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REVIEW ARTICLE

Methylglyoxal, glyoxalase 1 and the dicarbonyl proteome

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Abstract Methylglyoxal (MG) is a potent protein glycating agent. Glycation is directed to guanidino groups of arginine residues forming mainly hydroimidazolone N_{δ}-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H1) residues. MG-H1 formation is damaging to the proteome as modification is often directed to functionally important arginine residues. MG-H1 content of proteins is quantified by stable isotopic dilution analysis tandem mass spectrometry and also by immunoblotting with specific monoclonal antibodies. MG-glycated proteins undergo cellular proteolysis and release MG-H1 free adduct for excretion. MG-H1 residues have been found in proteins of animals, plants, bacteria, fungi and protoctista. MG-H1 is often the major advanced glycation endproduct in proteins of tissues and body fluids, increasing in diabetes and associated vascular complications, renal failure, cirrhosis, Alzheimer's disease, arthritis, Parkinson's disease and ageing. Glyoxalase 1 and aldo–keto reductase 1B1 metabolise >99% MG to innocuous products and thereby protect the proteome, providing an enzymatic defence against MG-mediated glycation. Proteins susceptible to MG modification with related functional impairment are called the "dicarbonyl proteome" (DCP). DCP includes albumin, haemoglobin, transcription factors, mitochondrial proteins, extracellular matrix proteins, lens crystallins and other proteins. DCP component proteins are linked to mitochondrial dysfunction in diabetes and ageing, oxidative stress, dyslipidemia, cell detachment and anoikis and apoptosis. Biochemical and physiological susceptibility of a protein to modification by MG and sensitivity of biochemical pathways and physiological systems to related functional impairment under challenge of physiologically relevant increases in MG exposure are key concepts. Improved understanding of the DCP will likely have profound importance for human health, longevity and treatment of disease.

Keywords Methylglyoxal · Glycation · Glyoxalase · Proteomics · Oxidative stress · Ageing · Diabetes · Renal failure

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Introduction: arginine-directed glycation

Damage to the proteome by glycation is a continual process in living systems. Glycation of amino groups pioneered by Maillard (1912) led to studies of glycation of proteins by mainly glucose in physiological applications. Protein glycation by glucose occurs by reaction with N-terminal and lysyl side chain amino groups forming N_{ε}-fructosyl-lysine (FL) and other fructosamine residues (Bookchin and Gallop 1968). Glycation by glucose of haemoglobin (Koenig et al. 1977), albumin (Dolhofer and Wieland 1979), other plasma proteins (Stevens et al. 1980) and proteins of tissues in vivo (Myint et al. 1995) have been studied. There are also reactive dicarbonyl metabolites in physiological systems—such as methylglyoxal (MG), glyoxal, 3-deoxyglucosone and others (Thornalley et al. 1999). Unlike glucose, dicarbonyl metabolites are glycating agents with modification directed mainly to the guanidino group of arginine residues. The discovery of dicarbonyl metabolites as important precursors of glycation adducts in physiological systems has broadened the focus of glycation to include arginine residues as loci of glycation at which hydroimidazolones are

the major adducts (Thornalley 2005; Fig. 1a, b). Hydroimidazolone residues in proteins and related free adducts in physiological fluids have been detected in all divisions or phyla of biological life: animals (Thornalley et al. 2003), plants (Bechtold et al. 2009), bacteria (Tajika et al. 1997), fungi (Gomes et al. 2008) and protoctista (Wendler et al. 2009; Table 1). They are also in food consumed by animals and other organisms—being also influenced by culinary processing of food for human subjects and domesticated animals (Henle et al. 1994; Ahmed et al. 2005c). The quantitative amounts of hydroimidazolone damage found and related physiological effects observed suggest that this is a type of glycation often linked to protein dysfunction and impairment.

