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Influence of glutathione fructosylation on its properties

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Abstract

Incubation of fructose and glutathione leads to the formation of *N*-2-deoxy-glucos-2-yl glutathione as the major glycation product, with characteristic positive ion at 470 Th in LC–MS spectra. Glutathione disulfide and fructose generate two compounds: *N*-2-deoxy-glucos-2-yl glutathione disulfide ($m/z = 775$ Th) and bis di-*N*,*N*′-2-deoxy-glucos-2-yl glutathione disulfide ($m/z = 937$ Th). *N*-2-deoxy-glucos-2-yl glutathione is 2.5-fold less effective than glutathione in reducing dehydroascorbic acid. Glutathione peroxidase and glutathione-*S*-transferase exhibit marginal activity toward *N*-2-deoxy-glucos-2-yl glutathione, while glyoxalase I shows 44.9% of the enzyme's specific activity. Glutathione reductase demonstrates 6.9% of the enzyme's specific activity with bis di-*N*,*N*′-2-deoxy-glucos-2-yl glutathione, while with mono-*N*-glucosyl glutathione disulfide retained 5 6.1% of the original activity. Glutathione reductase could not reduce *N*-2-deoxy-glucos-2-yl glutathione in mixed disulfide with γS-crystallin, but reduced glutathione in mixed disulfide with γS-crystallin by 90%. The presence of *N*-2-deoxy-glucos-2-yl glutathione in mixed disulfide with γS-crystallin makes this molecule more susceptible to unfolding than native γS-crystallin.

Keywords: Non-enzymatic glycation; Fructose; Glutathione; Glutathione mixed disulfides

Glutathione (GSH)[1] is essential for maintaining sulfhydryl groups of enzymes and lens proteins, thus preventing the formation of protein–protein disulfides [1–3]. It also keeps ascorbic acid in the reduced state and detoxifies oxygen-free radicals and xenobiotics [4]. In the newborn human lens, GSH levels reach 6.0 mM [5]. However, in the presence of persistent hyperglycemia, as seen in diabetes, a precipitous drop in GSH levels is the most common and earliest biochemical change, even before cataract develops [6–9]. Major factors thought to be involved in diabetes-related cataract formation include GSH oxidation by oxygen-derived species in combination with the inability of glutathione reductase (GR) to maintain the pool of reduced GSH [8], decreased levels of GSH-synthesizing enzymes [10], osmotic stress caused by intralenticular accumulation of polyols mediated by aldose reductase [8,11,12] and leakage of oxidized GSH from diabetic lens [6,8,10–12]. Conversely, Obrosova et al. [8,11] showed that while GSH levels dropped almost 5-fold in mildly diabetic rat lens, oxidized GSH levels remained unchanged. Other studies have demonstrated increased GR activity in diabetic lenses, both in humans [7] and in rats [8,11]. However, no significant change in the $NADP^+$/NADPH ratio has been observed, which argues against loss of GR as a cause of GSH depletion [8]. Significant leakage of oxidized GSSG has not been found in diabetic rat lenses [8]. Together, these results may indicate that oxidative stress does not play a significant role in GSH depletion in diabetic lens. Similar results were obtained in the mouse diabetic model

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[1] *Abbreviations used:* GSH, glutathione (reduced form); GSSG, glutathione (oxidized form); Fru, fructose; *N*-2-deoxy-glucos-2-yl glutathione; Glc-GSH, *N*-2-deoxy-glucos-2-yl glutathione disulfide; Glc-GSSG, bis di-*N*,*N*′-(2-deoxy-D-glucos-2-yl)-glutathione disulfide; Glc-GSSG-Glc, GR-glutathione reductase; GPx, glutathione peroxidase; GST, glutathione-*S*-transferase; GO1, glyoxalase I; CDNB, 1-chloro-2,4-dinitrobenzene; GLC-D, (−) glucose; TCEP, tricarboxyethyl phosphine; TCA, trichloroacetic acid; TMA, trimethylamine; DTNB, 5,5′-dithiobis (2-nitrobenzoic acid); TNBS, 2,4,6-trinitrobezene sulfonic acid; ROS, reactive oxygen species; γS-S-SG, mixed disulfide of GSH and γS-crystallin; γS-S-SG-Glc, mixed disulfide of *N*-2-deoxy-glucos-2-yl glutathione and γS-crystallin.

