Research Article

Journal of PeptideScience

Received: 6 May 2009 Revised: 2 June 2009 Accepted: 5 June 2009 Published online in Wiley Interscience: 03 July 2009

(www.interscience.com) DOI 10.1002/psc.1159

Screening for glucose-triggered modifications of glutathione

Ivanka Jerić and Štefica Horvat*

Nonenzymatic protein glycation is caused by a Schiff's base reaction between the aldehyde groups of reducing sugars and the primary amines of proteins. These structures may undergo further Amadori rearrangement and free radical-mediated oxidation to finally generate irreversible advanced glycation end products (AGEs). One of the factors known to modulate the glycation of proteins is glutathione, the most abundant nonprotein thiol tripeptide with the γ-linkage, H-Glu(Cys-Gly-OH)-OH (GSH). Screening for products formed by GSH with D-glucose is an essential step in understanding the participation of GSH in glycation (the Maillard) reaction. Under the conditions used in these studies we observed *N*-(1-deoxy-D-fructos-1-yl)-pyroglutamic acid as the major glycation product formed in the mixtures of GSH and glucose *in vitro*. A RP HPLC/MS and tandem MS analyses of the GSH/glucose mixtures revealed that cleavage of the *N*-terminal glutamic acid and the formation of pyroglutamic acid-related Amadori product were accompanied by generation of Cys-Gly-derived Amadori and thiazolidine compounds. Copyright © 2009 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: glutathione; glycation; Amadori; thiazolidine; mass spectrometry; Maillard; pyroglutamic acid

Introduction

Nonenzymatic glycation has been recognized as an important posttranslational modification underlying alterations of structure and function of extracellular and intracellular proteins during aging and diabetes [1,2]. Glycation of proteins occurs by means of a chemical reaction of reducing sugars with amino groups to form reversible Schiff base adducts. The reactive amino groups are either an *N*-terminal α-NH_2 or a lysyl ε-NH_2 group, depending on the accessibility and environment. The analysis of the glycation potential of the human proteome revealed that the proteins associated with the extracellular matrix could be less glycated than proteins that are parts of other biological cellular components [3]. Schiff base adducts may undergo further Amadori rearrangement and free radical-mediated oxidation to finally generate irreversible advanced glycation end products (AGEs) [4]. In diabetes, the level of Amadori compounds formed on long-lived proteins increases and there is a good correlation between blood glucose levels and the concentration of Amadori adducts [5]. The excess of glucose and elevated levels of reactive oxygen species (ROS) leads to depletion of low molecular weight antioxidants, such as glutathione (GSH) and ascorbic acid. GSH, the tripeptide with γ-linkage, H-Glu(Cys-Gly-OH)-OH (**1**), is the most abundant nonprotein thiol located in the cytosol and mitochondria of mammalian cells [6]. It plays an important role in the detoxification of electrophilic foreign compounds and chemically reactive intermediates, which may arise during the biotransformation of xenobiotics. GSH and glutathione disulfide (GSSG, **6**) constitute the most important redox buffer in animal cells both in the cytosol and in the organelles [7]. Since in the diabetic lenses the glucose concentration can increase 10-fold and higher, the formation of Amadori products from GSH and GSSG with this monosaccharide may also increase. This could contribute to a lowering of GSH levels and increase oxidative stress observed in diabetic lens. Previous studies demonstrated that Amadori modification at the α-amino group of glutamyl residue in GSH or GSSG profoundly influences their utilization by enzymes that are part of lens ROS-detoxifying system [8]. It was also suggested that, because of its ubiquity and high concentration, GSH may be the most important transglycating agent, able to regenerate unmodified proteins with a concomitant production of GSH–aldose transglycation byproducts [9].

The aim of this work is to present evidence for the formation of novel glycation and/or fragmentation products generated in aqueous or methanolic GSH–glucose model systems. HPLC coupled with diode array and mass spectrometry detectors (LC/DAD/MS) allowed a rapid screening of the solutions and identification of compounds. An unusual cleavage of *N*-terminal glutamic acid residue in GSH during the reaction with glucose was observed, resulting in the formation of pyroglutamic acid-related Amadori compound **3**, and accompanied by cysteinyl-glycine-derived Amadori and thiazolidine compounds **4** and **5** (Figure 1).

Materials and Methods

General

NMR spectra were recorded on a Bruker AV 600 spectrometer, operating at 150.91 MHz for ^{13}C and 600.13 MHZ for ^{1}H nuclei. The spectra were measured in D_2O solution at 25 °C. Chemical shifts in parts per million were referenced to dioxane. Spectra were assigned based on 2D homonuclear (COSY, NOESY, ROESY) and heteronuclear (HMQC, HMBC) experiments. RP HPLC was performed on a Varian Pro Star 230 HPLC system using a

* *Correspondence to: Štefica Horvat, Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Bijenička c. 54, POB 180, Zagreb, 10002-HR, Croatia. E-mail: shorvat@irb.hr*

Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Zagreb, 10002-HR, Croatia

Journal of PeptideScience

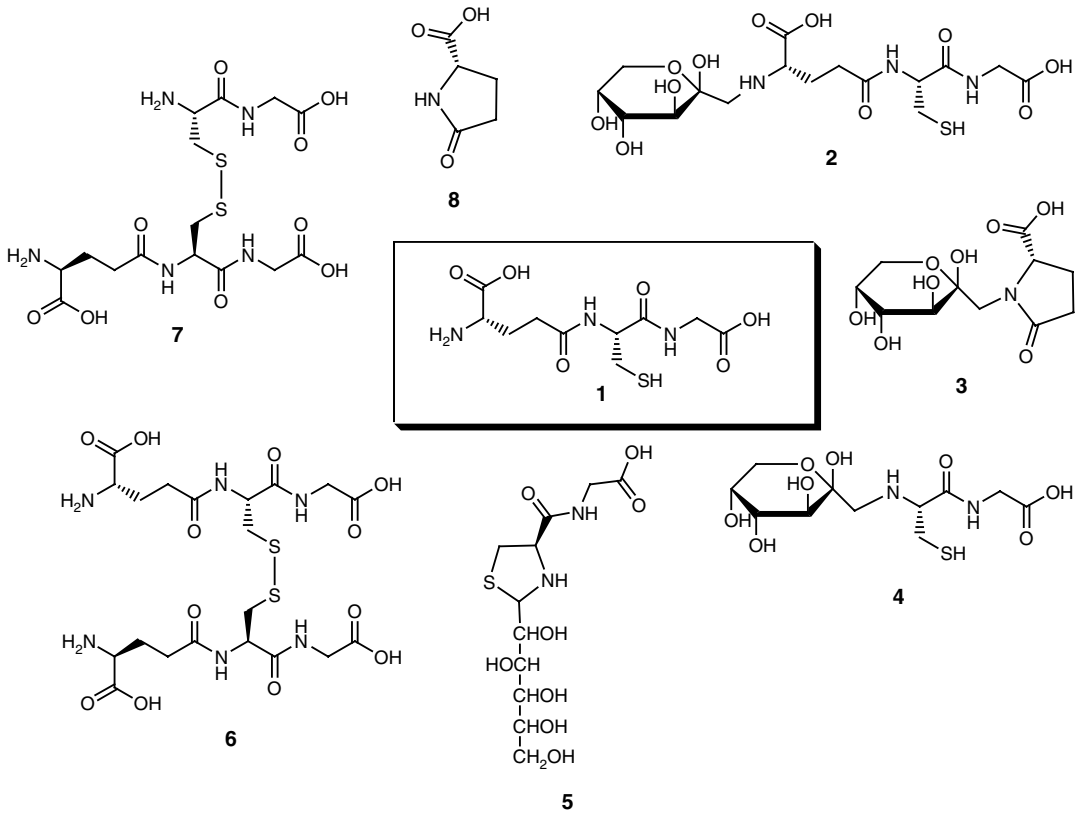


Figure 1. Structures of compounds derived from GSH (**1**).

Eurospher 100 RP C-18 semipreparative (250 × 8 mm ID, 5 µm) (flow rate: 1.0 ml/min) or analytical (150 × 4.5 mm ID, 5 µm) (flow rate: 0.5 ml/min) column under isocratic or gradient conditions using different concentrations of MeOH in 0.1% aqueous TFA. UV detection was performed at 215 nm using a Varian Pro Star 335 photodiode-array detector. GSH was purchased from Fluka.

Isolation of the Amadori Compound of Pyroglutamic Acid (3)

A solution of the GSH (**1**) (60 mg, 0.2 mmol) in MeOH–AcOH (9 : 1) (30 ml) was refluxed with D-glucose (540 mg, 3.0 mmol) for 16 h. The solvent was evaporated, the residue was dissolved in water (1 ml) and applied to a column (4 × 1 cm) of Amberlite IR-120 in H^+-form. The column was first washed with water and then with 10% formic acid. The fractions containing glycation product **3** were further purified by semipreparative RP-HPLC using 10% MeOH/0.1% TFA as eluent to yield 20 mg (34%) of a highly pure Amadori compound **3** as a white amorphous powder. The analytical RP HPLC analysis: 5% MeOH/0.1%TFA t_R = 12.84 min. ^{1}H NMR and ^{13}C NMR data in D_2O are given in Table 1. MW 291.10 ESI-MS: *m/z* 314.2 $[M + Na]^+$.

RP HPLC Analysis of Glycation Products Formed by Incubation of GSH (1) with D-glucose

Incubations in water or phosphate buffer

Solutions of GSH (**1**) (15 mg, 0.05 mmol) and D-glucose (135 mg, 0.75 mmol) in water or in 0.05 M phosphate buffer/0.1 M NaCl (pH 7.4) (PBS) (15 ml), containing the internal standard *o*-hydroxyphenylacetic acid (120 µg/ml) and NaN_3 (0.02%) were sterilized by passage through a 0.45 µm nylon filter and incubated in the dark at 37 or 50 °C. Aliquots were withdrawn from the incubation mixtures at appropriate time intervals and immediately frozen. The relative concentrations of the glycation products and/or starting compounds in the incubation mixtures were determined by analytical RP HPLC at a flow rate of 0.5 ml/min (for details see General Methods) using linear gradient from 5 to 75% MeOH/0.1% TFA over 30 min by directly injecting incubation mixture and UV monitoring at 215 nm.