Methylglyoxal: an important glycating agent in physiological systems

MG is formed in physiological systems by the spontaneous degradation of triosephosphates, glyceraldehyde-3-phosphate (GA3P) and dihydroxyacetonephosphate (DHAP). GA3P was ca. eightfold more reactive than DHAP in the degradation to MG but as the concentration ratio of DHAP/GA3P in cells in situ is ca. 20 or similar, both of these triosephosphates are important sources of MG formation in physiological systems in situ (Phillips and Thornalley 1993). MG formation is a minor fate of triosephosphates; cell studies suggested that only 0.089% glucotriose (2× glucose consumption) was converted to MG (Thornalley 1988). MG is also formed by the oxidation of acetone catalysed by cytochrome P450 2E1 in the catabolism of ketone bodies (Reichard et al. 1986), the oxidation of aminoacetone in the catabolism of threonine (Lyles and Chalmers 1992), the degradation of proteins glycated by glucose and the degradation of monosaccharides (Thornalley et al. 1999). The rate of total cellular formation of MG was estimated to be ca. 125 μmol/kg cell mass/day (Thornalley 1988) which for an adult human of 25 kg body cell mass (Ellis 2000) equates to a predicted whole body rate of formation of ca. 3 mmol MG/day. As total MG-derived glycation adduct excreted in urine of healthy human subjects was typically <10 μmol/day (Thornalley et al. 2003; Ahmed et al. 2005a), it can be inferred that less than 1% MG formed endogenously modifies the proteome. Most of the formed MG (>99%) is metabolised by glyoxalase 1 (Glo1) and aldo–keto reductase (AKRd) isozymes, which thereby constitute an enzymatic defence against MG glycation (see below).

MG modifies proteins to form advanced glycation end-product (AGE) residues. The major AGE formed is the arginine-derived hydroimidazolone N_{δ}-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H1)—formed via an intermediate dihydroxyimidazolidine. MG-H1 accounts for typically >90% adducts. Other minor adducts are: N_{ε}-carboxyethyl-lysine (CEL), the fluorophore argpyrimidine, lysine-derived 4-methylimidazolium crosslink (MOLD) and the arginine–lysine-derived crosslink 2-ammonio-6-([2-[(4-ammonio-5-oxido-5-oxopentyl)amino]-4-methyl-4,5-dihydro-1H-imidazol-5-ylidene]amino)hexanoate (MODIC) (Biemel et al. 2002; Ahmed et al. 2005b; Dobler et al. 2006; Fig. 1b, c). The concentration of MG in cells and tissues is

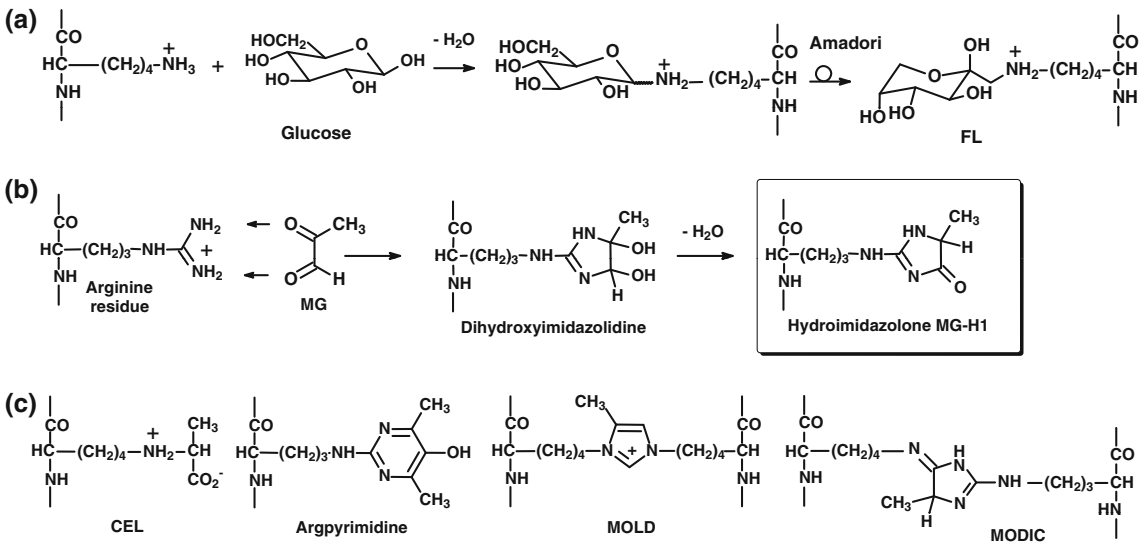

Fig. 1 Glycation by glucose and methylglyoxal in physiological systems. **a** Glycation of lysine residues by glucose forming N_{ε}-fructosyl-lysine residues via the Schiff's base intermediate and Amadori rearrangement. **b** Glycation of arginine residues by methylglyoxal forming hydroimidazolone N_{δ}-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H1) via intermediate dihydroxyimidazolidine. **c** Other glycation adducts formed by methylglyoxal

