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Biochem Soc Trans. Author manuscript; available in PMC 2009 February 11.

Published in final edited form as:
Biochem Soc Trans. 2008 October ; 36(Pt 5): 1045–1050. doi:10.1042/BST0361045.

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Dicarbonyls linked to damage in the powerhouse: glycation of mitochondrial proteins and oxidative stress

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Abstract

Protection of mitochondrial proteins from glycation by endogenous dicarbonyl compounds, methylglyoxal and glyoxal, was found recently to prevent increased formation of reactive oxygen species and oxidative and nitrosative damage to the proteome during aging and produce life extension in the nematode *Caenorhabditis elegans*. This suggests that dicarbonyl glycation damage to the mitochondrial proteome may be a preceding event to mitochondrial dysfunction leading to oxidative stress. Future research will address the functional charges in mitochondrial proteins that are the targets for dicarbonyl glycation.

Keywords

advanced glycation end-product (AGE); dicarbonyl; glycation; mitochondrion; oxidative stress

Dicarbonyl glycation of mitochondrial proteins as a cause of mitochondrial dysfunction and oxidative stress

In our recent research with collaborators, we found evidence of life extension of the nematode *Caenorhabditis elegans* with overexpression of the gene for glyoxalase 1 (*Glo1*) [1]. Silencing of *Glo1* decreased lifespan. *Glo1* is a further example of a 'vitagene', a gene which with manipulated change in expression produces life extension [2]. Glo1 is a glutathione-dependent enzyme that catalyses the metabolism of reactive dicarbonyl-glycating agents, MG (methylglyoxal) and glyoxal [3]. It thereby prevents the glycation of proteins by these dicarbonyl compounds. Proteins of mitochondria were found to be major targets of dicarbonyl glycation: increased glycation of mitochondrial proteins was associated with increased formation of ROS (reactive oxygen species) and increased proteome damage by oxidative and nitrosative processes. There was a decline of *Glo1* expression in *C. elegans* and increased formation of mitochondrial ROS in normal aging. Overexpression of *Glo1* in *C. elegans* decreased dicarbonyl glycation of mitochondrial proteins, decreased the formation of ROS and proteome markers of dicarbonyl glycation and also markers of oxidative and nitrosative damage (methionine sulfoxide and 3-nitrotyrosine residues respectively) with concomitant life extension. This indicated, for the first time, that dicarbonyl glycation may be the critical damage to the mitochondrial proteome that triggers increased ROS and oxidative damage in aging. It may therefore be important to prevent increased dicarbonyl glycation to achieve a decrease in formation of ROS by mitochondrial dysfunction. This has important implications for current understanding of ROS and oxidative damage in an attempt to achieve healthy aging and prevent vascular disease,

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particularly in diabetes and renal failure. An important future consideration is what are the likely targets of dicarbonyl glycation in mitochondria?

Protein glycation

Glycation is a major cause of spontaneous damage to cellular and extracellular proteins in physiological systems, affecting 0.1–0.2% of lysine and arginine residues [4,5]. For some proteins with limited protein turnover, such as lens fibre cells, the extent of protein glycation may be up to 10-fold higher [6]. Most mitochondrial proteins are short-lived, however, with turnovers from 10–30 min to 3–5 days [7]. Quality control of the mitochondrial proteome involves mitochondrial proteases, leading to release of peptides from mitochondria [8], ubiquitination and proteolysis of outer membrane, mitochondrial fission and fusion, and finally autophagy of damaged mitochondria (mitophagy) [9].

