Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 855 (2007) 59–65
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Review

Protein glutathionylation and oxidative stress☆

Toshimitsu Niwa*

Nagoya University Hospital, Department of Clinical Preventive Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8560, Japan

Received 25 August 2006; accepted 28 December 2006
Available online 3 January 2007

Abstract

Liquid chromatography/electrospray ionization-mass spectrometry (LC/ESI-MS) demonstrated that glutathionyl hemoglobin (Hb) levels are increased in patients with diabetes, hyperlipidemia, uremia and Friedreich's ataxia. Glutathionylation of Hb is enhanced by oxidative stress. High performance liquid chromatography (HPLC) and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS) have also been developed for the quantification of glutathionyl Hb. Glutathionyl-lens proteins were detected in uremic patients and cataractous aged subjects. Glutathionylation of numerous enzymes is induced by oxidative stress, reduces their catalytic activities and may be involved in protection from the damaging effects of oxidative agents. Thioredoxin, glutaredoxin (thioltransferase) and protein disulfide isomerase are the key enzymes in controlling cellular oxidative stress that catalyze reduction of glutathionyl protein disulfide bonds. Thus, protein glutathionylation is closely associated with oxidative stress.
© 2007 Elsevier B.V. All rights reserved.

Keywords: Glutathionyl hemoglobin; Oxidative stress; Diabetes mellitus; Uremia; Liquid chromatography/mass spectrometry; High performance liquid chromatography; Matrix-assisted laser desorption ionization-time of flight mass spectrometry

Contents

1. Introduction

Oxidative stress occurs when there is excessive free radical production in the face of defective anti-oxidant defenses. Oxidative stress produces profound alterations to cellular membrane lipids, proteins and nucleic acids, impairing cell metabolism and viability, and has been considered to be involved in aging [1] and such diseases as diabetes mellitus [2], uremia [3], atherosclerosis [4], hyperlipidemia [5], rheumatoid arthritis [6], adult respiratory distress syndrome [7], reoxygenation injury [8], human immunodeficiency virus infection [9], cystic fibrosis [10] and Friedreich's ataxia [11]. Oxidative stress corresponds to an imbalance between the production of reactive oxygen species,

☆ This paper was presented at the 31st Annual Meeting of the Japanese Society for Biomedical Mass Spectrometry, Nagoya, Japan, 28–29 September 2006.

* Tel.: +81 52 744 1980; fax: +81 52 744 1954.
E-mail address: tniwa@med.nagoya-u.ac.jp.

1570-0232/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2006.12.029

mainly the superoxide anion, hydroxyl radical, peroxyl radicals and hydrogen peroxide, and protective mechanisms. Several enzymatic systems can detoxify reactive oxygen species: superoxide dismutase catalyzes the conversion of superoxide anion to hydrogen peroxide and works concomitantly with catalases and glutathione peroxidase. The level of reduced glutathione (GSH) is a limiting factor in this enzymatic process, which requires the maintenance of high reduced to oxidized glutathione ([GSH]/[GSSG]) ratio as achieved by glutathione reductase. Some reducing agents act as free radical scavengers to non-enzymatically detoxify reactive oxygen species: GSH, vitamin E and vitamin C.

Glutathione (γ-L-glutamyl-L-cysteinylglycine) is the major intracellular non-protein thiol compound, and plays a major role in the protection of cells and tissue structures from oxidative injury. Glutathione can be reduced (GSH), oxidized (GSSG), or a protein–glutathione mixed disulfide. GSH inhibits free radical-mediated injury by eliminating reactive oxygen species, and protects protein thiol groups from oxidation by serving as a biological redox agent. Intracellular and blood concentrations of GSH are in millimolar range, while plasma concentration is in the micromolar range and accounts for approximately 0.4% of total blood GSH [12,13].

2. Glutathionylation of hemoglobin (Hb)

2.1. Formation and oxygen affinity of glutahionyl Hb

Human adult Hb (HbA) can react *in vitro* with GSSG with disulfide bond formation between Cys β93 and the cysteine of glutathione [14]. The glutathione adduct formation is associated with β chain but not α chain, because Cys β93 provides the only accessible thiol group at the surface of the Hb molecule [14]. The glutathionyl Hb produced *in vitro* shows increased oxygen affinity, a reduced cooperativity and a reduced alkaline Bohr effect [15]. Glutathionyl Hb was produced *in vitro* by thiol-disulfide exchange between mixed disulfides of Hb and GSSG to study its anti-sickling effect. It was possible to bind most of the intracellular GSSG to Hb by using a two-step reaction, the formation of a mixed disulfide, followed by a thiol-mixed disulfide exchange. By using this method, up to 25% of intracellular Hb could be obtained in the glutathionyl Hb form. However, glutathionyl Hb could not be detected in normal erythrocytes by using electrophoresis [16], because GSSG, which could form a mixed disulfide with Hb, is present at a very low concentration [17]. However, we could detect glutathionyl Hb in normal erythrocytes by using highly sensitive and specific liquid chromatography/electrospray ionization-mass spectrometry (LC/ESI-MS).