Table 1 Methylglyoxal-derived hydroimidazolone content of proteins and tissues and body fluids

Organism	Protein source	*n*	MG-H1 residue (mmol/mol arg)
Human	Plasma protein	10	0.31 ± 0.20
	Red blood cells	10	3.14 ± 0.72 (%Hb)
	Peripheral lymphocytes	3	7.46 ± 1.13
	Mesangial cells (in vitro)	3	0.60 ± 0.05
	Lens protein	55	15.0 ± 1.7
Rat	Plasma protein	13	1.29 ± 0.49
	Aortal collagen	6	0.22 ± 0.18
	Heart	13	2.52 ± 1.11
	Liver	13	3.34 ± 0.32
	Skeletal muscle	13	1.87 ± 0.59
	Brain	13	2.73 ± 0.39
	Renal glomeruli	13	2.42 ± 0.79
	Retina	13	1.79 ± 0.65
	Sciatic nerve	13	2.99 ± 1.12
Mouse	Brain stem	6	0.765 ± 0.166
	Brain cortex	6	0.382 ± 0.077
Plant (*Arabidopsis thaliana*)	Leaves	3	1.84–2.23
Nematode (*Caenorhabditis elegans*)	Whole organism	3	0.45–0.10
Protozoan (*Trypanosoma brucei*)	Whole organism	3	1.3–1.7

Data from Ahmed et al. (2003, 2005a), Thornalley et al. (2003), Morcos et al. (2008), Wendler et al. (2009), Bechtold et al. (2009), Karachalias et al. (2010), and Kurz et al. (2010). For tissues and cells, estimates are for cytoplasmic protein extracts

typically 2–4 μM (Phillips et al. 1993; Dobler et al. 2006; Nicolay et al. 2006) and the concentration of MG in human plasma is ca. 100 nM (Beisswenger et al. 1999; Nicolay et al. 2006). Estimates of MG concentrations that are markedly higher than this cannot be sustained given the reactivity of MG with the proteome, the level of MG-derived glycation adducts in the steady state and protein turnover (Thornalley 2005, 2008). Overestimates are likely due to interferences in analytical methodology—particularly degradation of derivatising agent and/or other sample components to MG during pre-analytic processing. Although the concentration of MG in plasma is ca. 50,000-fold lower than glucose, MG has much higher intrinsic reactivity towards glycation than glucose, 10,000–50,000-fold higher (Thornalley 2005). It is, therefore, predicted that the formation of glycation adduct residues in proteins by MG in vivo occurs at fluxes approaching those of glucose.

For the major glycation adduct of glucose, FL residues, there is a pathway of de-glycation and thereby repair of FL-modified proteins catalysed by fructosamine 3-phosphokinase (Delpierre et al. 2000). Currently, there is no known mechanism of de-glycation of hydroimidazolone-modified proteins. There is, however, slow dynamic reversibility of hydroimidazolone formation with de-glycation half-life of 12 days (Ahmed et al. 2002; Thornalley et al. 2003). This implies that when there is a sustained decrease of the MG concentration, e.g. by induction of increased expression of Glo1, there is expected to be a later commensurate decrease in hydroimidazolone content of proteins.