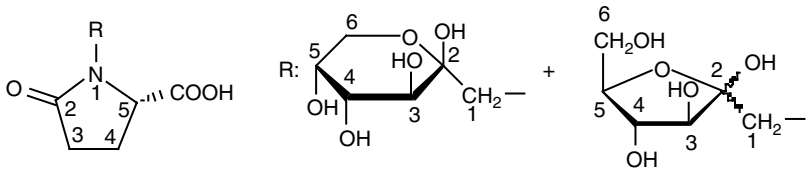
Incubations in MeOH or MeOH–AcOH (9 : 1)

Solutions of GSH (**1**) (15 mg, 0.05 mmol) and D-glucose (135 mg, 0.75 mmol) were prepared in MeOH or MeOH–AcOH (9 : 1) (15 ml). The reaction mixtures were kept at either 37 or 50 °C. The analysis of products was performed as described above.

HPLC-MS Analysis of the GSH Glycation Products

MS measurements were performed on a Agilent Technologies 1200 HPLC system consisting of binary pump, degasser, autosampler and diode-array detector and coupled with 6410 Triple Quadrupole mass spectrometer, operating in a positive ESI mode. Separation of incubation mixture components was achieved on a Zorbax Eclipse XDB-C18 column (4.6 × 150 mm, 5 µm). The mobile phase consisted of a 0.1% TFA in water (solvent A) and 10% MeOH in 0.1% TFA (solvent B) at a flow rate 0.5 ml/min. To achieve a better separation gradient elution was used: with 100% solvent A to 100% solvent B in 20 min and then with 100% solvent A in 10 min. Chromatograms were recorded at 215 nm. For the MS/MS experiments N_2 was used as collision cell gas and the collision energy was set to 10 eV. Spectra were acquired from

Table 1. NMR chemical shifts data (ppm) of the glutathione-derived Amadori compound of the pyroglutamic acid (Glp) (**3**)[a,b]



3 β-pyranose (β-*p*) α,β-furanose (α,β-*f*)

Residue	Atom	β-pyranose		β-furanose		α-furanose	
		δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
Sugar moiety	1	3.18/4.01	47.84	3.44/3.68	47.67	3.35/3.79	45.78
	2	–	98.35	–	101.14	–	104.59
	3	3.61	69.64	3.93	77.45	4.02	82.26
	4	3.83	69.61	4.03	74.69	3.99[c]	76.20
	5	3.94	69.18	3.74	80.46	4.04[c]	82.00
	6	3.64/3.94	63.39	3.74	62.57	ND	60.97
Glp	2	–	180.31	–	180.12	–	180.33
	3	2.50	29.20	2.50	29.23	ND	ND
	4	2.12/2.47	22.77	2.12/2.47	23.39	ND	22.89
	5	4.68	62.00	4.56	62.00	4.63	62.00
	6	–	176.30	–	175.85	–	176.13

[a] In D_2O at RT.
[b] Tautomeric composition: β-*p*:β-*f*:α-*f* = 64 : 31 : 5.
[c] Assignments of signals can be interchangeable.
ND, not determined.

the incubation mixture aliquots; solvent was evaporated and the residue dissolved in solvent B to obtain concentration of 1 mg/ml. Using autosampler 10 µl of solution was injected to the column.

Results and Discussion

The reaction of glucose and GSH (**1**) in MeOH–AcOH, 9 : 1, under reflux conditions for 16 h, afforded after RP HPLC purification Amadori rearrangement product of pyroglutamic acid (Glp) **3** in 34% yield. In spite of the large excess of glucose used in the glycation reaction (sugar to tripeptide molar ratio 15 : 1), GSH-related Amadori product **2** was not detected in the reaction mixture. Amadori product **3** was characterized by NMR and MS. The NMR spectra (D_2O) of Glp derivative **3** (Table 1) showed three sets of sugar resonances attributable to the 1-deoxy-D-fructosyl moiety in its α- and β-furanose and β-pyranose forms. The β-pyranose and β-furanose forms were the major tautomers, 64% and 31%, respectively, while the α-furanose form was minor component (5%). Characteristic large downfield shift ($\Delta\delta$ ~10 ppm) of the Glp 5-CH-N carbon is caused by the *N*-alkylation at this position. The mass spectrum of compound **3** gave an ion at *m/z* 314.20 corresponding to the pseudomolecular $[M + Na]^+$ ion.

In order to better understand the influence of the medium, the effect of four different solvents was evaluated under oxidative conditions on the reactivity of GSH in glycation reaction. As shown in Table 2, Amadori product formation was not observed in water, PBS and MeOH solution. The results indicate that glucose accelerates oxidation of GSH in aqueous solutions. Especially, much higher yields of oxidation product (GSSG) were obtained after 3 days in the GSH/glucose (Glc) water solution. In earlier studies Linetsky *et al*. [8] demonstrated that GSH/Glc molar ratio of 1 : 20, produced approximately 3% of the Amadori product of GSH (**2**) at 37 °C after 48 h of the incubation under argon in 50 mM Chelex-treated phosphate buffer (pH 7.0). It can be assumed that the formation of the substantial amounts of GSSG during incubations in PBS under our conditions prevented Amadori product **2** formation in detectable amounts. Incubation of GSH and Glc (molar ratio 1 : 15) in MeOH in the presence of the acid catalyst (acetic acid), at 50 °C or under reflux, leads to the formation of Amadori product of pyroglutamic acid (**3**) as the major reaction product. Under these conditions, formation of GSSG was very slow.