Murakami and Mawatari [18] studied biochemical consequences of oxidation of Hb in intact human erythrocytes. The incubation of washed erythrocyte with 1 mM *tert*-butylhydroperoxide induced an increase in glutathionyl Hb. The formation of glutathionyl Hb occurred linearly until 10 min in parallel with the formation of methemoglobin (metHb) after exhaustion of GSH. Thus, they demonstrated that metHb, but not normal Hb, reacts with GSSG to form glutathionyl Hb.

The glutathionyl Hb prepared *in vitro* by incubating Hb with GSH showed a marked increase in oxygen affinity and a marked decrease in a Hill coefficient as compared to Hb incubated without GSH. The increase in oxygen affinity was similarly observed in Hb treated with other sulfhydryl reagents such as *N*-ethylmaleimide [19,20]. The high oxygen affinity of glutathionyl Hb, and also probably those of the other *S*-modified Hb described above, is ascribed to the perturbation of the tertiary structure of β chain and the α1–β2 contacts in the T state of Hb [15], leading to the shift of the allosteric equilibrium toward the high affinity R-state. The increased levels of glutathionyl Hb with high oxygen affinity and low cooperativity in diabetes and uremia may lead to reduced tissue oxygen delivery.

2.2. Liquid chromatography/electrospray ionization-mass spectrometry

We measured glutathionyl Hb levels in type 2 diabetic patients, hyperlipidemic patients, hemodialysis patients, patients on continuous ambulatory peritoneal dialysis (CAPD) and healthy subjects by use of liquid chromatography/electrospray ionization-mass spectrometry (LC/ESI-MS) [21–23]. Hemoglobin α and β chains were separated in the reconstructed ion chromatogram of Hb fraction from a diabetic patient (Fig. 1A). The deconvoluted mass spectrum of Hbα (peak 1) demonstrated the presence of α chain (15,127 Da) and glycated α chain (15,289 Da), but no glutathionyl α chain (Fig. 1B). The deconvoluted mass spectrum of β chain (peak 2) demonstrated the presence of β chain (15,868 Da), glycated β chain (16,030 Da) and glutathionyl β chain (16,173 Da) (Fig. 1C).

The level of glutathionyl β chain expressed as the percent to intact β chain was markedly increased in diabetic patients ($7.9 \pm 0.5\%$, mean ± S.E., $p < 0.001$) and hyperlipidemic patients ($8.1 \pm 0.8\%$, $p < 0.001$) as compared with healthy subjects ($3.7 \pm 0.3\%$) [21]. The oral administration of vitamin E (tocopherol nicotinate) at a dose of 600 mg/day for 8 weeks to 10 diabetic patients markedly reduced the level of glutathionyl β chain (before vitamin E: $10.2 \pm 0.8\%$, mean ± S.E.; after vitamin E: $4.1 \pm 0.4\%$, $p < 0.001$) (Fig. 2), whereas it did not change HbA1c (glycohemoglobin) levels at all (before vitamin E: $7.6 \pm 0.3\%$; after vitamin E: $7.6 \pm 0.4\%$) [23].

Recently, Sampathkumar et al. [24] have analyzed glutathionyl Hbβ chain in erythrocyte lysates from diabetic subjects with ($n = 53$) and without ($n = 47$) microangiopathy by using LC/ESI-MS. The positivity for glutathionyl Hb in diabetic patients with microangiopathy was significantly higher (69%) compared to diabetics without microangiopathy (22%) and control subjects (14%) ($n = 30$). Glutathionyl Hb levels were significantly associated with the duration of diabetes, HbA1c and thiobarbituric acid substances (TBARS) levels. GSH levels were negatively correlated with glutathionyl Hb in diabetic subjects. Thus, they demonstrated that glutathionyl Hb levels are markedly increased in diabetic subjects with microangiopathy. Since diabetic subjects also exhibited increased lipid peroxidation and decreased GSH levels, it appears that enhanced oxidative stress may account for the