Glycation adducts are formed in non-enzymatic spontaneous reactions of monosaccharides with proteins. Glucose can enter mitochondria via GLUT1 (glucose transporter 1), which also transports vitamin C into mitochondria [10]. Probably the most important glycating agents to consider for glycation damage to the mitochondrial proteome are reactive dicarbonyls such as glyoxal, MG and 3DG (3-deoxyglucosone) (Figure 1). Dicarbonyls are formed endogenously by lipid peroxidation and the degradation of glycolytic intermediates and glycated proteins. Dicarbonyl compounds are potent glycating agents, 200–50 000-fold more reactive than glucose. The physiological concentrations of dicarbonyls are typically 10 000–50 000-fold lower than glucose, yet nevertheless dicarbonyl compounds remain important precursors of AGEs (advanced glycation end-products) in physiological systems. MG was suggested to have low permeability of mitochondrial membranes as it inhibited D-*β*-hydroxybutyrate dehydrogenase activity in inverted inner mitochondrial membrane vesicles better than in intact mitochondria [11], but this may have been be due to decreased inhibitory activity with intact mitochondria owing to binding to GSH of MG.

Glycation by glucose and other monosaccharides is directed to amino groups of lysine and N-terminal amino acid residues of proteins, whereas glycation by dicarbonyl compounds is mainly, but not exclusively, directed to arginine residues [12].

Early-stage glycation adducts and AGEs

The initial glycation adducts formed by glycation by glucose and other monosaccharides are called early-stage glycation adducts. The predominant early glycation adduct is FL (N^{ε}-fructosyl-lysine). Schiff base and FL adducts degrade slowly in further advanced reactions to form many different glycation adducts. These adducts are known collectively as AGEs. Dicarbonyls react with proteins directly to also form AGEs. Important AGEs quantitatively are hydroimidazolones derived from arginine residues modified by glyoxal, MG and 3DG: G-H1 [N^{δ}-(5-hydro-4-imidazolon-2-yl)ornithine], MG-H1 [N^{δ}-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine] and 3DG-H {N^{δ}-[5-hydro-5-(2,3,4-trihydroxybutyl)-4-imidazolon-2-yl]ornithine and related structural isomers}. Other important and widely-studied AGEs are CML (N^{ε}-carboxymethyl-lysine) and CEL (N^{ε}-carboxyethyl-lysine), and the protein cross-links, pentosidine and glucosepane [5,13–16]. Further AGEs and related derivatives of emerging importance are CMC (N^{ε}-carboxymethylcysteine) [17], CMA (N^{ε}-carboxymethylarginine) [18] and ornithine [19], the latter formed as a degradation product of hydroimidazolones (Figure 2).

Enzymatic defence against glycation

Protein glycation in physiological systems is prevented and repaired by components of the enzymatic defence against glycation [20]. It involves enzymatic activities that suppress the

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formation of glycation adducts and repair sites of early glycation: Glo1 and AKRds (aldo–keto reductases) and aldehyde dehydrogenases detoxify reactive dicarbonyl-glycating agents [21,22], and amadoriase and fructosamine 3-phosphokinase that catalyse the removal of FL residues and free adducts [23-25] (Figure 3). The enzymatic defence against glycation suppresses damage to biological macromolecules, but it is an imperfect defence; glycation adducts of proteins, nucleotides and basic phospholipids are formed in normal physiological states. The enzymatic defence against glycation is overwhelmed in some disease states, and glycation adduct concentrations increase. The decline of expression of enzymes of the enzymatic defence against glycation may be key to increased protein glycation in the aging phenotype.

Glycation of mitochondrial proteins

Proteins modified by AGE residues in mitochondria have been detected by immunoassay and by GC–MS. Immunoassay of AGEs suffers problems of incomplete characterization of antibody epitope recognition. For example, the 6D12 monoclonal antibody used to quantify the CML residues in rat liver mitochondrial protein [26,27] detects both CML and CEL residues with different affinities (the later with higher affinity than the former [28]) and cannot be used to quantify AGE residue content of proteins. Solutions of dried milk protein powder, a rich source of CML and other AGE residues [29], was also used to suppress non-specific antibody binding and hence probably produced interferences in these studies. Immunohistochemistry of tissues with 6D12 and anti-AGE antibodies 1F6, and 2A2 all showed mitochondrial immunoreactivity in human tissues [30].

