.9), and 20 μL of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) for 5 min, and the absorbance was read at 412 nm.

Assays for Enzymes. The activity of glutathione reductase was measured according to the method of Bellomo et al. (*15*). A mixture of 1.1 mM magnesium chloride, 5 mM GSSG, 0.1 mM NADP, 100 mM phosphate buffer (pH 7.0), and cell lysate (0.1 mL) in a final volume of 1 mL was incubated at 37 °C for 30 min. The change in absorbance at 340 nm was monitored for 3 min. The enzyme activity was calculated from the standard curve and expressed as micromoles of NADPH per minute per milligram of protein.

The glutathione peroxidase activity assay was performed according to the method of Lawrence et al. (*16*). The cell lysate (0.1 mL) was incubated at 37 °C for 5 min with 0.8 mL of 100 mM phosphate buffer (pH 7.0), containing 1 mM EDTA, 1 mM sodium azide, 1 mM GSH, 1 unit/mL glutathione reductase, and 0.2 mM NADPH. After 0.1 mL of 2.5 mM hydrogen peroxide had been added, the decrease of absorbance at 340 nm was recorded for 5 min. The glutathione peroxidase activity was calculated according to the standard curve and expressed as micromoles of NADPH per minute per milligram of protein.

Catalase activity was assayed according to the method of Cohen et al. (*17*). A mixture of 50 mM phosphate buffer (pH 7.0), 20 mM H_2O_2, and cell lysate (0.1 mL) in a final volume of 1 mL was incubated at 37 °C for 30 min. The decrease in absorbance was recorded at 240 nm for 2 min. The catalase activity was calculated from the standard curve and expressed as micromoles per minute per milligram of protein.

Determination of Lipid Peroxidation. Lipid peroxidation in cells was assayed according to the method of Chirico et al. (*18*). Briefly, at the end of the 30-min incubation period at 37 °C, cells were collected and washed with PBS. Cell homogenate was mixed with 0.6 M TBA and 10% TCA and then heated in boiling water for 10 min. After cooling, malondialdehyde (MDA) was measured at 532 nm and expressed as nanomoles per milligram of protein.

Determination of 8-OHdG in DNA. DNA from lymphocytes was isolated using the method described by Miller et al. (*19*) with a small modification. Briefly, lymphocytes were suspended in 3 mL of nuclei lysis buffer (10 mM Tris-HCl, 100 mM NaCl, 25 mM EDTA, pH 8.2) and following the addition of 0.2 mL of 10% SDS and 0.5 mL of solution containing 1 mg of proteinase K in 0.5% SDS, 2 mM EDTA was digested for 5 h in the dark. Then, proteins were precipitated by 1 mL of saturated NaCl, followed by centrifugation for 15 min at 2000*g* and 4°C. Supernatant containing nucleic acids was treated with 2 volumes of cold absolute ethanol to precipitate high molecular weight DNA. The precipitate was removed with a plastic spatula washed with 75% ethanol and, after centrifugation, dissolved in 300 μL of water. DNA samples were stored at −70 °C until hydrolysis was performed.

For DNA hydrolysis to nucleosides, DNA samples (200 μL) were mixed with 100 μL of 40 mM sodium acetate, 0.1 mM $ZnCl_2$ (pH 5.1), and 20 μL of nuclease P1 solution (20 μg of protein). Samples were incubated for 1 h at 37 °C. Thereafter, 30 μL of 1 M Tris-HCl (pH 7.4) and 5 μL of alkaline phosphate solution containing 1.5 units of the enzyme were added to each sample following 1 h of incubation at 37 °C. All DNA hydrolysates were ultrafiltered using an Ultrafree-MC filter unit (cutoff = 5000 kDa). 8-OHdG and 2′-dG in hydrolysates were determined using HPLC with electrochemical and UV detector systems. DNA hydrolysates were chromatographed isocratically using 20 mM ammonium acetate (pH 5.3)/MeOH (85:15, v/v). Detection of 2′-dG was performed at 290 nm (Hitachi, L-4200). 8-OHdG was determined using an electrochemical detector (ECD, Merck, L-3500A). The amount of 8-OHdG in DNA was expressed as 8-OHdG molecules per 10^5 dG.