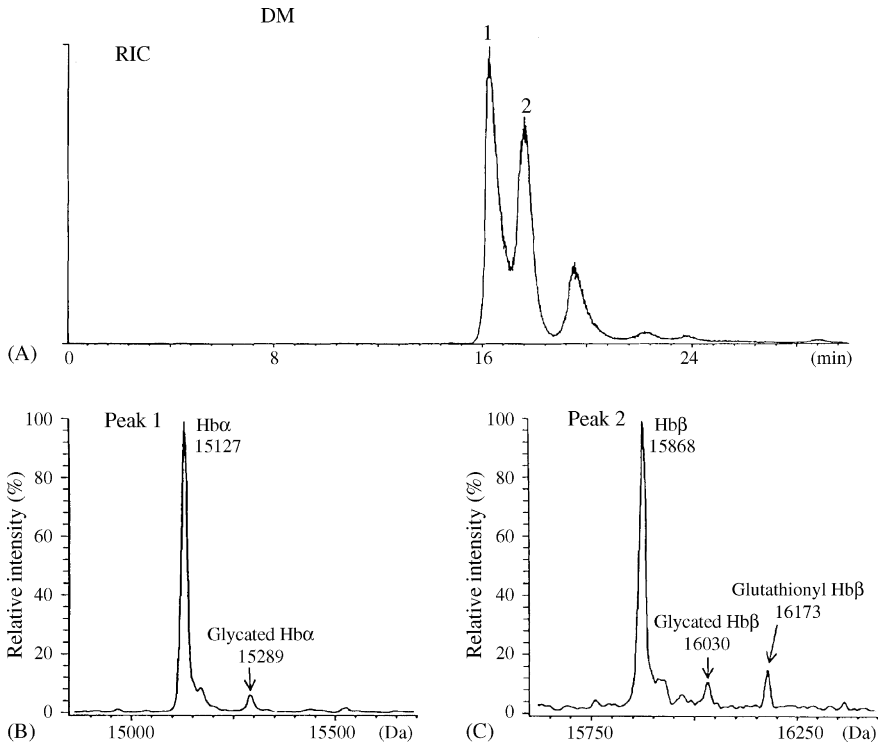

Fig. 1. Reconstructed ion chromatogram (RIC) of Hb from a diabetic patient (A), and deconvoluted ESI mass spectra of peak 1 (B) and peak 2 (C) in the RIC chromatogram. Glycated α chain and β chain could be detected, whereas glutathionyl β chain but no glutathionyl α chain could be detected (DM: diabetes mellitus). Blood samples were drawn using EDTA as an anti-coagulant. Whole blood samples (15 μl) were immediately diluted with distilled water (485 μl). The mixture was subsequently centrifuged at 12,000 × *g* for 10 min. The supernatant was kept at −40 °C until LC/ESI-MS analysis [21–23]. LC/ESI-MS was performed using a triple-stage quadrupole mass spectrometer (TSQ7000; Thermoquest, San Jose, CA, USA) equipped with a reversed phase column (TSKgel Phenyl-5PW RP 4.6 mm i.d. × 7.5 cm). A mobile phase consisting of solution (A) (2% acetonitrile in 0.2% acetic acid) and solution (B) (90% acetonitrile in 0.2% acetic acid) was delivered at a flow rate of 0.5 ml/min at ambient temperature. The mobile phase was linearly programmed from 15% of solution (B) to 45% of solution (B) in 30 min. The conditions for ESI-MS were as follows; electric field 4.5 kV, nitrogen sheath gas 70 psi, auxiliary gas 15 units, capillary temperature 275 °C. Samples (10 μl) were diluted with solvent A (90 μl), and subsequently were subjected to LC/ESI-MS. Molecular weights of proteins were determined by deconvoluted mass spectra of their peaks. The levels of glutathionyl β chain were expressed as the percents of the peak height ratios to intact β chain.

increased glutathionyl Hb concentrations and altered redox signaling.

Glutathionyl Hb levels were measured in hemodialysis and continuous ambulatory peritoneal dialysis (CAPD) patients and normal subjects by calculating the peak height ratios of glutathionyl β chain to intact β chain (Fig. 3). Glutathionyl Hb levels in hemodialysis patients ($8.0 \pm 3.6\%$; $n = 30$; $p < 0.0001$) and continuous ambulatory peritoneal dialysis patients ($5.9 \pm 2.7\%$; $n = 10$; $p < 0.05$) were significantly elevated as compared with normal subjects ($3.0 \pm 1.6\%$; $n = 20$) [25]. However, there were no significant differences in glutathionyl Hb between hemodialysis patients and continuous ambulatory peritoneal dialysis patients. There were also no significant differences in the glutathionyl Hb levels between before ($8.7 \pm 3.2\%$; $n = 12$) and after HD ($8.7 \pm 2.8\%$; $n = 12$).

Friedreich's ataxia is a neurodegenerative disease due to a GAA expansion in a gene coding for a mitochondrial protein (frataxin), implicated in the regulation of iron metabolism. Oxidative stress and mitochondrial dysfunction have long been considered to play a role in Friedreich's ataxia. Piemonte et al. [26] studied glutathione metabolism in the blood of 14 patients with Friedreich's ataxia by measuring total, free and protein-bound glutathione concentrations. Total and free glutathione concentrations were determined by reverse-phase liquid chromatography with fluorescence detection. They found a reduction of free glutathione levels in the blood of patients with Friedreich's ataxia, a total glutathione concentration comparable to the controls. Glutathionyl Hb in erythrocytes was measured by ESI-MS. They found a significant increase in glutathionyl Hb in the patients with Friedreich's ataxia as compared with healthy subjects ($15 \pm 1.5\%$ *versus* $8 \pm 1.8\%$, $p < 0.05$). Thus, this study also proves that glutathionyl Hb is useful as a clinical marker for oxidative stress in Friedreich's ataxia; and suggests that free radical cytotoxicity may play a relevant role in the pathophysiology of neurodegeneration.

