General Physiology and Biophysics
Revised manuscript #1

Title: Glutathione is the main endogenous inhibitor of protein glycation
Running title: Glutathione as main inhibitor of protein glycation
Create date: 2016-09-18

Name	*Affiliations*
Dr hab. Izabela Anna Sadowska-Bartosz	1. University of Rzeszow, Department Biochemistry and Cell Biology, Rzeszow, Poland
M. Sc. Sabina Galiniak	1. University of Rzeszow, Department Biochemistry and Cell Biology, Rzeszow, Poland
Prof. Grzegorz Bartosz	1. Department of Molecular Biophysics, Institute of Biophysics, University of Lodz, Lodz, lodzkie, Poland

Corresponding author: Dr hab. Izabela Anna Sadowska-Bartosz <isadowska@poczta.fm>

Abstract
Glycation is the reason for diabetic complications and contributes to the development of other diseases and aging. Numerous exogenous compounds have been tested for their anti-glycating activity. In this study we aimed to answer the question, which endogenous compounds can be important in preventing glycation at physiological concentrations. A set of endogenous compounds has been tested for the ability to protect albumin from glucose-induced glycation in vitro at a concentration of 1 mM and in a physiological concentration range. Only glutathione was found to protect against glycation at physiological concentrations. Glutathione depletion increased the rate of hemoglobin glycation in erythrocytes incubated with high glucose. These results indicate that the level of glutathione is the main determinant of glycation of intracellular proteins.

Keywords: glycation; advanced glycation end products; diabetes; albumin; glutathione

Response to reviews:
response to reviews file - download

Tables:
Tab. 1 - download
Tab. 2 - download
Tab. 3 - download

Glutathione is the main endogenous inhibitor of protein glycation

Sabina Galiniak [a], Grzegorz Bartosz [a, b], Izabela Sadowska-Bartosz [a] *

[a] Department of Biochemistry and Cell Biology, Faculty of Biology and Agriculture, University of Rzeszów, Poland

[b] Department of Molecular Biophysics, Faculty of Biology and Environmental Protection, University of Łódź, Poland

* Corresponding author. Department of Biochemistry and Cell Biology, Faculty of Biology and Agriculture, University of Rzeszow, Zelwerowicza St. 4, PL 35-601 Rzeszow, Poland.
E-mail address: isadowska@poczta.fm

Abstract

Glycation is the cause of diabetic complications and contributes to the development of other diseases and aging. Numerous exogenous compounds have been tested for their anti-glycating activity. The aim of this study was to answer the question, which endogenous compounds at physiological concentrations can effectively prevent glycation. A set of endogenous compounds has been tested for the ability to protect albumin from glucose-induced glycation *in vitro* at a concentration of 1 mM and in a physiological concentration range. Only glutathione was found to protect significantly against glycation at physiological concentrations. Glutathione depletion increased the rate of hemoglobin glycation in erythrocytes incubated with high glucose concentrations. These results indicate that the level of glutathione is the main determinant of glycation of intracellular proteins.

Keywords: glycation, advanced glycation end products, diabetes, albumin, glutathione

1. Introduction

Glycation is one of the most important non-enzymatic post-translational modifications of proteins. This process is initiated by binding of carbonyl groups of monosaccharides or aldehyde metabolic intermediates to free amino groups of proteins to form Schiff bases. Then the Schiff bases are subject to intramolecular rearrangements resulting in formation of early glycation products (Amadori products). Further transformations of Amadori products yield Advanced Glycation End Products (AGEs) (Tessier 2010; Schalkwijk and Miyata 2012). AGEs formation takes place under normal physiological conditions, but is accelerated in hyperglycemia (type 1 and 2 diabetes mellitus) and diseases involving oxidative stress such as cardiovascular diseases (Semba et al. 2015; Cao et al. 2014), chronic obstructive pulmonary disease (Hoonhorst et al. 2014), cystic fibrosis (Sadowska-Bartosz et al. 2014a) or multiple sclerosis (Sadowska-Bartosz et al. 2013).