Determination of Free Radical Generation. Free radical generation was measured according to the method of Hiramoto et al. (*20*) with a reaction mixture containing 0.2 mM 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) and different concentrations of MRPs. The EPR spectra were recorded on a Burker (EMX-10/12) spectrometer. All spectra were recorded at room temperature.

Effect MRPs on Hydrogen Peroxide Generation. The effect of MRPs on H_2O_2 generation was determined using the method described by Rinkus and Taylor (*21*). A solution containing horseradish peroxidase (HRPase) and phenol red was mixed with different concentrations of MRPs for 30 min. The reaction was terminated in an ice bath for 10

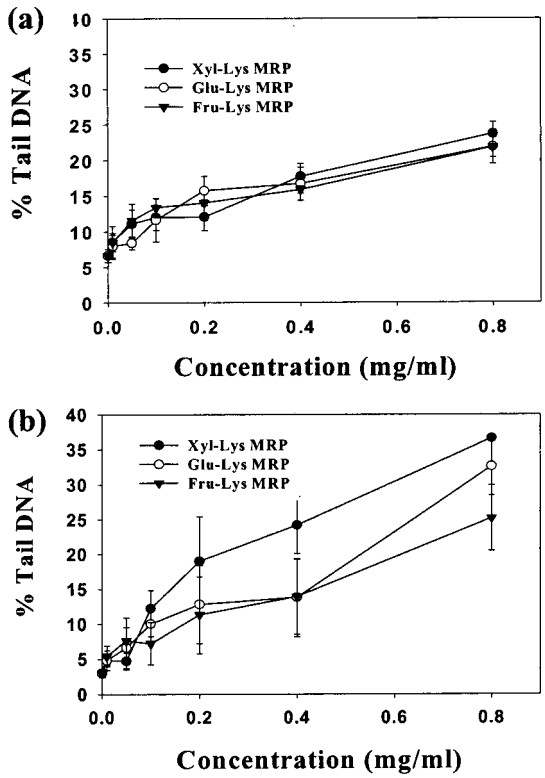

Figure 1. DNA damage of human lymphocytes treated with undialyzed (a) and undialyzable (b) Maillard reaction products. Results are mean ± SD for $n \geq 3$.

min, and then the amount of H_2O_2 was determined spectrophotometrically (Hitach U-2000, Japan) at 610 nm.

Statistical Analysis. All analyses were run in triplicate and the results averaged. Statistical analyses were performed according to the *SAS Institute User's Guide*. Analyses of variance were performed using the ANOVA procedure. Significant differences ($p < 0.05$) between the means were determined using Duncan's multiple-range test.

RESULTS AND DISCUSSION

Effect of MRPs on DNA Damage in Human Lymphocytes. In this study, human lymphocytes were used to study the effect of MRPs on DNA damage in human cells. The cytotoxicity of undialyzed and undialyzable MRPs to human lymphocytes was evaluated. The observed cell viability was >95% when undialyzed and undialyzable MRPs (0.05−0.8 mg/mL) were incubated with cells at 37 °C for 30 min (data not shown). Thus, MRPs showed no cytotoxicity to human lymphocytes under the tested concentrations.

Figure 1 shows DNA damage in human lymphocytes treated with different concentrations (0−0.8 mg/mL) of undialyzed and undialyzable MRPs at 37 °C for 30 min and measured using the comet assay. The results indicated that undialyzed Xyl-Lys MRP and Fru-Lys MRP showed obvious DNA damage at a concentration of 0.05 mg/mL, with a percent tail DNA of ~12%; however, Glu-Lys MRP showed the same percent tail DNA at a concentration of 0.1 mg/mL (**Figure 1a**). At a concentration of 0.1 mg/mL, undialyzable Xyl-Lys MRP and Glu-Lys MRP had 12 and 10% tail DNA, respectively; these values are significantly different ($p < 0.05$) from that of the control group (**Figure 1b**). The undialyzable Fru-Lys MRP showed 11% tail DNA at a concentration of 0.2 mg/mL (**Figure 1b**). At a concentration of 0.8 mg/mL, the DNA damage induced by MRPs was markedly increased; Xyl-Lys MRP caused the greatest damage, whereas Fru-Lys MRP caused the least. The results also indicated that undialyzed MRPs caused less DNA damage than did undialyzable MRPs. The reason for this finding might have been that the undialyzed MRPs contained some compounds with low molecular weights. Hiramoto et al. (*20*) reported that MRPs could generate reactive oxygen radicals and proposed that MRP-induced DNA damage was due to the high molecular weight polymer. Therefore, undialyzable MRPs with molecular weights >7000 might be the main substance causing DNA damage. Weijewickreme and Kitts (*5*) indicated that Glu-Lys and Fru-Lys MRPs induced damage in PM2 bacteriophage DNA at concentrations >0.01%. They attributed DNA strand breakage by MRPs to the metal ion derived Fenton reaction. Hiramoto et al. (*22*, *23*) reported that 4-hydroxy-2-(hydroxymethyl)-5-methyl-3(2*H*)-furanone (HHMF) and 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP), derived from the glucose/glycine Maillard reaction, could produce hydroxyl radical and induce DNA strand breakage.