2.3. High performance liquid chromatography

Glutathionyl Hb has been suggested as a biomarker of oxidative stress. To be widely used, a simple method such as high performance liquid chromatography (HPLC) should be developed for the measurement of glutathionyl Hb. Pastore et al. [27] reported a simple method to measure glutathionyl Hb level in erythrocytes, by using cation-exchange HPLC with UV detection. Glutathionyl Hb level was measured in erythrocytes of healthy subjects, with a mean value of

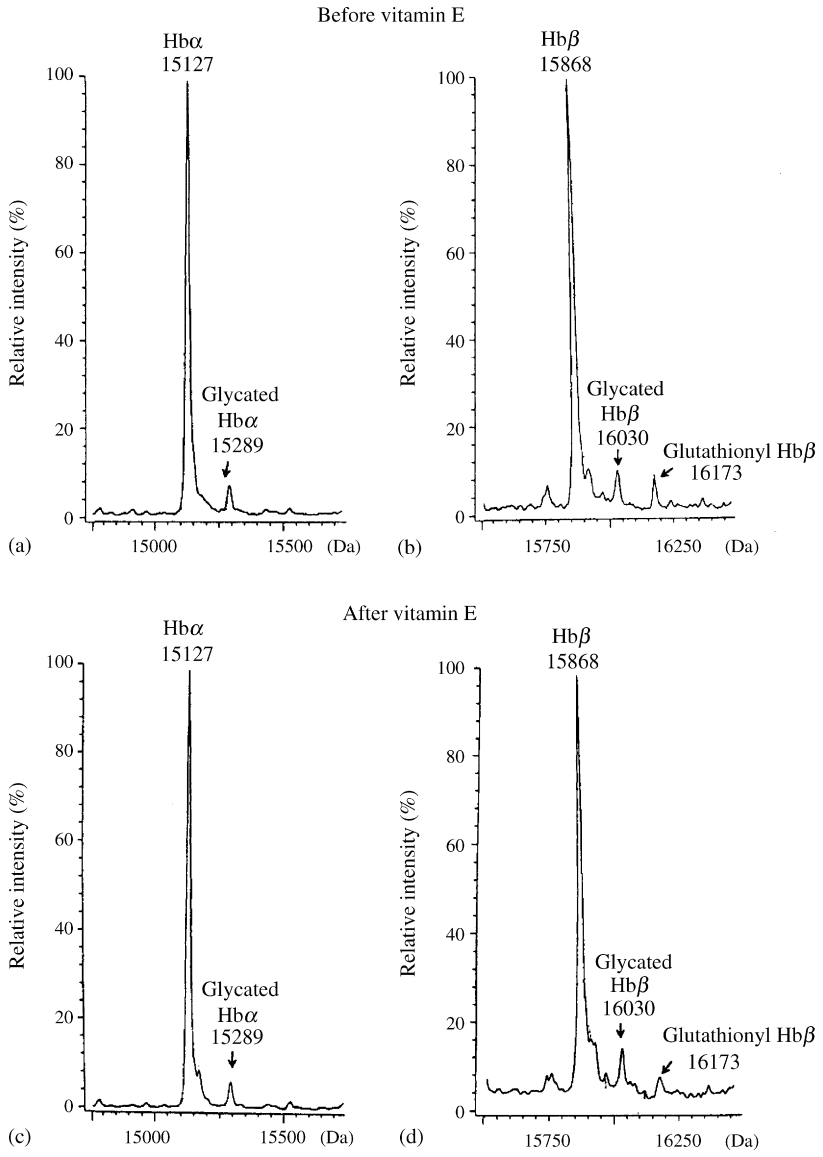

Fig. 2. Deconvoluted ESI mass spectra of peak 1 (a) and peak 2 (b) in the RIC chromatogram of Fig. 1 (before vitamin E administration), and of their respective peaks (c and d) in the same patient 8 weeks after administration of vitamin E. Glycated α chain and β chain could be detected, whereas glutathionyl β chain but no glutathionyl α chain could be detected. The peak height of glutathionyl β chain 8 weeks after administration of vitamin E was decreased as compared with that before vitamin E administration.

$2.58 \pm 0.7\%$, calculated as the percentage of its peak area ratio to that of total Hb. The availability of a simple and reproducible method to detect glutathionyl Hb concentration in blood could be useful in monitoring oxidative stress, and for investigating the efficacy of anti-oxidant therapies in clinical trials.