Glycation induces structural changes of proteins, which are believed to be responsible for diabetic complications and to contribute to the course of other diseases and aging (Drenth et al. 2016; Ramasamy et al. 2016; Simm et al. 2015). In particular, the accumulation of AGEs plays an important role in the formation of degenerative changes in the lens of the eye, leading to cataracts or vision loss (Kandarakis et al. 2014).

Therefore, prevention of glycation or acceleration of the removal of glycation products can be expected to ameliorate the course diabetes and other diseases and slow down aging, extending both lifespan and healthspan. Various exogenous substances have been tested for their ability to prevent glycation, including aminoguanidine, pyridoxamine, 2,3-diaminephenazine, pyridoxine, penicillamine, benfotiamine, thiazolidine derivatives, edaravone (Schalkwijk and Miyata 2012), gold and silver nanoparticles (Ashraf et al. 2016; Liu et al. 2014) and nitroxides (Sadowska-Bartosz et al. 2015a). Numerous food constituents, especially flavonoids and other polyphenols were found to be efficient inhibitors of glycation

68 *in vitro* and some of them were reported to be active *in vivo* (Sadowska-Bartosz and Bartosz
69 2015). However, attenuation of glycation by exogenous compounds is not easy to achieve.
70 Glycation is a non-enzymatic reaction and can be inhibited on a competitive basis. It is hardly
71 possible to achieve *in vivo* a concentration of an exogenous compound, which would compete
72 for reactive carbonyl groups with amino groups of endogenous compounds, present at
73 concentrations of 10-100 mM. Moreover, exogenous compounds may have side effects
74 precluding their safe use *in vivo*. It may be thus of interest to check, which of endogenous
75 compounds can be the most important in the protection of cellular or extracellular proteins
76 against glycation. The aim of this study was to answer this question by comparing protection
77 of a model protein (albumin) *in vitro* against glycation by relevant endogenous compounds
78 present at physiological concentrations.
79
80 **2. Materials and methods**
81 *2.1. Materials*
82 All basic reagents were from Sigma-Aldrich (Poznań, Poland), unless indicated
83 otherwise. Bovine serum albumin (BSA) was purchased from AppliChem GmbH (Darmstadt,
84 Germany). Spectrophotometric and fluorimetric measurements were made in an Infinite200
85 PRO multimode reader (Tecan Group Ltd., Männedorf, Switzerland). All measurements were
86 performed in triplicate and repeated at least three times.
87 *2.2. Sample preparation*
88 BSA was dissolved in 0.1 M sodium phosphate buffer, pH 7.4, at a final concentration
89 of 90 µM. Glucose (500 mM) was used to induce glycation. Samples with or without the
90 compounds tested at a concentration of 1 mM or at physiologically relevant concentrations
91 were incubated at closed vials with addition of 1 mM sodium azide as a preservative at 37^{o}C
92 for 6 days [16]. The use of the high glucose concentration allowed for shortening incubation

time necessary to obtain a significant level of glycation. The effects of physiological concentrations of the compounds, chosen on the basis of literature data, on BSA glycation were also tested.

2.3. Fluorescence measurements

Fluorescence of sample aliquots (200 µl) was measured in a microplate reader, at wavelengths of 325/440 nm (AGEs), 330/415 nm (dityrosine), 325/434 nm (*N*'-formylkynurenine) and 365/480 nm (kynurenine) (Sadowska-Bartosz et al. 2014b). Formation of amyloid beta-sheet structure was measured using thioflavin T. Shortly, 95 µl of sample aliquots were added with 5 µl of 640 µM thioflavin T in 0.1 M sodium phosphate buffer, pH 7.4, and incubated at room temperature for 1h. Then the fluorescence intensity was measured at wavelengths of 435/485 nm (LeVine et al. 1999).

2.4. AGE assay

AGE content was also evaluated by enzyme-linked immunosorbent assay (ELISA) Kit for Advanced Glycation End Products (USCN Life Science Inc., Product No. CEB353Ge), according to the protocol of the manufacturer.