Estimates of AGE residues of mitochondrial protein of improved analytical security came form the studies of Pamplona et al. [31] using stable-isotopic-dilution analysis/GC–MS. They found 1–2 mmol of CML/mol of lysine in rat heart mitochondrial protein [31]; CML residue content determined by 6D12 immunoassay was approx. 10-fold over estimates [26]. CEL residue content of mitochondrial protein was approx. 0.5 mmol/mol of lysine. Both CML and CEL residue content of mitochondrial protein decreased with caloric restriction [32]. Quantitative immunoblotting of AGE residue content of mitochondrial proteins has been performed with one of the best characterized monoclonal antibodies against AGE residues: monoclonal antibody 1H7G5 which recognizes MG-derived hydroimidazolone MG-H1 [33]. Quantitative immunoblotting with this antibody showed MG-H1 residues of mitochondrial protein in wild-type *C. elegans* and decreased MG-H1 residues of mitochondrial protein in transgenic *C. elegans* with overexpression of *Glo1* [1]. Similar immunoblotting showed MG-H1 residue content of protein of mitochondria isolated from rat renal cortex. MG-H1 residue content of mitochondrial proteins was increased in diabetic rats [34].

The proteins modified by AGE residues have, as yet, only been detected by immunoreactivity with anti-AGE antibodies. This has not been corroborated by MS analysis of glycated peptides after limited proteolysis. It has been suggested that glutamate dehydrogenase is a target for AGE modification by immunoblotting with antibody 6D12 [35]. Several other mitochondrial proteins were found to be susceptible to modification by MG [34] (Table 1).

Effect of physiological dicarbonyl compounds on mitochondrial function

Incubation of rat renal mitochondria with MG (10–200 μM) for only 5 min at 24 °C produced a concentration-dependent decrease in state 3 respiration, an increase and then a decrease in state 4 respiration and a decrease in respiratory control quotient [36]. With such a short incubation time, it is likely that these effects were due to cysteinyl thiol modifications [37]. Reactive surface thiols are present in proteins of complexes I, II and IV

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[38], and proteins susceptible to thiol oxidation have been identified [39] (Table 1). The cellular concentrations of MG is usually approx. 2–4 μM [40]. Approx. 25% of MG is expected to be bound reversibly to GSH and 50% to protein thiols (assuming cellular concentrations of GSH and protein thiols of 3 and 6 mM respectively [41] and a thiol/MG dissociation constant of 3 mM [3]). It is therefore unclear whether physiological concentrations of MG have an acute effect on mitochondrial respiration; any effect is likely to be a modest decrease of effect on state 3 respiration. Similarly, the acute effects of MG and glyoxal (200 μM–5 mM) on the mitochondrial transition pore protein are of unlikely physiological relevance [42]. The steady-state levels of MG-H1 residues of mitochondrial proteins, as found in *C. elegans* and rat renal cortical mitochondria [1,34], as MG-H1 is the most important AGE residue quantitatively in physiological systems [43] and is associated with functional impairment of modified proteins [40,44].

MG modification of the mitochondrial proteome

There are estimated to be approx. 1500 proteins in the mitochondrial proteome. Only 13 proteins involved in oxidative phosphorylation are encoded by the mitochondrial genome; the others are nuclear-encoded, synthesized in the cytosol and targeted to mitochondria [45]. Seven proteins have, to date, been suggested as susceptible targets of advanced glycation and AGE residue formation (Table 1). We do not yet understand the full consequences of increased modification of mitochondrial proteins by MG. Enoyl-CoA hydratase is involved in fatty acid β-oxidation. Core protein I of complex III was susceptible to S-carboxymethylation and modification inhibited electron-transport activity [46]. Other targets for dicarbonyl glycation are subunits of complex I and F_1-ATPase. Mitochondrial electron flavoprotein β-subunit was a further subject for dicarbonyl glycation. Modification of a single arginine residue is known to impede electron transfer [47]. Cytochrome c_1 is part of the cytochrome bc_1 complex of complex III, a 30 kDa membrane-bound *c*-type cytochrome protein of mitochondria that functions as an electron donor to cytochrome *c* [48].

