JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2004,** *52,* 3577−3582 **3577**

Redox-Related Cytotoxic Responses to Different Casein Glycation Products in Caco-2 and Int-407 Cells

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Sugar-casein glycation products (GPs) were generated by Maillard reaction (MR) with different monosaccharide sources [e.g., glucose (Glc), fructose (Fru), and ribose (Rib)] and prolonged heating (e.g., 27 days at 55 °C) to produce Maillard reaction products (MRPs) that varied in opponent (L, a, b) color measurement and changes in pH, available lysine, and amino-sugar ratio. Theses results signified different rates of three sugar and casein glycation. Sugar-casein GPs from aldohexose, ketohexose, and aldopentose sugar sources were recovered on day 18 of heating and compared for bioactive properties using human embryonic intestinal cell (Int-407) and adenocarcinoma cell (Caco-2) lines. Glu- and Fru-casein GPs produced significant ($p < 0.05$) decreases in antioxidant superoxide dismutase (SOD), glutathione peroxidase, and glutathione reductase enzyme activities in the Int-407 cell line, whereas no effect on antioxidant enzymes was obtained from Rib-casein GP. Moreover, the Caco-2 cell antioxidant enzyme status was not affected by the presence of sugar-casein GPs, regardless of sugar source. The reduction in antioxidant enzyme activity of Int-407 cells by Glu and Fru- casein GPs corresponded to a significant ($p < 0.05$) reduction in Int-407 cell viability. In contrast, no change in Caco-2 cell viability was observed with sugar-casein GP. This finding demonstrates that the noted variable cytotoxic, sugar specific effects of casein GP were related to reductions in critical antioxidant enzyme activities. Moreover, the source of intestinal cell line was an important factor to show the effect of sugar-casein GPs on redox-related cytotoxicity.

KEYWORDS: Sugar-casein glycation products; monosaccharides; colorimetric; available lysine; Caco-2 cell; Int-407 cell; cytotoxicity; antioxidant enzymes

1. INTRODUCTION

The Maillard reaction (MR), or nonenzymatic browning, occurs when reducing sugars condense with amino acids or proteins during food processing and storage conditions, and subsequent products progress into network reactions that form complex reaction products, known as Maillard reaction products (MRPs) or protein glycation products (GP) (*1*−*3*). Many factors, such as reactant source, pH, temperature, and time of heating influence MR (*4*, *5*). At the first stage of the reaction, condensation of amino residues of proteins and carbonyl groups of sugars forms a reversible Schiff base, which rearranges to colorless Amadori products. In the intermediate and advanced stages of the reaction, Amadori products undergo further transformation to colored intermediate products and protein oligolymers (*6*, *7*).

Browning development in MR is generally used as an indicator of MRP formation (*8*, *9*). In most cases, a single wavelength (420 nm) was used to measure browning intensity in model and food preparations. This measurement does not define the specific colors of the yellow to brown pigments derived from products formed at different stages of the MR. The Hunter Lab color parameters have been used as an alternative measurement to monitor colors derived from various products in the MR and to provide better characterization of the reaction products (*10*, *11*). In sugar-protein MR model studies, estimation of lysine loss has also provided a direct means of monitoring the extent of protein modification in the initial stages of the MRP (*12*, *13*).

MRPs generated from sugar-Lys models have been reported to have toxic effects (*14*, *15*). Cell growth inhibition has also been observed with mouse C6 glioma cells and Caco-2 cells treated with glucose (Glc)- and fructose (Fru)-lysine model MRPs (*16*, *17*) and with FT cells (Hela clone) treated with ribose (Rib)-lysine model MRPs (*18*). A recent study showed that Glc-modified bovine serum albumin also inhibited growth of bovine retinal capillary pericytes but did not inhibit growth of bovine aortic endothelial cells (*19*). Our previous study also showed that sugar-casein GPs had no effect on Caco-2 cell growth inhibition (*27*). These reports suggested that different cell types may respond differently in the presence of sugar-protein MRPs.