2.5. Glycation of hemoglobin in erythrocytes

Peripheral blood from one healthy donor (38-year-old women) was collected using 3% sodium citrate as an anticoagulant. The study was approved by the Bioethics Committee of the University of Łódź (Poland). The blood was centrifuged (2000 × *g*, 10 min, 4 °C), plasma and leukocyte buffy coat were aspirated and the erythrocyte pellet was washed three times with 3 volumes of phosphate buffered saline (PBS) per 1 volume of a suspension. Half of the of erythrocyte suspension was incubated on a shaker with 2,4-chlorodinitrobenzene (CDNB) at 37°C for 1 h, and washed thrice to remove excess CDNB; another half was not treated with CDNB. The hydrophobic compound CDNB enters cells via diffusion, is conjugated with

glutathione intracellularly and the conjugate formed (2,4-dinitrophenyl-*S*-glutathione) is pumped out by the multidrug resistance protein 1 (MRP1; ABCC1) (Keppler et al. 1998).

Erythrocytes were suspended to a final hematocrit of 10% in PBS containing 5, 50 or 100 mM glucose and 17 µM ampicillin at 37°C and incubated on a shaker for 24, 48 and 72 h.

2.6. Estimation of glutathione content

The erythrocyte content of reduced glutathione (GSH) was estimated with *o*-phthalaldehyde according to Senft et al. (2000). Hemoglobin was estimated according to Drabkin and Austin (1935).

2.7. Estimation of hemoglobin glycation

Glycated hemoglobin was assayed with ELISA Kit for Glycated Hemoglobin A1c (USCN Life Science Inc., Product No. CEA190Hu) according to the protocol of the manufacturer.

2.8. Statistical analysis

All the experiments were done at least in triplicate. Data were presented as mean values and standard deviations. Statistical analysis of the data was performed using STATISTICA software package (version 10, StatSoft Inc. 2010, Tulsa, OK, USA, www.statsoft.com). Differences between means were analyzed using Student's t-test for independent samples and were considered significant or highly significant at ^{a}p values<0.001, ^{b}p values <0.01 or ^{c}p values < 0.05.

3. Results

We have compared the influence of a set of potential glycation inhibitors on the extent of BSA glycation estimated with simple measurements of the fluorescence of glycoxidation products and the formation of amyloid structures, estimated with thioflavin. Fifteen amino acids or their derivatives and peptides, four organic acids, two polyamines, three B-group

vitamins and three nucleotides were used at 1 mM concentration (as in our previous studies) and at physiologically relevant concentrations (Table 1)

Arginine, glycine, tyrosine, serine, carnosine, urea as well as creatine, spermine and spermidine did not decrease glycation at a 1 mM concentration and in the physiological concentration range. All the nucleotides tested, both at physiological concentration for blood plasma and at a concentration of 1 mM (approximate physiological intracellular concentration) did not alter the glycation process. Cysteine inhibited glycation in a concentration-dependent manner; 1 mM cysteine significantly decreased formation of AGEs ($t=14.6^{a}$; k = 4 in all cases; $^{a}p<0.001$), dityrosine , ($t=40.3^{a}$), N'-formylkynurenine ($t=17.7^{a}$), kynurenine ($t=10.4^{a}$) and amyloid aggregates ($t=4,49^{b}$; $^{b}p<0.01$). However, physiological concentrations of cysteine had little effect: 25 µM cysteine decreased AGE formation ($t=4.62^{b}$), dityrosine content ($t=6.35^{b}$) and N'-formylkynurenine content ($t=14.7^{b}$), without affecting any other glycoxidation parameters. Lysine at a concentration 1 mM decreased formation of AGEs ($t=6.42^{b}$), dityrosine , ($t=7,64^{a}$) and N'-formylkynurenine ($t=3.49^{c;}$ $^{c}p<0.05$); 0.4 mM lysine slightly reduced formation of AGEs ($t=4.67^{b}$), dityrosine ($t=2.91^{c}$), N'-formylkynurenine ($t=6.8^{b}$), kynurenine ($t=2.89^{c}$) and amyloid β-structures ($t=3.65^{c}$). Histidine, methionine and tryptophan at a concentration of 1 mM slightly decreased glycation as evaluated by all parameters measured (AGEs: $t=19.9^{a}$, 9,92 ^{a} and 11.6^{a}, respectively; dityrosine: $t=32.0^{a}$, 26.0^{a} and 21.0^{a}, respectively; N'-formylkynurenine: $t=23.6^{a}$, 16.6^{a} and 16.3^{a}, respectively; kynurenine: $t=13.4^{a}$ for histidine and 10.6^{a} for tryptophan; amyloid formation: $t=7.18^{b}$, 7.31^{b} and 5.54^{b}, respectively), but physiological concentration of these amino acids had generally no effect either. Oxidized glutathione (GSSG) at physiological concentrations did not affect glycation; however, 1 mM GSSG caused an increase in AGEs ($t=5.67^{b}$), dityrosine ($t=12.0^{a}$), N'-formylkynurenine ($t=8.03^{b}$) and kynurenine ($t=3.04^{c}$) levels. Melatonin at a concentration of 1 mM significantly inhibited formation of AGE