Effect of MRPs on DNA Damage in Human Lymphocytes Induced by Hydrogen Peroxide. The effect of undialyzable MRPs on DNA damage in human lymphocytes induced by H_2O_2 (50 μM) was investigated. The undialyzable MRPs showed no inhibitory effect on DNA damage in human lymphocytes induced by H_2O_2 ($p > 0.05$) (data not shown). In addition, the DNA damage in human lymphocytes induced by H_2O_2 was increased by the undialyzable MRP treatment, but the damage was not obvious. This means that the undialyzable MRPs could not effectively scavenge the H_2O_2 in the lymphocytes. Hayase et al. (*24*) reported that glucose−glycine MRP has the ability to scavenge H_2O_2. However, Ookawara et al. (*25*) and Elgawish et al. (*26*) found that reactive oxygen species (ROS) or H_2O_2 formed during the Maillard reaction. Kim et al. (*27*) also pointed out that MRPs exhibited mutagenicity, and the ROS, especially singlet oxygen and H_2O_2, enhanced mutagenicity. Therefore, when MRPs, H_2O_2, and human lymphocytes were mixed together, H_2O_2 could increase the extent of DNA damage in cells induced by MRPs.

To determine whether the DNA damage in cells was caused by free radicals, free radical scavengers (superoxide dismutase, catalase, and mannitol) and metal chelator (EDTA) were added in the reaction mixture to study their effects on DNA damage in human lymphocytes treated with MRPs. As shown in **Figure 2**, the addition of mannitol and EDTA had no influence on DNA damage in lymphocytes as compared with the control group. However, the addition of SOD and catalase could reduce DNA damage by 22 and 19%, respectively. This means that the superoxide anion and hydrogen peroxide might have been formed during the reaction between MRPs and lymphocytes. The SOD and catalase could scavenge superoxide and hydrogen peroxide and reduce DNA damage. Namiki and Hayashi (*28*) and Roberts and Lloyd (*29*) reported that the Maillard reaction produced *N*,*N*-dialkylpyrazinium radical cations. Elgawish et al. (*26*) and Ookawara et al. (*25*) also found that ROS or H_2O_2 was produced during the Maillard reaction. Thus, free radicals might be the main mechanism by means of which MRPs can damage DNA in cells.

Effect of MRPs on the Activities of Antioxidant Enzymes. Glutathione (GSH) and antioxidant enzymes, such as glutathione peroxidase, glutathione reductase, and catalase, can protect cells or tissues against free radical induced damage (*30*). Thus, a decrease or loss of antioxidant enzyme activity in cells may cause oxidative stress and induce lipid peroxidation, DNA damage, and cell injury, resulting in cell death (*9*).

GSH in cells has many functions. When the GSH was oxidized to GSSG and the activity of glutathione reductase was