The main objective of this study was to determine the extent of casein modification associated with Glc, Fru, and Rib reactants during the MR and to evaluate potential alterations in

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10.1021/jf035512m CCC: $27.50
Published on Web 05/05/2004

cytotoxic properties in different human intestinal cell lines that could be related to changes in redox status resulting from antioxidant enzyme inhibition. Potential cytotoxicity of modified caseins was evaluated in two enterocyte cell lines of human origin, e.g., Caco-2 and Int-407 cells.

2. MATERIALS AND METHODS

2.1. Preparation of Sugar-Casein Model GPs. Sugar-casein MRP model systems were prepared as reported previously (*27*). These models consisted of casein (10 g) (Sigma-Aldrich Co., St Louis, MO) and one of three reducing sugars (Glc, Fru, or Rib) (5 g) dissolved in 100 mL of distilled deionzed water (dd water) and adjusted to pH 7.0. The solution was transferred to a 50 mL polypropylene tube with a lid sealed with Parafilm and placed in a water bath at 55 °C for up to 27 days under an aluminum foil covering to exclude light. The heated sugar-casein mixtures were termed as sugar-casein model MRPs. Samples of these sugar-casein model GPs were removed at two day intervals and kept at 4 °C before colorimetric and pH measurements. The samples from the 18 day heating treatment were lyophilized and used for further chemical and biological experiments.

2.2. Colorimetric Measurement. Colorimetric measurements were performed on all samples using a 5 cm diameter aperture in a HunterLab Labscan 600 spectrocolorimeter with Labscan II software (version 3.0) (Hunter Associates Laboratory Inc., Reston, Virginia). The spectrocolorimeter was calibrated with black and white tile plates. The system provided the values of three color components: L* (black and white components) and the chromatic coordinates a* (+ red to − green component) and b* (+ yellow to − blue component). A sample (30 mL) was measured in a plastic Petri dish (100 mm × 15 mm) covered in a black plastic box, which acted as a light shield.

2.3. Available Lysine Assay. Available lysine was determined using a 2,4,6-trinitrobenzenesulfonic acid (TNBS) method as outlined by Kakade and Liener (*21*). Briefly, 1 mL of 0.1% TNBS was added to the sample solution containing 1 mg of protein in 4% $NaHCO_3$ (pH 8.5). The reaction was allowed to proceed at 40 °C for 2 h, and then, 3 mL of concentrated HCl was added. The reaction mixture was autoclaved for 1 h at 121 °C (15 psi). After the mixture was cooled to room temperature, the hydrolysate was mixed with 5 mL of dd water and extracted twice with ethyl ether. The aqueous solution was read at 346 nm against a blank in a Shimadzu UV−visible recording spectrophotometer (UV-160, Kyoto, Japan). The amount of ϵ-TNP-lysine was calculated by using a value of 1.46×10^4 M^{-1} cm^{-1} for the molar absorptivity of ϵ-TNP-lysine.

The amino-sugar analysis of different sugar-casein GPs was performed on a Waters Alliance 2690 Separation Module (Advanced protein technology center, University of Toronto) after hydrolysis by 6 M HCI at 110 °C for 4 h. External calibration was carried out by use of standard amino-sugars: galactosamine and glucosamine.

2.4. Cell Growth Inhibition Assay. Human colon adenocarcinoma cell (Caco-2) and human embryonic intestinal cell (Int-407) lines were purchased from American Type Tissue Collection (ATCC, Manassas, VA). Cells were cultured with Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 100 units/mL of penicillin, and 100 μg/mL of streptomycin, hereafter referred as DMEM10. The cell culture was maintained in a CO_2 incubator (37 °C) with 5% CO_2 and 90% humidity. All experiments were performed with cells between passage 20 and 40.

Cell suspensions containing 7.5×10^4 cells/mL in DMEM10 were plated in a 96 well cell culture plate (100 μL per well) and maintained overnight to allow cell attachment. The cell culture medium was then replaced with phosphate buffer saline (PBS, pH 7.2), and MRPs were added at the final concentrations of 0.5, 1, or 2 mg/mL. After 3 h of incubation, the PBS medium containing MRPs was removed and replaced with DMEM10. The treated cells were cultured for a further 3 days before performing the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay (*27*). Culture media of monolayer cells were removed from the cell culture plates, and MTT solution (0.5 mg/mL DMEM) was added to each well and incubated at 37 °C for 3 h. The optical density of each well was measured at an absorbance of 570 nm, using a Microplate reader (BIO-RAD model 550). Cell MTT response (% control) was calculated from the equation:

$$\% \text{ control} = (\text{absorbance}_{\text{treatment}}/\text{absorbance}_{\text{control}}) \times 100\%$$