(t=16.2[a]), dityrosine (t=5.12[b]) and N'-formylkynurenine, but had no effect in the physiological range of concentrations. Panthothenic acid at a physiological concentration had no effect, with the exception of an increase in amyloid formation (t=11.6[a]) but a concentration of 1 mM intensified glycation. Uric acid promoted glycation in concentration-dependent manner.

Vitamin B1 at a concentration of 1 mM significantly increased AGE (t=34.1[a]), dityrosine (t=20.5[a]), N'-formylkynurenine (t=22.7[a]) and kynurenine formation (t=47.4[a]), and slightly enhanced tryptophan loss (t=5.49[b]). Vitamin B2 at a physiological concentration did not affect most of glycation markers; however, 1 mM riboflavin significantly decreased formation of AGE (t=93.6[a]), dityrosine (t=418.4[a]), N'-formylkynurenine (t=172.7[a]) and kynurenine (t=45.5[a]), had no effect on tryptophan loss and increased amyloid formation (t=10.3[a]). Vitamin B6 at a concentration of 1 mM inhibited glycation, judging on the basis of all parameters measured but kynurenine and amyloid formation; physiological concentration of vitamin B6 had no effect on glycation.

Pyruvic acid significantly decreased glycation at a supraphysiological concentration of 1 mM (AGE: t=12.4[a]; dityrosine: t=28.8[a]; N'-formylkynurenine: t=21.5[a]; kynurenine: t=9.72[a]; amyloid formation: t=8.52[a]) but was ineffective in the physiological concentration range. GSH significantly decreased the value of all indices of glycoxidation in a concentration-dependent manner, with the exception of kynurenine (AGE: t=19.3[a]; dityrosine: t=20.7[a]; N'-formylkynurenine: t=26.5[a]; t=9.72[a]; amyloid formation: t=14.6[a]) (Table 2).

The level of AGEs estimated by fluorimetric measurements was generally confirmed by estimation of AGEs with an ELISA Kit. Carnosine, GSSG, histidine, melatonin, methionine and vitamin B1at physiological concentration had no significant impact on AGEs formation. Tryptophan at a concentration of 80 μM and urea at a concentration of 10 mM slightly inhibited AGEs formation (t=3.18[c] and 2.84[c], respectively) but were ineffective at

lower concentrations. Uric acid in a physiological concentration range significantly increased AGEs generation assayed by ELISA ($t=32.2^{a}$ for 0.35 mM uric acid and 49.3^{a} for 0.7 mM uric acid). Cysteine at a concentration of 0.1 mM slightly reduced generation of AGEs ($t=4.71^{b}$) while lower concentrations did not affect the amount of AGEs. Lysine at concentrations of 0.2 and 0.4 mM slightly inhibited glycation ($t=3.91^{b}$ and 3.54^{c}, respectively). Pyruvic acid reduced glycation in a concentration-dependent manner but in a supraphysiological concentration range ($t=5.97^{b}$ and 6.25^{b} for 2.25 and 5 mM pyruvic acid, respectively). GSH protected against glycation in a concentration-dependent manner in the millimolar concentration range, corresponding to its intracellular concentrations ($t=2.99^{c}$, 4.65^{b} and 5.36^{b} for 5,10 1and 20 mM GSH, respectively; Table 3).