Pyroglutamic acid or 5-oxoproline (Glp, **8**) is an endogenous molecule derived from L-glutamate, being a major intermediate in the γ-glutamyl cycle. This cycle is necessary for the synthesis and breakdown of GSH and also to the intracellular transport of free amino acids [10]. High levels of Glp in cerebrospinal fluid, blood and urine are characteristically seen in glutathione synthetase deficiency, an inborn metabolic defect of the γ-glutamyl cycle. This disorder is clinically characterized by hemolytic anemia, metabolic acidosis and severe neurological disorders [11].

To our knowledge, nonenzymatic formation of pyroglutamic acid from GSH was not investigated so far. Having in mind above mentioned significance of Glp and GSH, we considered important to analyze GSH and GSH/Glc incubation mixtures in more detail. MS is always a method of choice when dealing with complex samples consisting of numerous analytes present in different concentrations. Therefore, RP HPLC-MS was used in this study as a starting point to achieve better insight into intermediates which may arise during transformation of GSH *in vitro* in the absence or presence of glucose.

First, an aliquot of GSH incubated in water for 5 days at 50 °C was analyzed by means of HPLC-MS (Figure 2A). Total ion current

Journal of PeptideScience

Table 2. Effect of solvent and glucose on the oxidation and Amadori compound formation from GSH[a]

Entry	Starting compounds	Solvent	Temp. (°C)	Incubation time (days)	Relative amount (%)		
					GSH (**1**)	GSSG (**6**)	Am-Glp (**3**)
1	GSH	Water	50	3	68	8	–
2	GSH/Glc	Water	50	3	15	60	–
3	GSH	PBS	50	1	–	70	–
4	GSH/Glc	PBS	50	1	–	86	–
5	GSH/Glc	PBS	37	1	–	100	–
6	GSH/Glc	MeOH	37	7	100	–	–
7	GSH/Glc	MeOH/AcOH	50	3	16	9	33
8	GSH/Glc	MeOH/AcOH	Reflux	1	21	6	47

[a] The relative amounts of the products in the reaction mixtures were determined by RP HPLC (for details see Materials and Methods).

(TIC) and DAD chromatograms clearly show presence of four products with molecular $[M + H]^+$ ions at *m/z* 130, 308, 484 and 613, respectively. Their structures have been confirmed by tandem MS. The MS/MS spectrum of the compound with *m/z* 130 revealed that collision-induced dissociation of the parent ion leads to ion at *m/z* 84, consistent with the cleavage of COOH group (46 Da loss). These data are consistent with the structure of pyroglutamic acid (Glp, **8**). The tandem mass spectrum of the molecular $[M + H]^+$ ion of the compound with *m/z* 308 is in agreement with the structure of GSH. The spectrum shows presence of b, y and z-type ions consistent with the tripeptide **1** structure. Fragmentation pattern of compound with *m/z* 484 confirmed structure of compound **7** (Figure 1) (GSSG missing one of the *N*-terminal glutamic acid residues). Cleavage of the glutamic acid residue yields ion at *m/z* 355, while cleavage of disulfide bridge generates low abundant ion at *m/z* 177. The MS/MS spectrum of the molecular $[M + H]^+$ ion of compound with *m/z* 613 shows consecutive losses of two glutamic acid molecules (129 Da loss) giving rise to ions at *m/z* 484 and 355, respectively, indicating that this component is oxidized GSH (GSSG, **6**). The obtained results indicate that in water solution, pyroglutamic acid is most probably formed from GSSG (**6**) leading to compound **7**. GSSG missing both *N*-terminal glutamic acid residues was not detected in the incubation mixture. Fragmentation patterns of all products obtained from GSH incubation in water are given in Figure 3A.

TIC and DAD chromatograms of GSH/Glc mixture incubated in MeOH–AcOH (9 : 1) for 5 days at 37 °C are presented on Figure 2B. The molecular $[M + H]^+$ ion at *m/z* 470 corresponds to GSH-derived Amadori compound **2** (t_R = 6.45 min). Even at first sight, the tandem mass spectrum of the molecular ion of the Amadori product **2** shows fragments originating from two base peaks, the molecular $[M + H]^+$ ion at *m/z* 470 and *m/z* 274. The spectrum exhibits presence of ions originating from consecutive losses of 18 Da (*m/z* 452, 434 and 416), corresponding to the elimination of one, two and three water molecules. Additionally, an ion corresponding to the M+78 modification of the parent peptide is also found at *m/z* 386, as marker of the Amadori type of compound [12,13]. Although not present, b-type ion at *m/z* 292 is precursor of ions at *m/z* 274, 256 and 234, corresponding to the losses of water molecules. The spectrum also exhibits the ion at *m/z* 208 with furylium-type of structure. Fragmentation pattern of Amadori compound **2** is given in Figure 3B. According to HPLC-MS data, the compounds eluting at 5.95 and 5.30 min are GSH and pyroglutamic acid, having molecular ions at *m/z* 308 and 130, respectively. DAD chromatogram also reveals presence of two peaks eluting at 3.60 and 3.16 min, both having molecular ions at *m/z* 341. However, TIC chromatogram indicates presence of multiple compounds eluted between 3 and 4 min, most probably originating from degradation of glucose. HPLC-MS/MS analysis of the compound eluted at 3.60 min with molecular $[M + H]^+$ ion at *m/z* 341 shows the presence of ions typical for the Amadori compounds (Figure 4A): elimination of two water molecules (*m/z* 305) and M+78 modification of Cys-Gly peptide (*m/z* 257). Ions at *m/z* 179 and 162 correlate with free Cys-Gly dipeptide and x-type of fragment, respectively. These data are consistent with the structure of Cys-Gly-derived Amadori compound **4** (Figure 1). However, low abundant ions at *m/z* 221 and 203 present in the spectrum cannot be correlated with the Amadori type of structure. Next, in the MS/MS spectrum (Figure 5) of the compound that elutes at 3.16 min, ions at *m/z* 221 and 203 are of the highest intensities. Thus, we proposed that in addition to Amadori compound **4** another product with *m/z* 341 is formed in the GSH/Glc incubation mixture with thiazolidine structure **5** presented in Figure 1. This finding is supported by the fragmentation pathway presented in Figure 4B. The loss of 120 Da from the *m/z* 341 ion gives rise to the ion at *m/z* 221. Sugar residue cleavage can proceed through the loss of either formaldehyde or HOCH═CHOH moieties (30 and 60 Da). Consecutive losses are not found in the MS/MS spectrum of the *m/z* 341, indicating low stability of these fragments or cleavage of 120 Da sugar part in a single step. Ion at *m/z* 221 can be considered as M+42 modification of the Cys-Gly dipeptide. It is interesting to mention that 42 Da mass gain of the parent peptide was found for the glycation-generated imidazolidinone-type modification of the peptide *N*-terminus [14]. Further elimination of the water molecule gives rise to the most abundant peak at *m/z* 203 carrying methylenethiazolidine moiety, while cleavage of the peptide backbone generates ion at *m/z* 100. These two ions correspond to M+24 modifications of the peptide and Cys-derived immonium ion, and can be used as diagnostic ions for the glycation-derived thiazolidine-type modification of peptides with *N*-terminal Cys residue.