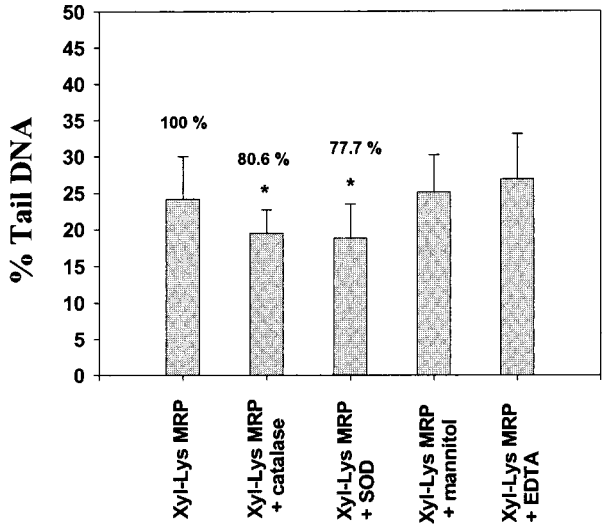

Figure 2. Effects of scavengers of active oxygen radicals and metal chelator on DNA damage in human lymphocytes induced by undialyzable Maillard reaction products (treated without hydrogen peroxide). A mixture of 0.4 mg/mL Xyl-Lys MRP with 0.5 mg/mL catalase, 150 units/mL SOD, 0.1 M mannitol, or 20 mM EDTA was incubated at 37 °C. Results are mean ± SD for $n = 3$. * = Means significantly different by comparison with the control ($p < 0.05$).

Table 1. Effect of Undialyzable Maillard Reaction Products on Glutathione Content in Human Lymphocytes

MRP concn (mg/mL)	glutathione[a] (nmol/mg of protein)		
	Xyl-Lys MRP	Glu-Lys MRP	Fru-Lys MRP
0	30.2 ± 1.2^{ab}	30.2 ± 1.2^{a}	30.2 ± 1.2^{a}
0.05	26.4 ± 6.3^{a}	28.9 ± 5.3^{a}	27.5 ± 2.1^{a}
0.1	26.3 ± 3.4^{a}	26.0 ± 3.3^{a}	23.6 ± 3.4^{b}
0.2	25.9 ± 2.3^{b}	26.7 ± 1.9^{b}	24.9 ± 3.4^{b}
0.4	24.7 ± 3.2^{b}	27.1 ± 8.8^{b}	25.7 ± 1.5^{b}
0.8	23.0 ± 2.2^{b}	23.7 ± 4.2^{b}	24.9 ± 2.6^{b}

[a] Values are means ± SD of triplicate determinations of DTNB conjugated formed. [b] Values in a column with different superscripts are significantly different ($p < 0.05$).

also inhibited or decreased, the reduction of GSSG in cells was limited, the cells were exposed to strong oxidative stress, and they could easily die (*9*). **Table 1** shows that all three undialyzable MRPs significantly ($p < 0.05$) decreased the GSH content in human lymphocytes at concentrations of 0.1−0.8 mg/mL.

When the GSH in cells was oxidized to GSSG, glutathione reductase could reduce the GSSG to GSH and keep the glutathione redox cycle in balance in cells (*9*). **Table 2** shows the effect of undialyzable MRPs on glutathione reductase activity in human lymphocytes. All three undialyzable MRPs significantly ($p < 0.05$) decreased the glutathione reductase activity at a concentration >0.4 mg/mL.

There was no significant difference ($p > 0.05$) in glutathione peroxidase activity in the human lymphocytes following treatment with undialyzable MRPs as compared with the control group (data not shown). This means that the undialyzable MRPs did not change the glutathione peroxidase activity in the human lymphocytes.

Catalase exists in peroxisomes, lysomes, and mitochondria; it plays an important role in protecting cells against oxidative damage by catalyzing hydrogen peroxide to water and oxygen (*31*). As shown in **Table 3**, Xyl-Lys and Fru-Lys MRP could

Table 2. Effect of Undialyzable Maillard Reaction Products on Glutathione Reductase Activity in Human Lymphocytes

MRP concn (mg/mL)	activity[a] (μmol/min/mg of protein)		
	Xyl-Lys MRP	Glu-Lys MRP	Fru-Lys MRP
0	30.2 ± 4.3^{ab}	30.2 ± 4.3^{a}	30.2 ± 4.3^{a}
0.05	29.9 ± 0.6^{a}	29.5 ± 2.5^{a}	29.4 ± 3.2^{a}
0.1	28.8 ± 1.5^{a}	28.3 ± 4.7^{a}	28.5 ± 0.9^{a}
0.2	29.1 ± 5.8^{a}	29.6 ± 2.5^{a}	27.8 ± 0.9^{a}
0.4	25.8 ± 1.7^{b}	28.3 ± 2.1^{a}	25.8 ± 2.1^{b}
0.8	22.4 ± 2.4^{b}	24.6 ± 3.2^{b}	23.0 ± 2.3^{b}

[a] Values are means ± SD of triplicate determinations of NADPH. The concentration of the NADPH was calculated by using a molar absorption coefficient of 6220 M^{-1} cm^{-1}. [b] Values in a column with different superscripts are significantly different ($p < 0.05$).