Incubation of erythrocytes in the presence of various glucose concentrations (5, 50 and 100 mM) at 37°C for up to 72 h led to gradual loss of GSH, attenuated by high glucose concentrations. Incubation with CDNB reduced drastically the GSH level (Fig. 1). Incubation of these erythrocytes with glucose did not restore the GSH content. The content of glycated hemoglobin increased with increasing incubation time and increasing glucose concentration. Preincubation of erythrocytes with CDNB promoted hemoglobin glycation as compared with non-treated cells (Fig. 2).

4. Discussion

We examined the protective effect of endogenous compounds on protein glycation *in vitro* using BSA as a model protein. BSA has a 76% similarity in amino acid sequence to human serum albumin (HSA), which is the most abundant human plasma protein (Arasteh et al. 2014). Glycated albumin may be a useful and specific marker of glycemia for pediatric diabetic patients (Lee et al. 2013), diabetic hemodialysis patients (Inaba et al. 2007), diabetic patients with cardiovascular complications (Sato et al. 2013), diabetic patients with advanced

chronic kidney disease (Vos et al. 2011) and patients with coronary artery disease (Ma et al. 2015).

Our results confirm that some potential anti-glycating agents, even if active at higher concentrations, are ineffective at physiological concentrations. Administration of L-arginine to rats with experimental diabetes decreased hemoglobin glycation (Ma et al. 2015; Méndez and Balderas 2001); however in these experiments animals were treated with 10 mM arginine, what suggest that only supraphysiological concentrations may be effective. In our study, arginine was effective at 1 mM concentration and not at lower concentrations. Pyruvate has been reported to prevent cataract development (Hegde and Varma 2005) and protect against fructose-induce formation of high molecular weight aggregates of crystallin (Ramamurthy et al. 2001). However, in our studies its protective activity was evident only at supraphysiological concentrations. GSSG at a high concentration (1 mM) had a pro-glycating effect. Such GSSG concentration is high but may be attained under oxidative stress conditions and may contribute to the pro-glycating effect of oxidative stress. It should be kept in mind that, due to differences in conditions and thus kinetics of glycation, the effect of endogenous compounds on protein glycation *in vivo* may be different than in the artificial *in vitro* system used. Nevertheless, the compounds not effective *in vitro* can hardly be expected to have significant effects *in vivo*. In particular, GSH can be expected to be a better antiglycating agent *in vivo* than *in vitro* since under *in vitro* conditions GSH is partly oxidized to GSSG during 6-day incubation.

From among the compounds studied, only GSH showed an anti-glycating effect *in vitro*, in a cell-free system. In order to check whether prediction from such a system holds within a cell, we studied the effect of glutathione depletion on glycation of hemoglobin in erythrocytes incubated with elevated glucose concentrations for up to 72 h. Incubation with

CDNB led to a drastic decrease in erythrocyte GSH level. Hemoglobin glycation proceeded at a higher rate in cells depleted of GSH.

Our results are in accordance with several earlier findings. Ramamurthy et al. observed that glutathione (10 mM) reverses the effect of glucose on myosin function (Zhao et al. 2000). Jain found a negative correlation between the level of GSH and that of glycated hemoglobin following experimental modulation of GSH level in erythrocytes (Jain 2008). Huby and Harding reported that galactosylation of lens proteins is inhibited by GSH (Huby and Harding 1988).

However, the anti-glycating action of GSH may be not free of undesired effects. Reaction of GSH with glucose may lead to glycation of the α-NH_2 group of the glutamate residue or –SH group of the cysteine residue glutathione, in GSH. N-1-Deoxyfructos-1-yl glutathione was identified as the major glycation product of GSH glycation by glucose *in vitro*. This compound is a poor substrate for glutathione peroxidase, glutathione reductase and glutathione *S*-transferase (Linetsky et al. 2005).