2.5. Antioxidant Enzyme Assays. Caco-2 and Int-407 cells were grown in tissue culture dishes (100 mm × 20 mm). After cells reached confluence ($1 \times 10^6 \sim 10^7$ cells/mL), cell culture medium was replaced with PBS and MRPs (2 mg/mL) were added for a further 3 h incubation. The treated cell monolayer was rinsed with ice-cold PBS and then scraped into a 1.5 mL tube. The cell samples were exposed three times to a freeze (−80 °C, 2 h)−thaw (37 °C, 5 min) cycle to release cytosol and centrifuged at 4 °C at 15 800*g* for 10 min. The supernatants were kept for antioxidant enzyme activity assay. The protein contents of the supernatants were measured using Bradford's method (*51*).

A working solution (1.2 mL) of 10 mM hydrogen peroxide in 50 mM phosphate buffer (pH 7.0) was added to a cuvette, followed by 10 μL of the cell supernatant to initiate the reaction. Absorbance readings were monitored at 240 nm and 25 °C for 2 min, using a Shimadzu UV−visible recording spectrophotometer UV-160, with a reference containing the working solution. Sample catalase (CAT) activity in sample was expressed as micromoles of hydrogen peroxide consumed per minute per milligram of protein (*22*, *23*).

Superoxide dismutase (SOD) activity assay was carried out based on the method of Umeik et al. (*24*). The treated cell supernatant sample was incubated at 25 °C for 30 min with a reaction mixture containing 0.1 mM xanthine, 0.01 U/mL xanthine oxidase, and 0.025 mM nitro blue tetrazolium (NBT). Absorbance readings at 560 nm were recorded using a Shimadzu UV−visible recording spectrophotometer UV-160. One unit of SOD activity was defined as the amount of enzyme required to inhibit the standard rate of NBT reduction by 50% (in the presence of SOD).

The glutathione peroxidase (GSH-Px) activity assay was executed by adding cell supernatant sample into a working solution that includes 1 mM GSH, 2 mM sodium azide, 1 U/mL glutathione reductase (GSH-R), 0.1 mM β-nicotinamide adenine dinucleotide phosphate (NADPH), and 0.025 mM hydrogen peroxide. The absorbance readings at 340 nm were monitored for 2 min at 37 °C, using a Shimadzu UV−visible recording spectrophotometer UV-160. One unit of GSH-Px activity was defined to be equivalent to the oxidation of 1 μM NADPH per minute per milligram protein (*25*).

A modified GSH-R activity assay was performed in a reaction mixture containing the cell supernatant sample, 3 mM glutathione of oxidized form (GSSH), and 0.1 mM NADPH. The absorbance at 340 nm was monitored for 2 min at 37 °C, using a Shimadzu UV−visible recording spectrophotometer UV-160. One unit of GSH-R activity was defined to be equivalent to the oxidation of 1 μM NADPH per minute per milligram protein (*26*).

2.6. Statistical Analysis. Each experiment was performed in triplicate generated from three tubes and repeated twice on different batches in separate experiments. Collected data were expressed as means ± SD. Means were compared by one way analysis of variance, followed by Tukey's pairwise comparisons, using Minitab software (Version 13, Minitab Inc., State College, PA). The level of confidence required for significance was selected at $p < 0.05$.

3. RESULTS

3.1. pH and Colorimetric Measurements. pH in sugar-casein GP models changed from pH 7.0 at day 0 to 6.2, 6.0, and 5.0 for Fru-, Glc-, and Rib-caseins, respectively, at 18 days of thermal (55 °C) treatment (**Figure 1A**).

L* values decreased ($p < 0.05$) in all Glc-, Fru-, and Rib-casein GPs with increased heating time (**Figure 1B**). Rib-casein L* values decreased dramatically ($p < 0.05$) within the first 3 days before reaching a plateau level (**Figure 1A**), whereas a slower decrease of L* values was observed for both Glc- and Fru-casein models. A significant ($p < 0.05$) difference between Glc-casein and Fru-casein L* values was observed after 15 days of heating time.