Rossi et al. [28] reported that oxidation of GSH to form GSSG occurs during sample preparation. To minimize the artifact oxidation of GSH to form glutathionyl Hb during sample preparation, *N*-ethylmaleimide that passes freely through cytoplasma membrane and quenches SH-groups, should be added immediately into blood, followed by centrifugation and hemolysis procedures for HPLC analysis. EDTA, a metal chelator that is able to decrease the oxidation process, should be used as an anti-coagulant.

2.4. Matrix-assisted laser desorption ionization-time of flight mass spectrometry

Recently, Biroccio et al. [29] have developed a technique based on linear mode matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS) for quantitative analysis of Hb species. This method was applied to the quantification of glycated and glutathionyl Hb. Glutathionyl Hb was investigated in 184 subjects (101 males and 83 females), indicating a bimodal distribution of this species. In fact 65.22% of screened individuals had glutathionyl Hb levels lower than 0.50% while 34.78% had glutathionyl Hb levels higher than 0.50%. A semiautomatic robotic procedure was developed for fast analysis of a large number of samples. This is the first report of a quantitative application of linear MALDI-TOF-MS for the

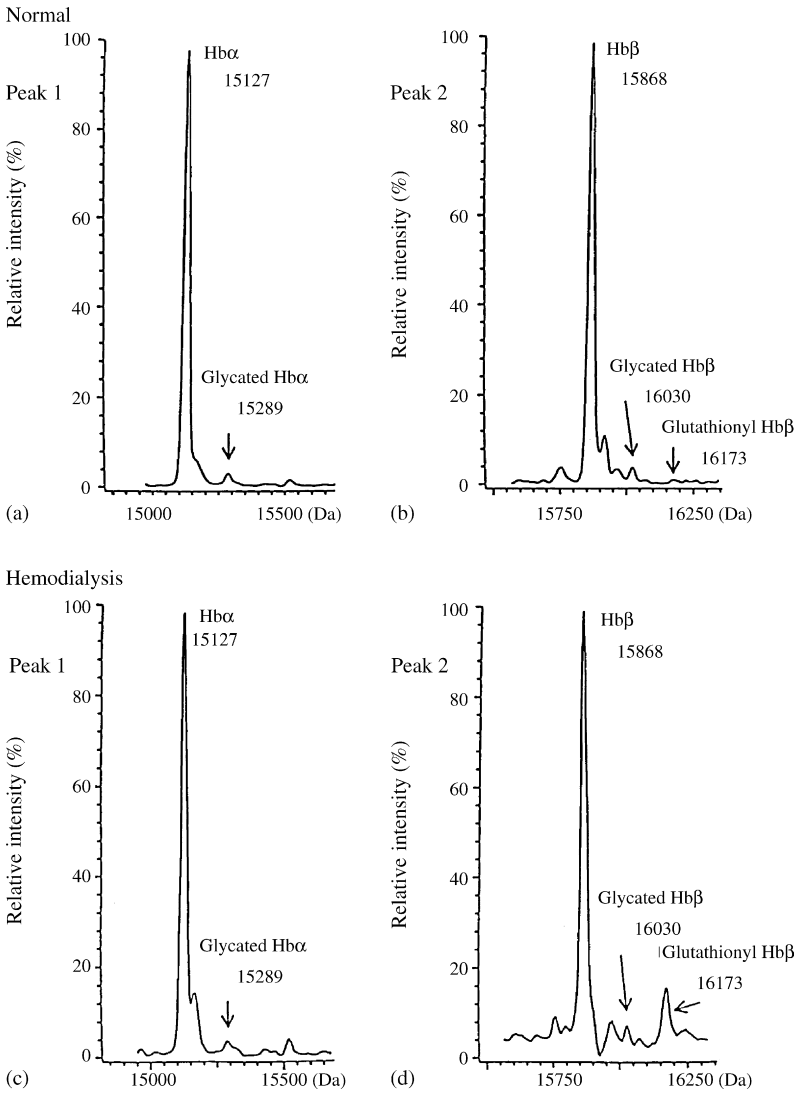

Fig. 3. Deconvoluted mass spectra of peak 1 (a) and peak 2 (b) from a normal subject and peak 1 (c) and peak 2 (d) from a hemodialysis patient. The α chain was detected at a molecular weight of 15,127 Da, and glycated α chain at 15,289 Da (15,127 + 162). The β chain was detected at 15,868 Da, and glycated β chain at 16,030 Da (15,868 + 162). Glutathionyl β chain was detected at 16,173 Da (15,868 + 305). Interestingly, glutathionyl β chain was markedly increased in the HD patient as compared with the normal subject.

determination of glutathionyl Hb in blood samples. This method allows fast screening of glutathionyl Hb, therefore opening the route to explore its specificity and sensitivity as a molecular biomarker.