Table 3. Effect of Undialyzable Maillard Reaction Products on Catalase Activity in Human Lymphocytes

MRP concn (mg/mL)	activity[a] (μmol/min/mg of protein)		
	Xyl-Lys MRP	Glu-Lys MRP	Fru-Lys MRP
0	73.0 ± 5.6^{ab}	73.0 ± 5.6^{a}	73.0 ± 5.6^{a}
0.05	68.6 ± 7.1^{ab}	71.7 ± 7.1^{a}	75.1 ± 5.3^{a}
0.1	63.9 ± 0.8^{abc}	67.7 ± 2.8^{ab}	77.5 ± 12.5^{a}
0.2	63.4 ± 7.3^{abc}	64.1 ± 1.6^{ab}	68.0 ± 4.2^{ab}
0.4	60.5 ± 5.1^{bc}	62.2 ± 7.3^{ab}	65.2 ± 1.7^{b}
0.8	56.5 ± 3.2^{c}	58.1 ± 10.1^{b}	63.4 ± 5.1^{b}

[a] Values are means ± SD of triplicate determinations by following the decomposition of hydrogen peroxide. [b] Values in a column with different superscripts are significantly different ($p < 0.05$).

significantly ($p < 0.05$) decrease the catalase activity at a concentration >0.4 mg/mL. Glu-Lys MRP also markedly decreased the catalase activity at a concentration of 0.8 mg/mL. As the concentration of the MRPs increased, the activity of catalase decreased.

Ukeda et al. (*32*) pointed out that Maillard reaction intermediate products or glycation products can reduce the activity of Cu,Zn-superoxide dismutase. Ueda et al. (*33*) also indicated that pentosidine, an advanced glycation end product, was negatively correlated with the activity of glutathione peroxidase. Methylglyoxal, a Maillard reaction product, has been reported to decrease the GSH content and the activities of SOD, catalase, GST, and glyoxalases I and II and to increase lipid peroxidation (*34*). This indicates that the complicated Maillard reaction products might contain many substances which can reduce the activities of antioxidant enzymes. The results shown above reveal that undialyzable MRPs not only caused DNA damage but also decreased the GSH content and the activities of glutathione reductase and catalase in human lymphocytes.

Effect of MRPs on Lipid Peroxidation in Cells. The effect of undialyzable MRPs on changes of lipid peroxidation in human lymphocytes was also studied to understand the possible mechanism of MRP-induced genotoxicity. The TBARS values in human lymphocytes showed no significant change ($p < 0.05$) following treatment with all three undialyzable MRPs (data not shown). Yang and Schaich (*35*) indicated that lipid hydroperoxides and aldehyde could induce DNA damage. However, Halliwell and Gutteridge (*36*) indicated that oxidative stress induced cell membrane injury was due not just to lipid peroxidation. Cell injury induced by other molecules, such as proteins, on membranes was more severe than that caused by lipid peroxidation. In this study, MRPs did not change lipid peroxidation in human lymphocytes following reaction at 37

Table 4. Formation of 8-OHdG in Human Lymphocyte DNA Exposed to Undialyzable Maillard Reaction Products

MRP concn (mg/mL)	8-OHdG/10^5dG[a]		
	Fru-Lys MRP	Glu-Lys MRP	Xyl-Lys MRP
0	2.3 ± 0.4^{ab}	2.3 ± 0.4^{a}	2.3 ± 0.4^{a}
0.05	2.2 ± 0.4^{a}	2.8 ± 0.4^{a}	3.5 ± 0.5^{a}
0.2	3.5 ± 0.6^{a}	4.0 ± 0.4^{b}	5.9 ± 0.7^{b}
0.4	6.7 ± 1.1^{b}	6.8 ± 1.2^{c}	9.1 ± 2.1^{c}
0.8	11.6 ± 2.5^{c}	11.9 ± 2.9^{d}	15.6 ± 3.7^{d}

[a] 8-OHdG and dG in hydrolysates were determined in duplicate using HPLC with electrochemical and UV detectors. Each value is the mean ± SD derivation of three replicate analyses. [b] Values in a column with different superscripts are significantly different ($p < 0.05$).