Another compound which may contribute to prevention of glycation *in vivo* is ascorbic acid. This compound was not included in the present study as it shows a pro-glycating activity *in vitro* (Sadowska-Bartosz and Bartosz 2015). In our previous experiments 1 mM ascorbate did not affect hemoglobin glycation in erythrocytes incubated with high glucose (Sadowska-Bartosz and Bartosz 2015; Sadowska-Bartosz et al. 2015b). However, it cannot be excluded that in cells expressing SVCT transporters, in which intracellular ascorbate concentration may reach millimolar levels, ascorbate may also be a significant anti-glycating agent.

We checked also the effect of uric acid (0.7 mM) on hemoglobin glycation of erythrocytes finding a tendency for an increase, but without statistical significance (not shown).

These results demonstrate that the level of intracellular glutathione may be an important determinant of the rate of glycation of intracellular proteins, though being of no importance for blood plasma proteins due to its low extracellular concentrations. It has been suggested that the action of exogenous antioxidants is based principally on the induction of endogenous antioxidant defense via activation of Nrf2 factor rather than direct antioxidant action of exogenous compounds (Forman et al. 2014). A similar situation may exist for glycation of intracellular proteins: keeping high glutathione level may be more important for limiting glycation than exogenous additives, reaching much lower levels *in vivo*. It would be of interest to examine epidemiological data for a possible correlation between the erythrocyte glutathione level and the level of glycated hemoglobin, and between the uric acid level and albumin glycation.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgement

This study was supported by Project 2015/17/B/NZ3/03731 from the National Science Center of Poland.

References

Arasteh A., Farahi S., Habibi-Rezaei M., Moosavi-Movahedi A. A. (2014): Glycated albumin: an overview of the *In Vitro* models of an *In Vivo* potential disease marker. J. Diabetes Metab. Disord. **13,** 49

289 Ashraf J. M., Ansari M. A., Khan H. M., Alzohairy M. A., Choi I. (2016): Green synthesis of
290 silver nanoparticles and characterization of their inhibitory effects on AGEs formation
291 using biophysical techniques. Sci. Rep. **6,** 20414
292 Cao W., Chen J., Chen Y., Chen X., Liu P. (2014): Advanced glycation end products promote
293 heart failure through inducing the immune maturation of dendritic cells. Appl. Biochem.
294 Biotechnol. **172,** 4062-4077
295 Drabkin D. I., Austin L. H. (1935): Spectrophotometric studies. II. Preparations from washed
296 blood cells nitric oxide hemoglobin and sulfhemoglobin. J. Biol. Chem. **112,** 51–65
297 Drenth H., Zuidema S., Bunt S., Bautmans I., van der Schans C., Hobbelen H. (2016): The
298 contribution of advanced glycation end product (AGE) accumulation to the decline in
299 motor function. Eur. Rev. Aging Phys. Act. **13,** 3
300 Forman H. J., Davies K. J., Ursini F. (2014): How do nutritional antioxidants really work:
301 nucleophilic tone and para-hormesis versus free radical scavenging *in vivo*, Free Radic.
302 Biol. Med. **66,** 24-35
303 Hegde K. R., Varma S. D. (2005): Prevention of cataract by pyruvate in experimentally
304 diabetic mice. Mol. Cell. Biochem. **269,** 115-120
305 Hoonhorst S. J., Lo Tam Loi A. T., Hartman J. E., Telenga E. D., van den Berge M.,
306 Koenderman L., Lammers J. W., Boezen H. M., Postma D. S., Ten Hacken N.H.
307 (2014): Advanced glycation end products in the skin are enhanced in COPD.
308 Metabolism **63,** 1149-1156
309 Huby R., Harding J. J. (1988): Non-enzymic glycosylation (glycation) of lens proteins by
310 galactose and protection by aspirin and reduced glutathione. Exp. Eye Res. **47,** 53-59
311 Inaba M., Okuno S., Kumeda Y., Yamada S., Imanishi Y., Tabata T., Okamura M., Okada S.,
312 Yamakawa T., Ishimura E., Nishizawa Y., Osaka CKD Expert Research Group (2007):
313 Glycated albumin is a better glycemic indicator than glycated hemoglobin values in

hemodialysis patients with diabetes: effect of anemia and erythropoietin injection. J. Am. Soc. Nephrol. **18,** 896-903