3. Glutathionylation of lens proteins

Glutathionyl α-crystalline was detected in the lens of uremic patients but not in the lens of normal subjects [30]. α-Crystallins from the water-soluble and the water-insoluble, guanidine-soluble portions of lenses from four uremic patients and two normal subjects of similar age were isolated and enzymatically digested into peptides. Fast atom bombardment mass spectrometry (FAB-MS) of the peptides demonstrated that molecular weights of the peptides indicated modifications specifically associated with uremia. The only modifications observed in the α-crystallins from uremic patients, but not in the normal old lenses, were glutathione adducts to Cys 131 and Cys 142. The formation of glutathionyl crystalline is considered to be due to oxidative stress associated with uremia, and may be involved in the development of cataract. The lenses from the uremic patients were searched for evidence of carbamylation at lysyl or cysteinyl residues. However, carbamylation was not detected. Thus, carbamylation is not a major modification of the lens α-crystallins of uremic patients [30].

Linetsky and LeGrand [31] investigated whether modification of lens proteins by glutathione could proceed by the formation of a non-reducible thioether bond between protein and glutathione. Direct enzyme linked immunoassay (ELISA) of the reduced water-soluble and water-insoluble lens proteins from human cataractous, aged lenses showed a concentration-dependent immunoreactivity toward human non-reducible glutathionyl-lens proteins only. The reduced water-insoluble cataractous lens proteins showed the highest

immunoreactivity. The level of this modification ranged from 0.7 to 1.6 nmol/mg protein in water-insoluble proteins from aged and cataractous lenses. Since disulfides were not detected in the reduced and alkylated human lens proteins, glutathione is most likely attached to lens proteins through thioether bonds. Thus, glutathionylation of human lens proteins can occur through the formation of non-reducible thioether bonds.

4. Glutathionylation of enzymes

There are a number of reports that enzymes can undergo glutathionylation, often followed by a reduced catalytic efficiency. The followings are a few examples of glutathionylation of enzymes. Importantly, glutathionylation of thioredoxin-1 that catalyzes reduction of glutathionyl protein (protein-SSG), contributes to the regulation and functions of thioredoxin-1 [32].

Bovine lens aldose reductase (ALR2) is readily modified by GSSG to glutathionyl ALR2 exhibiting a reduced catalytic efficiency [33,34]. The modification is completely reversed by GSH or dithiothreitol. Thus, the rate and the maximal extent of ALR2 inactivation are proportional to the redox ratio of [GSH]/[GSSG]. Glutathionylation of ALR2 might be part of a cell strategy to preserve reducing power in conditions of oxidative stress.

Glutathionylation of carbonic anhydrase III (CAIII) occurs rapidly in hepatocytes under oxidative stress [35]. The crystal structure of glutathionyl CAIII from rat liver reveals covalent adducts on Cys 183 and Cys 188. Glutathionylation of CAIII may be involved in the protection/recovery from the damaging effects of oxidative agents.

The control of mitochondrial redox balance and oxidative damage is one of the primary functions of mitochondrial NADP(+)-dependent isocitrate dehydrogenase (IDPm) [36]. GSSG led to enzyme inactivation with simultaneous formation of a mixed disulfide between GSH and Cys 269 in IDPm. The inactivated IDPm was reactivated enzymatically by glutaredoxin 2 in the presence of GSH, indicating that the inactivated form of IDPm is a glutathionyl mixed disulfide. Glutaredoxin (thioltransferase) is a specific and efficient catalyst of protein-SSG reduction. The glutathionyl IDPm appeared to be significantly less susceptible than native protein to peptide fragmentation by reactive oxygen species and proteolytic digestion, suggesting that glutathionylation plays a protective role presumably through the structural alterations. Thus, IDPm activity appears to be modulated through enzymatic glutathionylation and deglutathionylation during oxidative stress.

5. Protein disulfide oxidoreductases

Glutathionylation of proteins has been proposed as a reversible means of storing GSH during oxidative stress, and has been regarded as a protective mechanism against irreversible protein thiol-oxidation. In the past, reversible oxidation of protein cysteines such as glutathionylation, disulfide formation and *S*-nitrosation were considered as protein damage in the context of oxidative stress. However, oxidoreduction of protein thiols/disulfides is now considered as a regulatory mechanism. Thioredoxin, glutaredoxin and protein disulfide isomerase are the key enzymes in cellular redox regulation that catalyze the reduction of these disulfide bonds [37]. Peroxiredoxins protect against oxidative stress, and modulate intracellular signaling cascades that apply hydrogen peroxide as a second messenger, and regulation of cell proliferation [38]. Peroxiredoxins are a family of anti-oxidant thioredoxin-dependent peroxidases. Methionine residues in proteins can be oxidized to methionine sulfoxide. Methionine sulfoxide can be repaired by methionine sulfoxide reductases. The methionine residues could act as endogenous anti-oxidants of proteins [39].