Summary

Dicarbonyl glycation of proteins and the glyoxalase system has been overlooked as an influential factor on oxidative stress. Life extension and decreased mitochondrial ROS with overexpression of *Glo1* in *C. elegans* has suggested that non-oxidative dicarbonyl glycation may be a critical upstream event of mitochondrial dysfunction and oxidative stress. This has implications for current hypotheses of the cause of mitochondrial dysfunction and oxidative stress in vascular cells during hyperglycaemia in diabetes, linked to chronic vascular complications, and to mitochondrial dysfunction and oxidative stress in aging. Modification of mitochondrial proteins with associated functional impairment may have a critical role in the formation of mitochondrial ROS and also in the activation of the mitochondrial pathway of apoptosis in some circumstances. It is now important to characterize proteins that are susceptible to dicarbonyl glycation and functional impairment associated with the ‘dicarbonyl proteome’ of mitochondria.

Acknowledgments

We thank the BBSRC (Biotechnology and Biological Sciences Research Council) (U.K.), Wellcome Trust, British Heart Foundation, Diabetes UK, Cancer Research UK, British Council and European Community 6th Framework Programme for support for our dicarbonyl glycation-related research.

Abbreviations used

AGE	advanced glycation end-product

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AKRd	aldo–keto reductase
CEL	N^{ε}-carboxyethyl-lysine
CML	N^{ε}-carboxymethyl-lysine
3DG	3-deoxyglucosone
FL	N^{ε}-fructosyl-lysine
Glo1	glyoxalase 1
MG	methylglyoxal
MG-H1	MG-derived hydroimidazolone, N^{δ}-(5-hydro-5-methyl-4-imidazolon-2-yl)ornithine
ROS	reactive oxygen species

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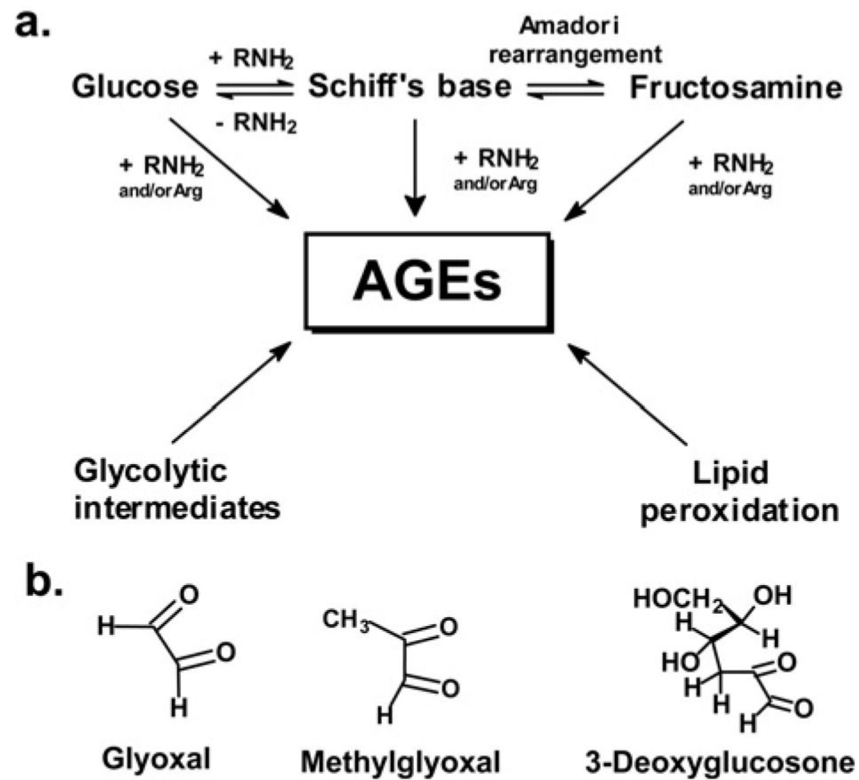