HPLC-MS studies of the GSH/Glc mixture incubated in MeOH–AcOH (9 : 1) for 2 days under reflux conditions (Figure 2C), showed the presence of the compound with *m/z* 314 as the major product, which eluted at 6.08 min. The tandem mass spectrum of the molecular $[M + Na]^+$ ion of this compound is in agreement with the structure of Amadori rearrangement product of pyroglutamic acid (**3**). HPLC-MS data of the compounds eluted at 5.95, 5.30, 3.60 and 3.16 min, and the specific fragmentation pattern

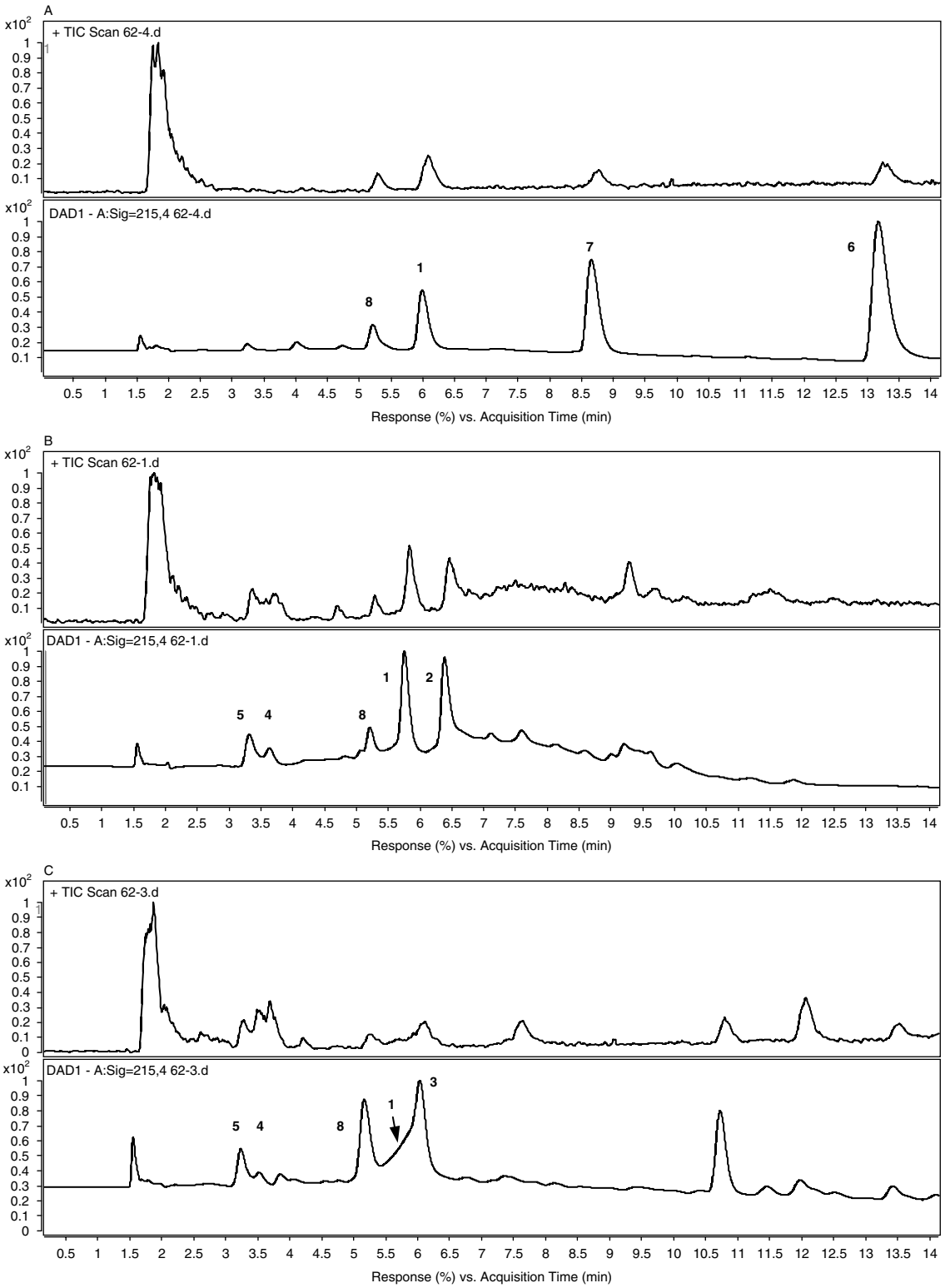


Figure 2. Total ion current chromatograms (TICs) and DAD chromatograms ($\lambda = 215$ nm) of incubation mixtures generated from GSH (**1**) in the