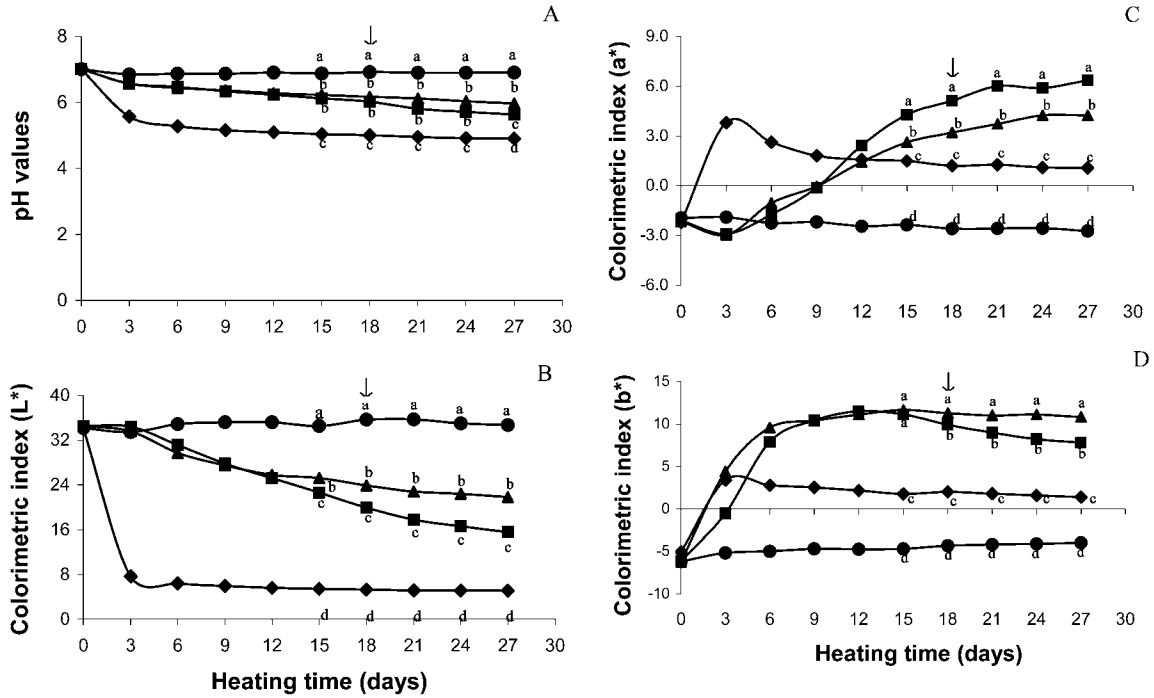

Figure 1. Temporal changes in pH and HunterLab colorimetric values. (**A**) pH, (**B**) lightness (L*), (**C**) redness (a*), and (**D**) yellowness (b*) for different sugar-casein GPs. Color readings were expressed as means ± SD ($n = 6$). Rib-casein (◆), Glc-casein (■), Fru-casein (▲), and casein (●). The mean values with different letters (a–d) in the same heating time are significantly ($p < 0.05$) different. The arrow represents the time (days) of heating (55 °C) at which sugar-casein GPs were taken and evaluated for their biochemical properties.

Table 1. Available Lysine and Amino Sugar Content of Different Sugar-Casein Model MRPs[a]

	available lysine	amino sugar		
	g/100 g sample	Gal (%)	Glu (%)	Gal/Glu
casein	6.9 ± 0.2[a]			
Glc-casein	4.3 ± 0.4[c]	75	25	2.9
Fru-casein	5.4 ± 0.3[b]	74	26	2.8
Rib-casein	1.3 ± 0.2[d]	85	15	5.6

[a] The values represent means ± SD ($n = 6$). Gal, galactosamine; Glu, glucosamine; Glc-C, Fru-C, and Rib-C, glucose-, fructose-, and ribose-casein model MRPs (55 °C, 18 days). The mean with different superscript letters (a–d) represents values that are significantly ($p < 0.05$) different.