5.1. Thioredoxin

Thioredoxin-1 consists of a redox regulatory domain containing the active cysteine residues 32 and 35, and three additional non-active cysteine residues at positions 62, 69 and 73 for human thioredoxin-1. Biological functions of thioredoxin-1 are anti-oxidative, anti-apoptotic and pro-proliferative properties. Thioredoxin-1 is regulated by the ability of the thioredoxin reductase to reduce oxidized thioredoxin-1 at cysteines 32 and 35. However, post-translational modifications of thioredoxin-1, including glutathionylation, thiol-oxidation and *S*-nitrosation, at the non-active cysteines importantly contribute to the regulation and functions of thioredoxin-1 [32].

5.2. Glutaredoxin

Glutaredoxins (GRx) or thioltransferases are a family of small molecular weight proteins with thiol-disulfide exchange activity. Glutaredoxins catalyze reduction of glutathionyl protein (protein-SSG). Glutaredoxins are a versatile catalyst, also facilitating glutathione-thiyl radical scavenging and *S*-glutathionylation of redox signal mediators, consistent with a critical role in cellular regulation [40]. Thus, glutaredoxins are a multifunctional enzyme with oxidoreductase, peroxidase and glutathione-*S*-transferases activity, and detoxify wide range of oxidants [41].

Glutaredoxin (thioltransferase) with a molecular weight of 11,300 was identified in human red blood cells [42,43]. The thioltransferase–GSH–GSSG reductase system catalyze the regeneration of hemoglobin from glutathionyl hemoglobin. However, in diabetic and dialysis patients, glutathionyl hemoglobin levels are increased, presumably due to highly extensive oxidative stress in the erythrocytes of these patients.

5.3. Protein disulfide isomerase

Protein disulfide isomerase (PDI) catalyzes formation, reduction and isomerization of disulfide bonds of proteins in the endoplasmic reticulum of mammalian cells [44]. Native disulfide bonds are essential for the structure and function of many membrane and secretory proteins. Cellular functions hinge on the ability of proteins to adopt their correct folds, and mis-folded proteins can lead to disease. Catalysis of disulfide bond formation is a step in the oxidative folding pathway that takes place in specialized cellular compartments. In the endoplasmic retic-

ulum of eukaryotes, disulfide formation is catalyzed by PDI; by contrast, prokaryotes produce a family of disulfide bond proteins [45].

6. Conclusion

Glutathionyl Hb levels are increased in diabetic patients, hyperlipidemic patients, uremic patients and patients with Friedreich's ataxia. The enhanced oxidative stress in these diseases accounts for the increased glutathionyl Hb. Not only hemoglobin but also lens proteins and numerous enzymes are glutathionylated by enhanced oxidative stress. Thus, protein glutathionylation appears to be a rather common phenomenon associated with oxidative stress.