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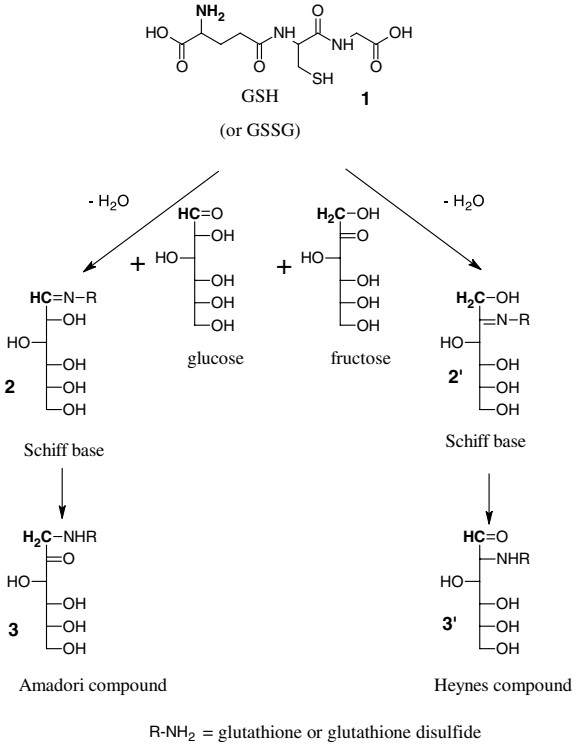
developed by Hegde et al. [9,13], who found that GSH levels in diabetic mice lens fell 2-fold and coincided with an 8-fold increase in glucose levels and a 6-fold increase in the accumulation of early glycation products in water-insoluble (WS) lens protein. In addition, sorbitol levels were insignificant in this diabetic mouse model, excluding osmotic stress as an influence on the decreased GSH levels [9–13].

An alternative explanation of how GSH levels may decrease in blood or in the lens of diabetic mammals was described by Szwergold [14] and Linetsky et al. [15], who showed that glucose (Glc) can form Amadori compounds with the α-NH_2-group of glutamyl in GSH, with a relatively high rate constant and at relatively low GSH-to-Glc ratio.

However, not only Glc increases in diabetic human lens. Depending on the severity of diabetes, the fructose (Fru) concentration increases along with the Glc level, and can range from 5.8 to 17.8 mM [16–20]. In diabetic human lenses Fru is formed by sorbitol oxidation, driven by unusually active polyol dehydrogenase [18]. Similar to Glc, Fru has been shown to react with the amines, which results the formation of Schiff bases, which is followed by the formation of Heynes compounds [21] (Scheme 1, structures 3′). Covalent incorporation of Fru in albumin Lys proceeds at a rate similar to that of Glc incorporation in albumin Lys. With more advanced stages of albumin glycation by Fru, there is at least a 3-fold increase in non-Trp fluorescence in these proteins [21], as compared to the rate at which albumin is glycated by Glc. McPherson et al. [21], found that proteins in human diabetic lenses contained fructose-derived early glycation products along with Amadori compounds. The level of Heyns modifications in these proteins has been found to reach 5–8 mmol per mmol of protein, and there is 3- to 4-fold increase in such modifications in lens proteins from diabetic persons as compared to healthy, age-matched individuals.



Scheme 1. Formation of Amadori (structures #1–3) and Heynes compounds (structures #1′–3′) of glutathione under physiological conditions.

Given the significance of Fru-mediated modification of proteins in diabetic lens, we conducted this project to examine whether Fru can modify GSH or GSSG in a facile manner during short-term (1- to 3-day) incubation under physiological pH and temperature.

We also attempted to identify the structures of Fru-mediated early glycation products of GSH/GSSG and to elucidate how the presence of 2-deoxyglucosyl modification at α-NH_2-group of GSH (Scheme 1, structure 3′) affects function as a reducing agent for dehydroascorbic acid (DHA). We also performed experiments to determine whether glutathione peroxidase (GPx), glutathione-*S*-transferase (GST), and glyoxalase I (GO1) can utilize fructose-derived Heyns compounds of GSH as a substrate and whether the Fru-derived mono-or di-substituted Heyns compounds of GSH disulfide can be utilized as substrates for glutathione reductase (GR). Because glycated forms of GSH are sulfur-containing compounds, we also studied whether the presence of such molecules as mixed disulfides within γ-crystallins influences their ability to withstand GnHCL- and heat-induced denaturation.

Materials and methods

Reagents

De-ionized water (18 MΩ or greater) was used throughout this project. *N*-(1-deoxy-D-fructos-1-yl)-glutathione was synthesized as described by Linetsky et al. [15,22]. D(−)fructose, (anhydrous; >99.5 purity), GSH (reduced), glutathione disulfide, *tert*-butyl hydroperoxide, 1-chloro-2,4-dinitrobenzene (CDNB), and methylglyoxal were supplied by Sigma–Aldrich (St. Louis, MO). D_2O was obtained from Cambridge Isotope Labs (Andover, MA), NADPH was obtained from Alexis (San Diego, CA) and tricarboxyethyl phosphine (TCEP) was obtained from Molecular Probes, (Eugene, OR). Acetonitrile (HPLC grade), methanol (HPLC grade), ethyl acetate, dimethylsulfoxide (DMSO), formic acid, glacial acetic acid, trichloroacetic acid (TCA), trimethylamine (TMA), and all of the salts used in this project were the highest quality available from Acros Organics (New Jersey, NJ) or Fischer Scientific (Pittsburgh, PA). Prodigy ODS 3 (C_{18}, 100 Å, 5 μ, 4.6 × 250), Prodigy ODS 3 (C_{18}, 100 Å, 5 μ, 10.0 × 250), and Prodigy ODS 3 (C_{18}, 100 Å, 5 μ, 22.0 × 250) columns were purchased from Phenomenex (Torrance, CA). All phosphate buffers were treated with Chelex resin to lower the concentration of transient metal ion contaminants, according the method of Dikalov et al. [23].