Table 2. Effect of Different Sugar-Casein Model MRPs on GSH-Px and GSH-R Activities of Caco-2 and Int-407 Cells[a]

	GSH-Px		GSH-R	
treatment[b]	Caco-2	Int-407	Caco-2	Int-407
control	45 ± 1	59 ± 2	29 ± 1	35 ± 1
casein	46 ± 2	58 ± 1	28 ± 2	35 ± 1
Glc-casein	43 ± 1	53 ± 2[c]	29 ± 1	31 ± 1[c]
Fru-casein	44 ± 1	54 ± 1[c]	30 ± 3	32 ± 1[c]
Rib-casein	45 ± 2	60 ± 2	30 ± 2	36 ± 2

[a] The values represent enzyme activity, expressed as means ± SD ($n = 9$); the units of the enzyme activities are mU/mg protein for GSH-Px and GSH-R. [b] Glc-, Fru-, and Rib-casein, heated glucose-, fructose-, and ribose-casein mixtures (55 °C, 18 days); the MRPs (2 mg/mL) were incubated in cell cultures containing PBS for 3 h prior to assessing enzyme activities. [c] $p < 0.05$, in comparison with the corresponding control group.

Rib-casein GP a* values reached a maximum value rapidly within the first 3 days before gradually decreasing. This contrasted to a decrease in a* values within 3 days for both Glc- and Fru-casein GPs. The Glc- and Fru-casein a* values increased gradually thereafter and were greater than the Rib-casein GP after 12 days (**Figure 1C**).

Similarly, Rib-casein GP b* values reached a maximum value rapidly within the first 3 days before gradually decreasing. A dramatic increase in b* values for both Glc- and Fru-casein GPs was observed within the first 6 days. Increases in b* values for Glc- and Fru-casein GPs were greater than that observed for the Rib-casein GP (**Figure 1D**).

3.2. Available Lysine. The available lysine contents of different sugar-casein GPs were significantly ($p < 0.05$) lower after heating at 55 °C for 18 days (**Table 1**). The relative loss of available lysine residues was greater ($p < 0.05$) for Rib-casein GP than for Glc-casein GP, which in turn was greater ($p < 0.05$) than Fru-casein GP.

Amino-sugars (i.e., galactosamine and glucosamine) were analyzed for sugar-casein GPs. Rib-casein GP also contained a relatively higher percent of galactosamine, which paralleled a higher galactosamine and glucosamine ratio, as compared to Glc- and Fru-casein GPs.

3.3. Cellular Antioxidant Enzymes. Glc-, Fru-, and Rib-casein GPs had no significant effect on Caco-2 cellular SOD, CAT, GSH-Px, and GSH-R activities, even at a relatively high concentration of 2 mg/mL (**Tables 2** and **3**). On the other hand, Int-407 cellular GSH-Px, GSH-R, and SOD activities, but not CAT activity, were significantly ($p < 0.05$) decreased in the presence of both Glc- and Fru-casein GPs. In contrast, Rib-casein GPs did not alter GSH-R, GSH-Px, CAT, and SOD activities in cultured Int-407 cells.

3.4. Cell MTT Response. The Caco-2 cell MTT response was not significantly affected after exposure to different sugar-casein GPs (**Table 4**). However, a significant ($p < 0.05$) reduction of cell MTT response occurred in Int-407 cells after exposure to both Glc- and Fru-casein model GPs, while Rib-casein GPs did not decrease Int-407 cell MTT response.

Table 3. Effect of Different Sugar-Casein Model MRPs on CAT and SOD Activity of Caco-2 and Int-407 Cells[a]

	CAT		SOD	
treatment[b]	Caco-2	Int-407	Caco-2	Int-407
control	2.0 ± 0.1	3.3 ± 0.1	4.2 ± 0.1	5.3 ± 0.1
casein	2.0 ± 0.1	3.2 ± 0.1	4.2 ± 0.1	5.5 ± 0.1
Glc-casein	2.1 ± 0.1	3.2 ± 0.1	4.1 ± 75	4.7 ± 0.2[c]
Fru-casein	2.0 ± 0.1	3.3 ± 0.1	4.2 ± 0.1	4.8 ± 0.1[c]
Rib-casein	2.0 ± 0.1	3.3 ± 0.1	4.1 ± 0.1	5.5 ± 0.1

[a] The values represent enzyme activity, expressed as means ± SD ($n = 9$); the units of the enzyme activities are U/mg protein for CAT and SOD. [b] Glc-, Fru-, and Rib-casein, heated glucose-, fructose-, and ribose-casein mixtures (55 °C, 18 days); the MRPs (2 mg/mL) were incubated in cell cultures containing PBS for 3 h prior to assessing enzyme activities. [c] $p < 0.05$, in comparison with the corresponding control group.