°C for 30 min but did induce DNA damage. Thus, DNA damage induced by MRPs might be indirectly related to the lipid peroxidation.

Effect of MRPs on 8-OHdG Formation. 8-OHdG is a good marker for the measurement of oxidative DNA damage in in vitro or in vivo tests (*36*). 8-OHdG also plays an important role in mutagenesis or carcinogenesis, causing miscoding on DNA templates and resulting in G−T and A−C substitutions (*37*). This causes misreading during DNA replication and synthesis (*38*); therefore, 8-OHdG is also a marker for carcinogenesis. The formation of 8-OHdG in lymphocytes by incubation with Glu-Lys MRPs, Fru-Lys MRPs, and Xyl-Lys MRPs for 30 min was analyzed by means of HPLC-UV-ECD. The retention times for 2-dG and 8-OHdG were 6.65 and 8.72 min, respectively. The standard curves of 2-dG and 8-OHdG show a linear relationship with $r^2 = 0.9807$ and 0.9600, respectively (data not shown). The formation of 8-OHdG in lymphocyte DNA after reaction with MRPs was expressed as an 8-OHdG/10^5dG ratio for indexing of oxidative DNA damage. **Table 4** shows the formation of 8-OHdG in human lymphocytes DNA exposed to undialyzable MRPs. There was no significant increase ($p < 0.05$) in the formation of 8-OHdG for all three MRPs at a concentration <0.05 mg/mL. However, the formation of 8-OHdG increased significantly when the concentration of MRPs was in the range of 0.2−0.8 mg/mL. The contents of 8-OHdG in lymphocyte following incubation with 0.8 mg/mL of Fru-Lys, Glu-Lys, and Xyl-Lys were 11.6, 11.9, and 15.6 8-OHdG/10^5dG, respectively. Xyl-lys MRPs resulted in the highest content of 8-OHdG. Floyd (*38*) reported that a large amount of 8-OHdG was formed in the Fe/ascorbate system or Fenton-type reaction, leading to DNA damage. The hydroxyl radical was also found to be involved in the formation of 8-OHdG because it attacked the C-8 position of guanine, after which hydrogen atoms were lost and 8-OHdG was formed (*36*, *38*). Thus, MRPs may have caused the formation of 8-OHdG because of the free radicals.

Effect of MRPs on the Formation of Free Radicals. Many studies have indicated that free radicals were involved in the Maillard reaction and that the formation of free radicals occurred in the early stage of the reaction (*28*, *29*). Roberts and Lloyd (*29*) demonstrated using ESR spectroscopy that free radicals were formed during the Maillard reaction with glycoaldehyde or glyceraldehyde and *N*,*N*′-dialkylethylenediamines (alkyl = methyl, ethyl, isopropyl, and *tert*-butyl). To determine the existence of free radicals in the prepared MRPs, the free radical was measured by means of EPR spectroscopy (*20*).

As shown in **Figure 3**, all three MRPs had weak free radical signals at a low concentration of 0.1 mg/mL (only Xyl-Lys MRP presented); however, the signal intensity increased at a concentration of 0.8 mg/mL, but there is no significant difference among the three samples. This result indicates that free radicals existed in the undialyzable MRPs. Thus, when the MRPs were incubated with the cells, the free radicals could directly attack the cell DNA or cause a free radical chain reaction, leading to DNA damage.