absence or presence of glucose. The molar ratio of the reactants and the reaction conditions were as follows: (A) GSH (0.05 M) (water, 50 °C, 5 d); (B) GSH/Glc (1 : 15) (MeOH–AcOH, 9 : 1, 37 °C, 5 d); (C) GSH/Glc (1 : 15) (MeOH–AcOH, 9 : 1, reflux, 2 d).

Journal of PeptideScience

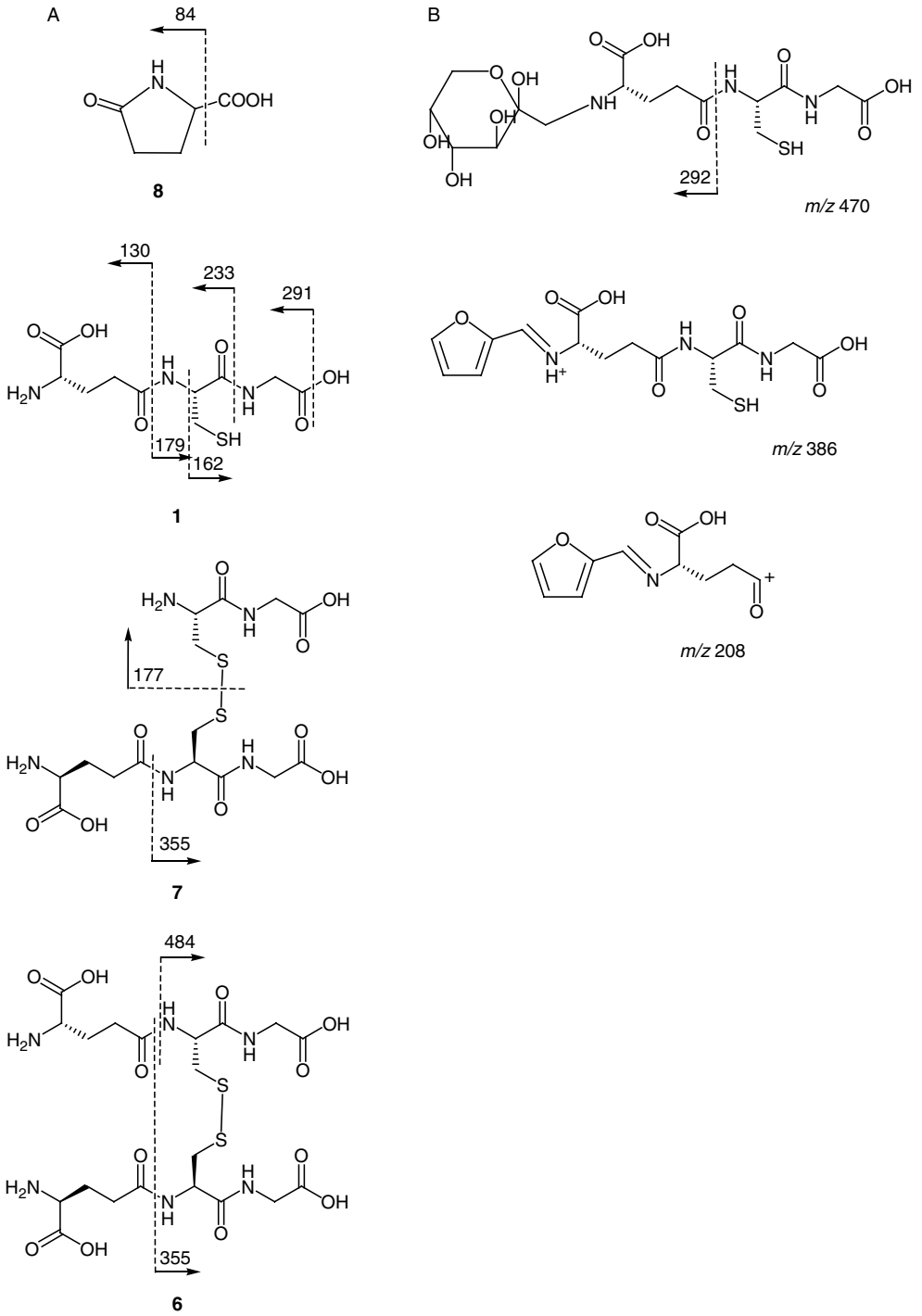


Figure 3. Structures and fragmentation assignments of ions observed in the MS/MS spectra of the molecular $[M + H]^+$ ions of (A) products obtained from GSH incubated in water, and (B) Amadori compound **2**.

of their molecular ions, indicate presence of GSH, pyroglutamic acid, as well as Cys-Gly-related glycation products **4** and **5,** in the incubation mixture.