Table 4. Potential Cell Growth Inhibition of Heated and Unheated Sugar-Casein Mixtures on Caco-2 and Int-407 Cells[a]

		cell type	
treatment	mg/mL	Caco-2	Int-407
control	0	100 ± 7	100 ± 6
casein	0.5	101 ± 8	97 ± 5
casein	1.0	96 ± 7	98 ± 6
casein	2.0	101 ± 7	98 ± 4
Glc/casein	0.5	97 ± 9	92 ± 5
	1.0	86 ± 10	96 ± 4
	2.0	100 ± 4	97 ± 3
Fru/casein	0.5	104 ± 6	96 ± 7
	1.0	97 ± 11	97 ± 4
	2.0	99 ± 4	97 ± 6
Rib/casein	0.5	101 ± 5	102 ± 7
	1.0	103 ± 9	101 ± 1
	2.0	105 ± 7	99 ± 3
Glc-casein	0.5	90 ± 4	75 ± 6[b]
	1.0	88 ± 6	65 ± 1[b]
	2.0	88 ± 4	56 ± 1[b]
Fru-casein	0.5	87 ± 8	62 ± 2[b]
	1.0	86 ± 9	53 ± 2[b]
	2.0	90 ± 5	46 ± 1[b]
Rib-casein	0.5	91 ± 6	96 ± 5
	1.0	97 ± 7	90 ± 2
	2.0	111 ± 6	92 ± 1

[a] The values represent cell MTT response (% control), expressed as means ± SD ($n = 9$); Glc/, Fru/, and Rib/casein, unheated sugar-casein mixtures (1:2, w/w), with concentrations corresponding to caseins; Glc-, Fru-, and Rib-casein, heated sugar-casein mixtures (55 °C, 18 days). [b] $p < 0.05$, in comparison with the corresponding controls.

4. DISCUSSION

4.1. pH and Color Changes. Decreases in pH as a consequence of MRP formation have been reported by many investigators as an index of monitoring the MR rate (*28*, *29*). A decrease in pH has been related to the formation of formic and acetic acids in sugar-casein model systems (*30*). A decrease from pH 7, an adjusted pH in the sugar-casein MR model, to 5.0, 6.0, and 6.2 was observed for Rib-, Glc-, and Fru-casein, respectively, in the present study. This observation is further confirmation that different MR reaction rates occurred for the three sugar-casein MR models, which likely resulted in generation of different amounts and types of acidic products.

All sugar-casein GPs demonstrated a characteristic pattern of color development with L* values decreasing and both a* and b* values increasing during the heating process. A similar color development was also observed as heating time and temperature progressed (*11*). Color development toward a dark (i.e., L* value decrease) brown (i.e., a* and b* values increase, respectively) corresponds to the formation of brown pigments and intermediate and late stage products of the MR (*31*, *32*). Pyrazine products and protein oligomers are among the MRPS formed (*7*, *33*). A darker color in the Rib-casein GP indicates a faster reaction rate and formation of possible late stage GP, as compared to Glc- and Fru-casein GPs. These results are consistent with our previous study that reported a higher browning Rib-casein GP as compared to Glc- and Fru-casein GPs (*27*).

4.2. Available Lysine. Free lysine and peptide bound lysine residues are actively involved in the MR due to the presence of the lysine ϵ-amino group (*34*). Available lysine content of protein is decreased during the heating process in the presence of reducing sugar (*35*), and the extent of modification of protein lysine residues depends on MR conditions (*36*, *37*). The demonstration in the present study that loss of available lysine was greater for the Rib-casein, as compared to the Glc-casein GP, which in turn was greater than the Fru-casein GP, is additional evidence that casein glycation occurs differently depending on the sugar source.