Figure 1. Major pathways for the formation of AGEs in physiological systems and precursor dicarbonyl metabolites

(**a**) Formation of early and advanced glycation adducts from glucose and glycolytic intermediates and products of lipid peroxidation. (**b**) Physiological reactive dicarbonyl-glycating agents.

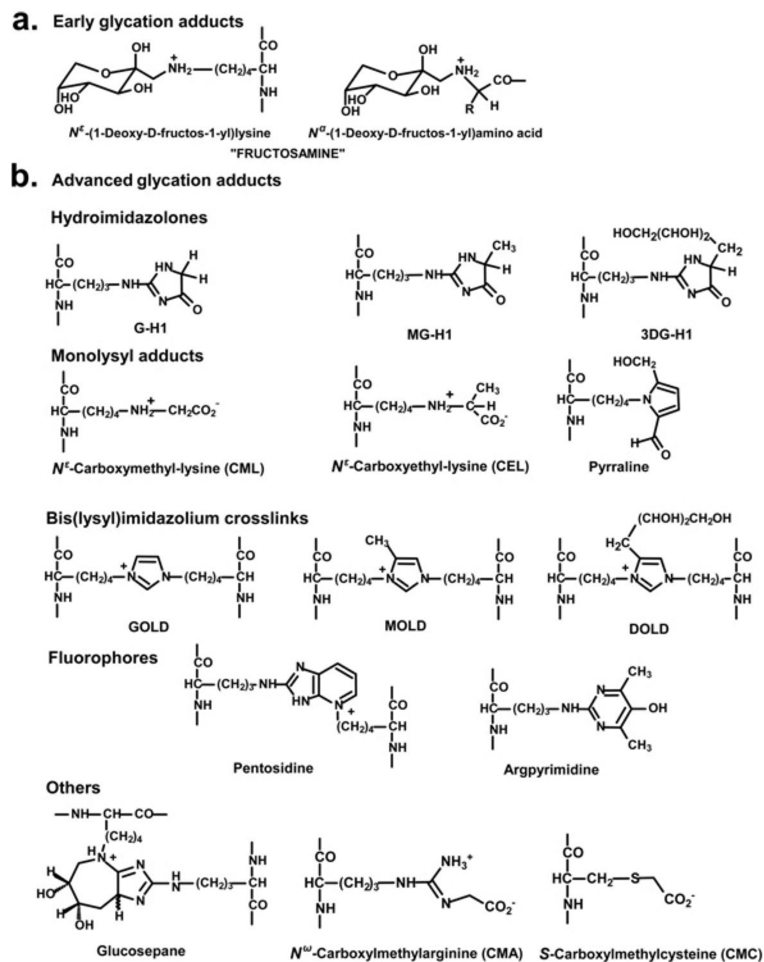

Figure 2. Protein glycation adduct residues

(**a**) Early glycation adducts. (**b**) AGEs. For the corresponding free adducts at physiological pH, the N-terminal amino group is protonated $-NH_3^+$ and the C-terminal carbonyl is a carboxylate $-CO_2^-$ moiety. DOLD, 3-deoxyglucosone-derived lysine dimer; GOLD, glyoxal-derived lysine dimer; MOLD, methylglyoxal-derived lysine dimer.