Recently, Szwergold *et al*. [15] identified glucose-derived thiazolidine compound of cysteine (Glc-Cys) in human urine and its elevation in diabetes. It was suggested that conjugation of glucose to GSH, probably by transglycation, and its subsequent elimination and metabolism *in vivo* at least partly lead to thiazolidine-like compounds such as glycated Cys-Gly derivative **5** and Glc-Cys.

Conclusion

RP HPLC-MS/MS method allowed identification of products formed in GSH or in GSH/Glc incubation mixtures under different conditions. The chemical degradation of GSH *in vitro* to Amadori derivative of pyroglutamic acid, accompanied by the Cys-Gly-derived Amadori and thiazolidine compound formation, was determined. Glycation of GSH by glucose was observed only in MeOH/AcOH (9 : 1, $\geq$50 °C) but not in water, PBS and MeOH.

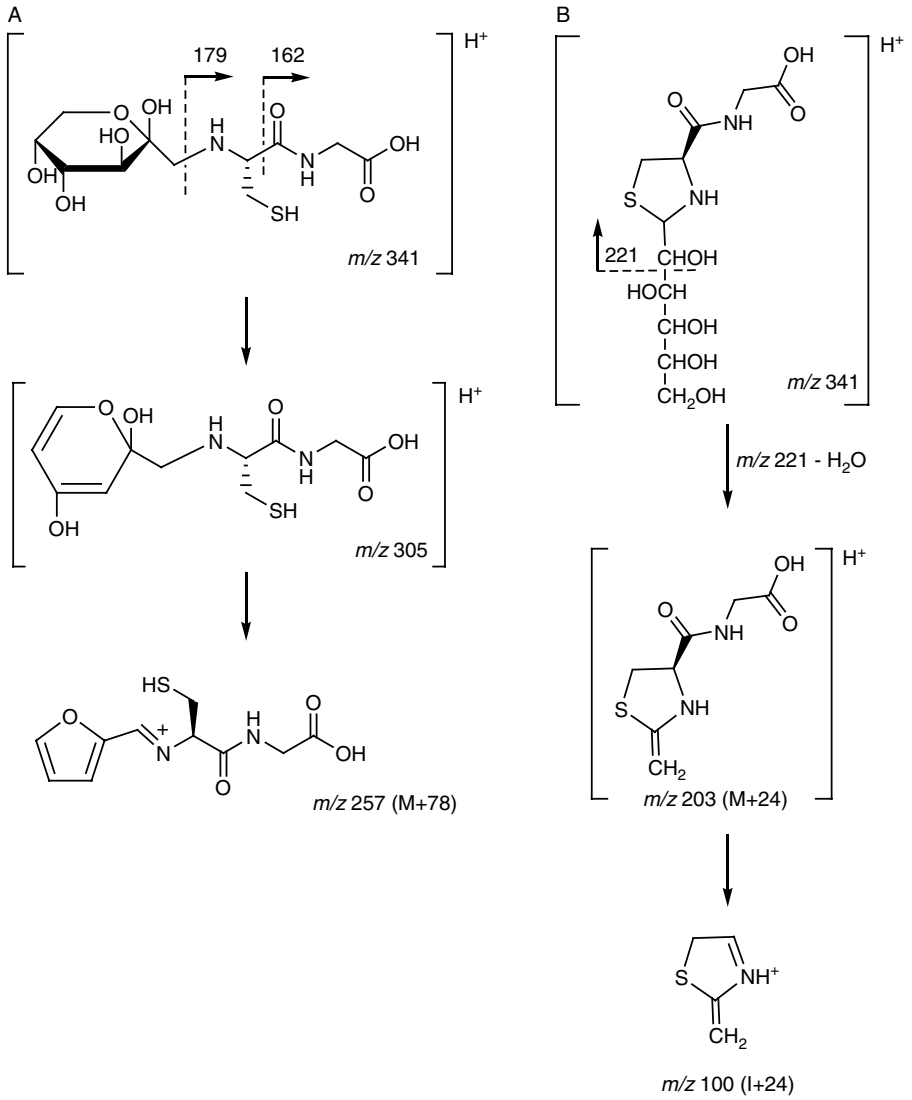


Figure 4. Structures and fragmentation assignments of ions observed in the MS/MS spectra of the molecular $[M + H]^+$ ion at *m/z* 341: (A) related to Amadori compound **4**, and (B) related to thiazolidine structure **5**.