Jain S.K. (1998): Glutathione and glucose-6-phosphate dehydrogenase deficiency can increase protein glycosylation. Free Radic. Biol. Med. **24,** 197-201

Kandarakis S. A., Piperi C., Topouzis F., Papavassiliou A. G. (2014): Emerging role of advanced glycation-end products (AGEs) in the pathobiology of eye diseases. Prog. Retin. Eye Res. **42,** 85-102

Keppler D., Leier I., Jedlitschky G., König J. (1998): ATP-dependent transport of glutathione S-conjugates by the multidrug resistance protein MRP1 and its apical isoform MRP2. Chem. Biol. Interact. **111-112**, 153-161

Lee J. W., Kim H. J., Kwon Y. S., Jun Y. H., Kim S. K., Choi J. W., Lee J. E. (2013): Serum glycated albumin as a new glycemic marker in pediatric diabetes. Ann. Pediatr. Endocrinol. Metab. **18,** 208-213

LeVine 3rd H. (1999): Quantification of beta-sheet amyloid fibril structures with thioflavin T. Methods Enzymol. **309,** 274-284

Linetsky M. D., Shipova E. V., Legrand R. D., Argirov O.O. (2005): Glucose-derived Amadori compounds of glutathione. Biochim. Biophys. Acta **1724,** 181-193

Liu W., Cohenford M. A., Frost L., Seneviratne C., Dain J.A. (2014): Inhibitory effect of gold nanoparticles on the D-ribose glycation of bovine serum albumin. Int. J. Nanomed. **9**, 5461-5469

Ma X., Hu X., Zhou J., Hao Y., Luo Y., Lu Z., Bao Y., Jia W. (2015): Glycated albumin is more closely correlated with coronary artery disease than 1,5-anhydroglucitol and glycated hemoglobin A1c. Cardiovasc. Diabetol. **14,** 16

337 Méndez J. D., Balderas F. L. (2006): Inhibition by L-arginine and spermidine of hemoglobin
338 glycation and lipid peroxidation in rats with induced diabetes. Biomed. Pharmacother.
339 **60,** 26-31
340 Méndez J. D., Balderas F. (2001): Regulation of hyperglycemia and dyslipidemia by
341 exogenous L-arginine in diabetic rats. Biochimie **83,** 453-458
342 Ramamurthy B., Höök P., Jones A. D., Larsson L. (2001): Changes in myosin structure and
343 function in response to glycation. FASEB J. 15, 2415-2422
344 Ramasamy R., Shekhtman A., Schmidt A. M. (2016) The multiple faces of RAGE -
345 opportunities for therapeutic intervention in aging and chronic disease. Expert Opin.
346 Ther. Targets **220,** 431-446
347 Sadowska-Bartosz I., Adamczyk-Sowa M., Galiniak S., Mucha S., Pierzchała K., Bartosz G.
348 (2013): Oxidative modification of serum proteins in multiple sclerosis. Neurochem. Int.
349 **63,** 507-516
350 Sadowska-Bartosz I., Bartosz G. (2015): Prevention of protein glycation by natural
351 compounds. Molecules **20,** 3309-3334
352 Sadowska-Bartosz I., Galiniak S., Bartosz G., Rachel M. (2014a): Oxidative modifications
353 of proteins in pediatric cystic fibrosis with bacterial infections. Oxid. Med. Cell.
354 Longev. **2014,** 389629
355 Sadowska-Bartosz I., Galiniak S., Bartosz G. (2014b): Kinetics of glycoxidation of bovine
356 serum albumin by glucose, fructose and ribose and its prevention by food components.
357 Molecules **19,** 18828-18849
358 Sadowska-Bartosz I., Galiniak S., Skolimowski J., Stefaniuk I., Bartosz G. (2015a):
359 Nitroxides prevent protein glycoxidation *in vitro*. Free Radic. Res. **49,** 113-121