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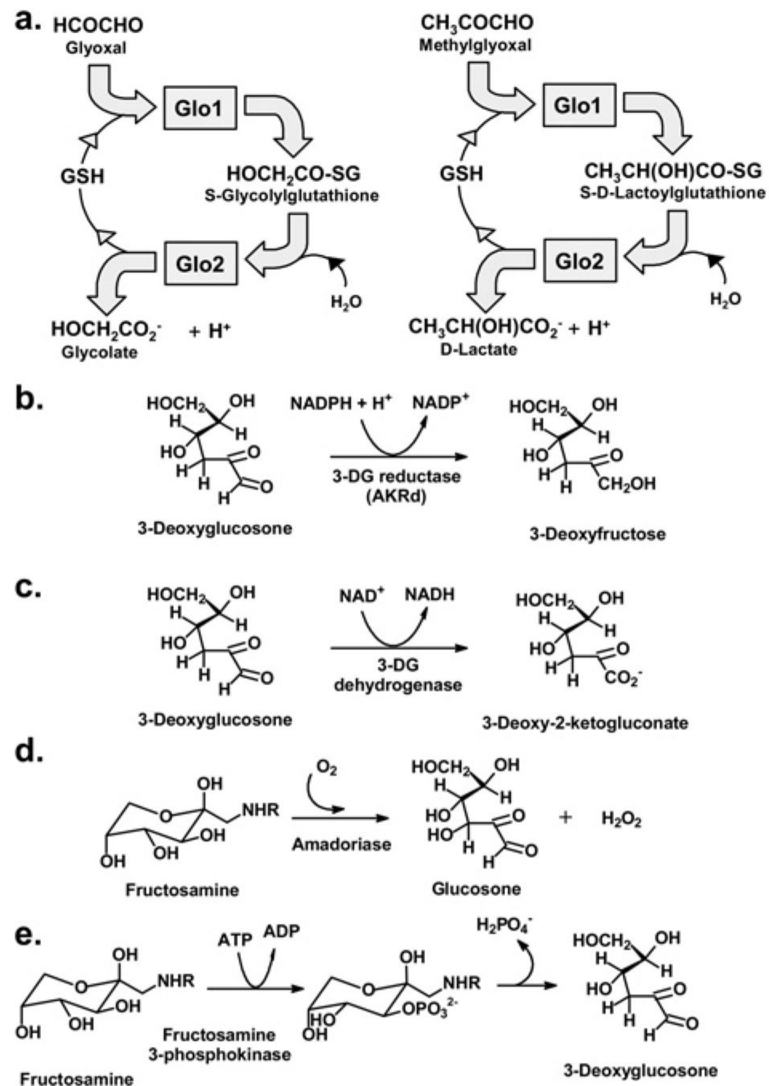

Figure 3. The enzymatic defence against glycation

(**a**) Metabolism of glyoxal and MG by the glyoxalase system. (**b**) Metabolism of 3DG by 3-deoxyglucosone reductase, an AKRd. (**c**) Metabolism of 3DG by 3-deoxyglucosone dehydrogenase. (**d**, **e**) Repair of early glycated proteins by Amadoriase and fructosamine-3-phosphokinase.

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Table 1

Mitochondrial proteins susceptible to modification by advanced glycation and thiol oxidation

Mitochondrial proteins modified by AGEs	AGE	Mitochondrial proteins susceptible to thiol oxidation
Enoyl-CoA hydratase, mitochondrial	MG-H1 [34]	Enoyl-CoA hydratase, short chain 1, mitochondrial [39]
Complex III, core protein I		Complex III, core protein I
NADH-ubiquinone oxidoreductase 30 kDa subunit, complex I		Acyl-CoA dehydrogenase, very long chain
F_1-ATPase, chain G		Carnitine acetyltransferase
Electron flavoprotein β-subunit		Mitochondrial acyl-CoA thioesterase 2
Cytochrome c_1, complex III		Mitochondrial trifunctional protein, α-subunit
Glutamate dehydrogenase	CML/CEL [35]	Propionyl-CoA carboxylase, α-chain
		Pyruvate dehydrogenase kinase, isoenzyme 2