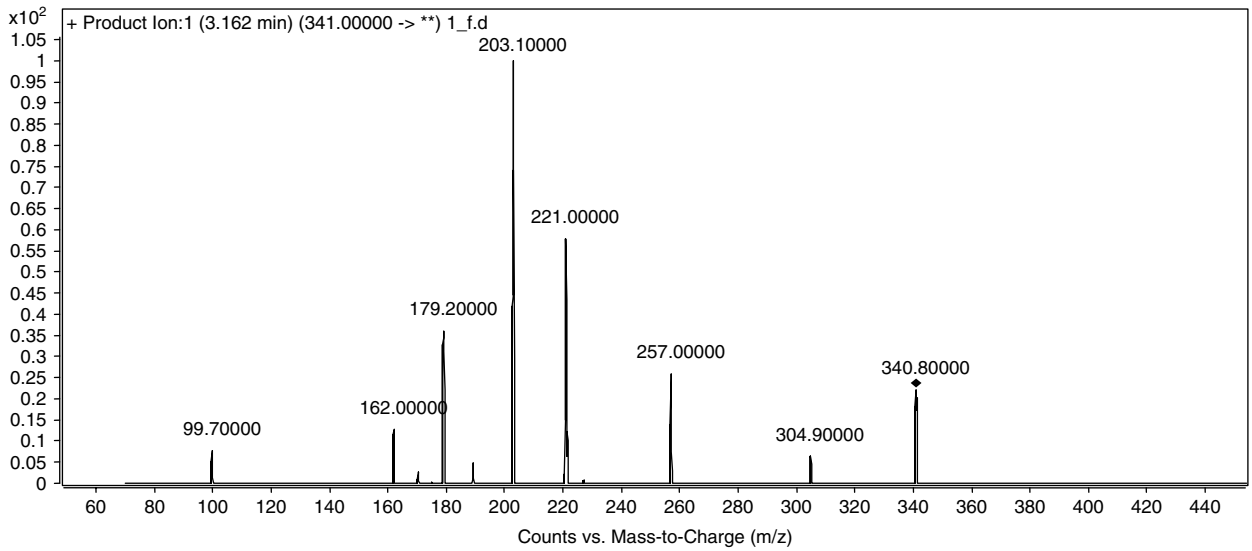


Figure 5. MS/MS spectrum of the *m/z* 341 ion (t_R = 3.16 min).

Journal of PeptideScience

Although solvents and reaction temperatures under which degradation products are formed from Glc-GSH adducts are not physiological, the present results suggest that GSH, in addition to its antioxidant function, could also participate in nonenzymatic glycation reaction, by formation of the relatively stable novel compounds whose physiological significance is yet unknown. Results presented here and obtained from model GSH/Glc systems represent tools that can be further used for the analysis of complex matrixes, such as biological fluids.

Acknowledgements

This work was supported by the Croatian Ministry of Science, Education and Sports, grant no. 098-0982933-2936. Authors thank Mrs. Milica Perc for excellent technical assistance.

References

1 Brownlee M. Advanced protein glycosylation in diabetes and aging. *Annu. Rev. Med.* 1995; **46**: 223–234.
2 Baynes JW. From life to death – the struggle between chemistry and biology during aging: the Maillard reaction as an amplifier of genomic damage. *Biogerontology* 2000; **1**: 235–246.
3 Johansen MB, Kiemer L, Brunak S. Analysis and prediction of mammalian protein glycation. *Glycobiology* 2006; **16**: 844–853.
4 Yan SF, Ramasamy R, Schmidt AM. Mechanisms of disease: advanced glycation end-products and their receptor in inflammation and diabetes complications. *Nat. Clin. Pract. Endocrinol. Metab.* 2008; **4**: 285–293.
5 Jaleel A, Halvatsiotis P, Williamson B, Juhasz P, Martin S, Nair KS. Identification of Amadori-modified plasma proteins in type 2 diabetes and the effect of short-term intensive insulin treatment. *Diabetes Care* 2005; **28**: 645–652.
6 Meister A, Anderson ME. Glutathione. *Annu. Rev. Biochem.* 1983; **52**: 711–760.
7 Csala M, Fulceri R, Mandl J, Benedetti A, Banhegyi G. Ryanodine receptor channel-dependent glutathione transport in the sarcoplasmic reticulum of skeletal muscle. *Biochem. Biophys. Res. Commun.* 2001; **287**: 696–700.
8 Linetsky MD, Shipova EV, Legrand RD, Argirov OO. Glucose-derived Amadori compounds of glutathione. *Biochim. Biophys. Acta* 2005; **1724**: 181–193.
9 Szwergold BS. Intrinsic toxicity of glucose due to nonenzymatic glycation is controlled in vivo by deglycation systems including FN3K-mediated deglycation of fructosamines and transglycation of aldosamines. *Med. Hypotheses* 2005; **65**: 337–348.
10 Meister A. Glutathione deficiency produced by inhibition of its synthesis, and its reversal; applications in research and therapy. *Pharmacol. Ther.* 1991; **51**: 155–194.
11 Ristoff E, Mayatepek E, Larsson A. Long-term clinical outcome in patients with glutathione synthetase deficiency. *J. Pediatr.* 2001; **139**: 79–84.
12 Frolov A, Hoffmann P, Hoffmann R. Fragmentation behavior of glycated peptides derived from D-glucose, D-fructose and D-ribose in tandem mass spectrometry. *J. Mass Spectrom.* 2006; **41**: 1459–1469.
13 Jerić I, Versluis C, Horvat Š, Heck AJR. Tracing glycoprotein structures: electron ionization tandem mass spectrometric analysis of sugar-peptide adducts. *J. Mass Spectrom.* 2002; **37**: 803–811.
14 Roščić M, Versluis C, Kleinnijenhuis AJ, Horvat Š, Heck AJR. The early glycation products of the Maillard reaction: mass spectrometric characterization of novel imidazolidinones derived from an opioid pentapeptide and glucose. *Rapid Commun. Mass Spectrom.* 2001; **15**: 1022–1029.
15 Szwergold BS, Howell SK, Beisswenger PJ. Transglycation-A potential new mechanism for deglycation of Schiff's bases. *Ann. N. Y. Acad. Sci.* 2005; **1043**: 845–864.