References

[1] E.R. Stadtman, Science 257 (1992) 1220.
[2] S.M. Son, M.K. Whalin, D.G. Harrison, W.R. Taylor, K.K. Griendling, Curr. Diab. Rep. 4 (2004) 247.
[3] N.D. Vaziri, Semin. Nephrol. 24 (2004) 469.
[4] R. Stocker, J.F. Keaney Jr., Physiol. Rev. 84 (2004) 1381.
[5] A. Warnholtz, H. Mollnau, M. Oelze, M. Wendt, T. Munzel, Curr. Hypertens. Rep. 3 (2001) 53.
[6] C.A. Hitchon, H.S. El-Gabalawy, Res. Ther. 6 (2004) 265.
[7] C.W. Chow, M.T. Herrera Abreu, T. Suzuki, G.P. Downey, Am. J. Respir. Cell. Mol. Biol. 29 (2003) 427.
[8] D.R. Janero, Crit. Rev. Food Sci. Nutr. 35 (1995) 65.
[9] A.P. Bautista, Free Radic. Biol. Med. 31 (2001) 1527.
[10] A. van der Vliet, C.E. Cross, Curr. Opin. Pulm. Med. 6 (2000) 533.
[11] J.M. Cooper, A.H. Schapira, Biofactors 18 (2003) 163.
[12] F. Michelet, R. Gueguen, P. Leroy, M. Wellman, A. Nicolas, G. Siest, Clin. Chem. 41 (1995) 1509.
[13] J.P. Richie, L. Skowronski Jr., P. Abraham, Y. Leutzinger, Clin. Chem. 42 (1996) 64.
[14] M.C. Garel, C. Domenget, J. Caburi-Martin, C. Prehu, F. Galacteros, Y. Beuzard, J. Biol. Chem. 261 (1986) 14704.
[15] C.T. Craescu, C. Poyart, C. Schaeffer, M.C. Garel, J. Kister, Y. Beuzard, J. Biol. Chem. 261 (1986) 14710.
[16] M.C. Garel, Y. Beuzard, J. Thillet, C. Domenget, J. Martin, F. Galacteros, J. Rosa, Eur. J. Biochem. 123 (1982) 513.
[17] I. Ceballos-Picot, V. Witko-Sarsat, M. Merad-Boudia, A.T. Nguyen, M. Thevenin, M.C. Jaudon, J. Zingraff, C. Verger, P. Jungers, B. Descamps-Latscha, Free Radic. Biol. Med. 21 (1996) 845.
[18] K. Murakami, S. Mawatari, Arch. Biochem. Biophys. 417 (2003) 244.
[19] A. Riggs, J. Biol. Chem. 236 (1961) 1948.
[20] R. Benesch, R.E. Benesch, J. Biol. Chem. 236 (1961) 405.
[21] T. Niwa, C. Naito, A.H.M. Mawjood, K. Imai, Clin. Chem. 46 (2000) 82.
[22] C. Naito, M. Kajita, T. Niwa, J. Chromatogr. B 731 (1999) 121.
[23] C. Naito, T. Niwa, J. Chromatogr. B 746 (2000) 91.
[24] R. Sampathkumar, M. Balasubramanyam, S. Sudarslal, M. Rema, V. Mohan, P. Balaram, Clin. Biochem. 38 (2005) 892.
[25] F. Takayama, S. Tsutsui, M. Horie, K. Shimokata, T. Niwa, Kidney Int. 59 (Suppl. 78) (2001) S155.
[26] F. Piemonte, A. Pastore, G. Tozzi, D. Tagliacozzi, F.M. Santorelli, R. Carrozzo, C. Casali, M. Damiano, G. Federici, E. Bertini, Eur. J. Clin. Invest. 31 (2001) 1007.
[27] A. Pastore, A. Francesca Mozzi, G. Tozzi, L. Maria Gaeta, G. Federici, E. Bertini, A. Lo Russo, L. Mannucci, F. Piemonte, Anal. Biochem. 312 (2003) 85.
[28] R. Rossi, A. Milzani, I. Dalle-Donne, D. Giustarini, L. Lusini, R. Colombo, P. Di Simplicio, Clin. Chem. 48 (2002) 742.
[29] A. Biroccio, A. Urbani, R. Massoud, C. di Ilio, P. Sacchetta, S. Bernardini, C. Cortese, G. Federici, Anal. Biochem. 336 (2005) 279.
[30] J.B. Smith, G.A. Shun-Shin, Y. Sun, L.R. Miesbauer, Z. Yang, Z. Yang, X. Zhou, J. Schwedler, D.L. Smith, J. Protein Chem. 14 (1995) 179.
[31] M. Linetsky, R.D. LeGrand, Mol. Cell. Biochem. 272 (2005) 133.
[32] J. Haendeler, Antioxid. Redox Signal. 8 (2006) 1723.
[33] M. Cappiello, M. Voltarelli, M. Giannessi, I. Cecconi, G. Camici, G. Manao, A. Del Corso, U. Mura, Exp. Eye Res. 58 (1994) 491.
[34] M. Cappiello, P. Amodeo, B.L. Mendez, A. Scaloni, P.G. Vilardo, I. Cecconi, M. Dal Monte, S. Banditelli, F. Talamo, V. Micheli, F.J. Giblin, A.D. Corso, U. Mura, Chem. Biol. Interact. 130–132 (2001) 597.
[35] R.J. Mallis, B.W. Poland, T.K. Chatterjee, R.A. Fisher, S. Darmawan, R.B. Honzatko, J.A. Thomas, FEBS Lett. 482 (2000) 237.
[36] I.S. Kil, J.W. Park, J. Biol. Chem. 280 (2005) 10846.
[37] P. Ghezzi, Free Radic. Res. Jun. 39 (2005) 573.
[38] S. Immenschuh, E. Baumgart-Vogt, Antioxid. Redox Signal. 7 (2005) 768.
[39] R.L. Levine, B.S. Berlett, J. Moskovitz, L. Mosoni, E.R. Stadtman, Mech. Ageing Dev. 107 (1999) 323.
[40] D.W. Starke, P.B. Chock, J.J. Mieyal, J. Biol. Chem. 278 (2003) 14607.
[41] E.J. Collinson, C.M. Grant, J. Biol. Chem. J. 278 (2003) 22492.
[42] J.J. Mieyal, D.W. Starke, S.A. Gravina, C. Dothey, J.S. Chung, Biochemistry 30 (1991) 6088.
[43] W.W. Wells, Y. Yang, T.L. Deits, Z.R. Gan, Adv. Enzymol. Relat. Areas Mol. Biol. 66 (1993) 149.
[44] C.E. Jessop, S. Chakravarthi, R.H. Watkins, N.J. Bulleid, Biochem. Soc. Trans. 32 (2004) 655.
[45] C.W. Gruber, M. Cemazar, B. Heras, J.L. Martin, D.J. Craik, Trends Biochem. Sci. 31 (2006) 455.