Galactosamine and glucosamine products have been detected in many sugar and protein GP mixtures (*38*, *39*). In the present study, a larger ratio of galactosamine/glucosamine was observed in the Rib-casein GP, which corresponded to the faster reaction rate for Rib-casein MR, as compared to Glc- and Fru-casein MR, respectively. Thus, formation of galactosamine and glucosamine in MR is affected by the type of reducing sugar present.

4.3. Cellular Antioxidant Enzymes. Effective activities of antioxidant enzymes, such as SOD, GSH-Px, GSH-R, and CAT, are important to protect cells from challenges induced by exogenous and endogenous oxidants (*40*, *41*). The effect of MRPs on intestinal cell antioxidant enzyme activities has not been fully explored. The present study showed that sugar-casein GP had no significant impact on Caco-2 cell antioxidant enzyme activity. In contrast, Glc- and Fru-casein, but not Rib-casein GP, significantly decreased GSH-Px, GSH-R, and SOD activities in Int-407 cells. A change in CAT activity was not detected, which supports previous findings that MRPs have a selective effect on different detoxification enzymes (*42*, *43*). The interaction between reactive oxygen species (ROS) and SOD and GSH-Px activities is important for detoxification of free radicals and protection of cellular membranes from oxidative stress. For example, generation of superoxide radical reduces GSH-Px activity (*44*) and lowers removal of hydrogen peroxide due to suppressed GSH-Px activity, which in turn adversely affects SOD activity (*45*). Thus, antioxidant enzymes are important in maintaining cellular redox status required for normal cell function and proliferation (*46*). The observed reduced responses of Int-407 cell GSH-Px, GSH-R, and SOD activities to MRPs corresponded to noted cell MTT changes in the same cell line. This result also indicates that Glc- and Fru-casein GP-induced suppression of Int-407 cellular antioxidant enzyme activities was related to the eventual decrease in cell viability.

4.4. GP Cytotoxicity. Cytotoxic activities of sugar-amino acid MRPs have been demonstrated using different cultured cell lines (*16*−*18*). Some glycated proteins, such as bovine serum albumin and β-lactoglobulin, also showed cytotoxic properties (*19*, *47*, *48*). In the present study, sugar-casein GPs did not produce a significant change in cell MTT response on Caco-2 cells; however, cell MTT response of the Int-407 cell, an intestinal embryonic cell, was decreased by Glc- and Fru-casein GPs, indicating a decrease in cell viability. Caco-2 cells appear to be more resistant than other cells to xenobiotics, probably due

to specific cellular metabolic compensation mechanisms (*43*, *49*). A recent study on cytotoxicity of glycated β-lactoglobulin (BLG) also showed that BLG was more effective on COS-7 cells than on HL-60 cells (*48*). Moreover, the cytotoxic effect varied with glycated β-lactoglobulins modified with different sugars (*48*). In contrast to a decreased cell MTT response by Glc- and Fru-casein, the Rib-casein GP did not change cell MTT response of either Caco-2 or Int-407 cells. The advanced Rib-casein GP resulted in a high molecular weight polymer with no apparent biological activity. This observation is consistent with other reports that more advanced forms of MRPs are generally regarded as biologically inert (*50*). Our results demonstrated that potential cytotoxicity of different sugar-casein GPs varied with both the source of MR reactants and the cell type.

In conclusion, a greater MR rate was observed for Rib than Glc followed by Fru, which was evidenced by color development and pH change in sugar-casein MR models. The extent of casein modification with three different sugars, confirmed by loss of available lysine, corresponded to different MR rates initiated by individual sugars. Glc- and Fru-modified caseins significantly ($p < 0.05$) decreased viability of Int-407 cells, but this effect was not observed with Rib-modified casein GP, possibly due to formation of late stage, inert products. It was also found that Int-407 cellular antioxidant enzyme activities of GSH-Px and GSH-R were significantly ($p < 0.05$) decreased for Glc- and Fru-casein GP treatments. Reduction in GSH-Px/GSH-R redox enzyme activity corresponded to an impaired cell viability in a cell line that has limited capacity to detoxify ROS as evidenced by GP-induced impaired SOD activity.

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Received for review December 24, 2003. Revised manuscript received March 9, 2004. Accepted March 17, 2004.

JF035512M