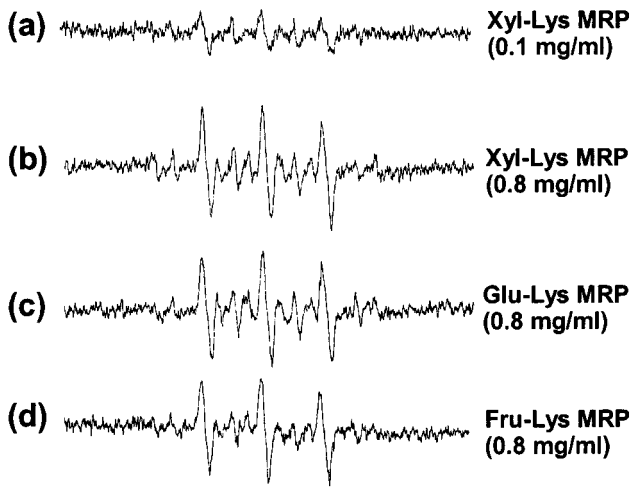

Figure 3. EPR spectra of the mixture of undialyzable Maillard reaction products with DMPO.

Table 5. Effect of Undialyzable Xyl-Lys Maillard Reaction Products on Hydrogen Peroxide Generation at Different Reaction Time in Peroxidase–Phenol Red System

reaction time (min)	H_2O_2[a] (μM)	reaction time (min)	H_2O_2[a] (μM)
10	152.7 ± 0.1^{ab}	30	367.8 ± 0.2^{c}
20	226.9 ± 0.2^{b}	40	487.3 ± 0.1^{d}

[a] The concentration of Xyl-Lys MRP was 0.8 mg/mL. Values are means ± SD of triplicate determinations of hydrogen peroxide generation. [b] Values in a column with different superscripts are significantly different ($p < 0.05$).

Table 6. Effect of Undialyzable Maillard Reaction Products on Hydrogen Peroxide Generation in Peroxidase–Phenol Red System

MRP concn (mg/mL)	H_2O_2[a] (μM)		
	Fru-Lys MRP	Glu-Lys MRP	Xyl-Lys MRP
0.05	3.1 ± 0^{ab}	–[c]	5.1 ± 0^{a}
0.1	24.5 ± 0^{b}	25.5 ± 0.1^{b}	31.6 ± 0^{b}
0.2	64.3 ± 0^{c}	47.0 ± 0^{c}	85.8 ± 0.1^{c}
0.4	141.9 ± 0.1^{d}	97.0 ± 0.1^{d}	171.5 ± 0.1^{d}
0.8	374.7 ± 0.2^{e}	242.0 ± 0.2^{e}	383.9 ± 0.2^{e}

[a] Values are means ± SD of triplicate determinations of hydrogen peroxide generation. MRP reaction with peroxidase–phenol red system for 30 min. [b] Values in a column with different superscripts are significantly different ($p < 0.05$). [c] Undetectable.

Effect of MRPs on the Formation of Hydrogen Peroxide. **Table 5** shows the generation of hydrogen peroxide in the peroxidase−phenol red system at different reaction times in the presence of undialyzable Xyl-Lys MRP (0.8 mg/mL). The results show that the generation of hydrogen pereoxide increased significantly ($p < 0.05$) with increasing reaction time, indicating that the MRP promoted hydrogen peroxide generation in the reaction system. **Table 6** shows the generation of hydrogen peroxide in the peroxidase−phenol red system with the three MRPs at a reaction time of 30 min. The content of hydrogen peroxide increased with increasing concentration for all three MRPs at 0.05−0.8 mg/mL. Xyl-Lys MRP generated the greatest

amount of hydrogen peroxide. At 0.8 mg/mL, the contents of hydrogen peroxide for Xyl-Lys MRP, Fru-Lys-MRP, and Glu-Lys MRP were 384, 375, and 242 μM, respectively. From these results, it can be concluded that the free radicals in the prepared MRPs may have caused a chain reaction, which formed reactive oxygen species, hydrogen peroxide, and/or other free radicals, thus leading to 8-OHdG formation and DNA damage (*36*).

On the basis of the results obtained in this study, both undialyzed MRPs and undialyzable MRPs caused DNA damage in human lymphocytes under the tested concentrations. The three undialyzable MRPs caused a decrease in the GSH content and in the activities of GSH reductase and catalase in human lymphocytes. The undialyzable MRPs had free radicals and promoted the generation of hydrogen peroxide. The superoxide may have been formed in the reaction system because the DNA damage induced by the MRPs decreased in the presence of superoxide dismutase or catalase. These three MRPs also caused 8-OHdG formation in human lymphocytes. Therefore, the free radicals might play an important role in the DNA damage in human lymphocytes induced by these MRPs in this reaction system.

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