360 Sadowska-Bartosz I., Stefaniuk I., Galiniak S., Bartosz G. (2015b): Glycation of bovine
361 serum albumin by ascorbate *in vitro:* Possible contribution of the ascorbyl radical?
362 Redox Biol. **6**, 93-99
363 Sato Y., Nagao M., Asai A., Nakajima Y., Takaya M., Takeichi N., Takemitsu S., Sudo M.,
364 Kano-Wakakuri T., Ishizaki A., Harada T., Tanimura-Inagaki K., Okajima F., Tamura
365 H., Sugihara H., Oikawa S. (2013): Association of glycated albumin with the presence
366 of carotid plaque in patients with type 2 diabetes. J. Diabetes Investig. **4**, 634-639
367 Schalkwijk C. G., Miyata T. (2012): Early- and advanced non-enzymatic glycation in diabetic
368 vascular complications: the search for therapeutics. Amino Acids **42,** 1193-1204
369 Semba R. D., Sun K., Schwartz A. V., Varadhan R., Harris T. B., Satterfield S., Garcia M.,
370 Ferrucci L., Newman A. B. (2015): Health ABC Study, Serum carboxymethyl-lysine,
371 an advanced glycation end product, is associated with arterial stiffness in older adults. J.
372 Hypertens. **33**, 797-803
373 Senft A., Dalton T., Shertzer H. (2000): Determining glutathione and glutathione disulfide
374 using the fluorescence probe *o*-phthalaldehyde. Anal. Biochem. **280**, 80–86
375 Simm A., Müller B., Nass N., Hofmann B., Bushnaq H., Silber R. E., Bartling B. (2015):
376 Protein glycation - Between tissue aging and protection. Exp. Gerontol. **68,** 71-75
377 Tessier F. J. (2010): The Maillard reaction in human body. The main discoveries and factors
378 that affect glycation. Pathol. Biol. **58,** 214–219
379 Vos F. E., Schollum J. B., Walker R. J. (2011): Glycated albumin is the preferred marker for
380 assessing glycaemic control in advanced chronic kidney disease. NDT Plus **4**, 368-375
381 Zhao W., Devamanoharan P. S., Varma S. D. (2000): Fructose-mediated damage to lens
382 alpha-crystallin: prevention by pyruvate. Biochim. Biophys. Acta **1500,** 161-168
383 Sadowska-Bartosz I., Bartosz G. (2015): Ascorbic acid and protein glycation *in vitro*. Chem.
384 Biol. Int. **240,** 154-162

385
386

FIGURE LEGENDS

Fig. 1. Effect of CDNB treatment on the glutathione content of erythrocytes. Statistical significance of differences: [a] $p < 0.001$, [b] $p < 0.01$, [c] $p < 0.05$ (corresponding CDNB-treated vs non-treated samples).

Fig. 2. Effect of GSH depletion and glucose concentration on hemoglobin glycation in erythrocytes incubated *in vitro*; [a] $p < 0.001$, [c] $p < 0.05$.

Fig. 1 Download full resolution image

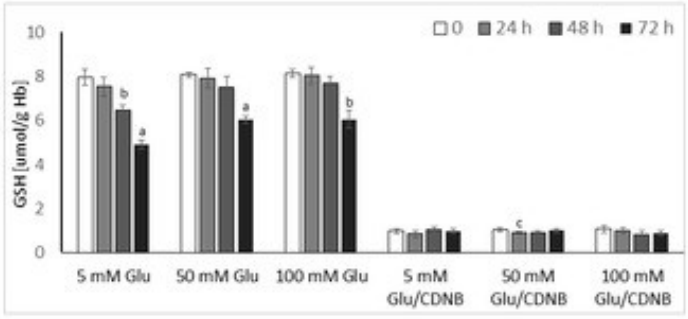

Fig. 2 Download full resolution image