MG-H1 adduct residues are released from proteins by cellular proteolysis. The MG-H1 free adduct thereby formed was the major quantitative glycation free adduct excreted in human and rat urine (Thornalley et al. 2003; Karachalias et al. 2010). Proteins containing MG-H1 residues were predicted to have distorted or damaged structures (Ahmed et al. 2005b; Dobler et al. 2006) and therefore may be targeted for proteolysis by the proteasome (Grune et al. 1996; Dudek et al. 2005; Hernebring et al. 2006). Lysosomal proteolysis is also important for degradation of long-lived cellular proteins, endocytosed extracellular proteins (Goldberg et al. 1997) and chaperone-mediated autophagy of cellular proteins (Franch et al. 2001). Release of MG-H1 free adduct from cells and tissues (Thornalley et al. 2003) and decrease of glycated proteins with increased cellular 20S proteasome activity (Hernebring et al. 2006) are consistent with targeting of MG-H1-modified proteins for proteasomal degradation. This remains to be confirmed by direct measurements of protein dynamics in situ. MG-H1 free adduct was detected in plasma, urine, cerebrospinal fluid, synovial fluid and peritoneal dialysis (Thornalley et al. 2003; Ahmed et al. 2004a, 2006; Agalou et al. 2005). In rats, loss of renal clearance imposed by bilateral nephrectomy produced a profound increase in MG-H1 free adduct but not MG-H1 residue content of plasma proteins—indicating that it is mainly MG-H1 free adduct rather than MG-H1-containing protein that is cleared from plasma and excreted from the body in urine (Rabbani et al. 2007).

Renal clearance of MG-H1 free adduct was 35 ± 3 ml/min in healthy human subjects (Ahmed et al. 2005a) and 0.57 ± 0.08 ml/min in healthy Sprague-Dawley rats (Karachalias et al. 2010). A biodistribution model of MG-H1 formation and elimination with free adduct excretion in urine is envisaged in mammals (Fig. 2) for which future multi-compartmental mathematical modelling will help rationalise and predict steady-state levels and fluxes of MG-H1 in health and disease.

Measurement of methylglyoxal-derived hydroimidazolone

MG-H1 and other AGEs formed by protein glycation by MG have been quantified by stable isotopic dilution analysis tandem mass spectrometry (LC–MS/MS). We developed a protocol of exhaustive enzymatic hydrolysis with stable isotopic dilution analysis LC–MS/MS for quantitation of MG-H1 and other MG-derived AGEs. Corresponding free adducts were assayed in ultrafiltrates of plasma and urine (Thornalley et al. 2003). MG-H1 residues in proteins may also be assessed by immunoblotting with specific monoclonal antibodies (Yao and Brownlee 2009; Queisser et al. 2010a). The analysis of human cells in culture, tissues of rodent models of human disease and human subjects revealed that MG-H1 was often the major AGE residue in proteins. The analysis of medium of cultured cells and body fluids revealed the presence of MG-H1 free adduct. We quantified protein damage of these types in disease—including diabetes and associated vascular complications, renal failure, cirrhosis, Alzheimer's disease, arthritis, Parkinson's disease and ageing (Ahmed et al. 2003, 2004a, b, 2005a, 2006; Agalou et al. 2005; Morcos et al. 2008; Karachalias et al. 2010; Kurz et al. 2010). There are particularly marked increases in urinary excretion of MG-H1 free adduct in experimental and clinical diabetes—27-fold and 15-fold, respectively (Ahmed et al. 2005a; Karachalias et al. 2010) and increased plasma MG-H1 free adduct accumulation in renal failure—18-fold in peritoneal dialysis and 40-fold in haemodialysis (Agalou et al. 2005). This was related to disease progression and quality of therapeutic intervention—suggesting a critical role in disease mechanisms.

MG glycation of the proteome is particularly damaging because (a) modification is directed to arginine residues which have the highest probability of any amino acid residue for location at functional sites of proteins (predicted probability that a functional site amino acid residue is arginine = 20%) (Gallet et al. 2000); (b) functionally important arginine residues are often hotspots for dicarbonyl glycation (Ahmed et al. 2005b; Dobler et al. 2006; Yao et al. 2007); and (c) MG-H1 is one of the most quantitatively important, spontaneous irreversible modifications of the proteome in health and disease—increasing where MG formation is enhanced and/or MG metabolism is decreased.

Even so, the extent of MG-H1 modification of proteins in the steady state in vivo rarely increases beyond 10% total protein—except in lens crystallins where there is

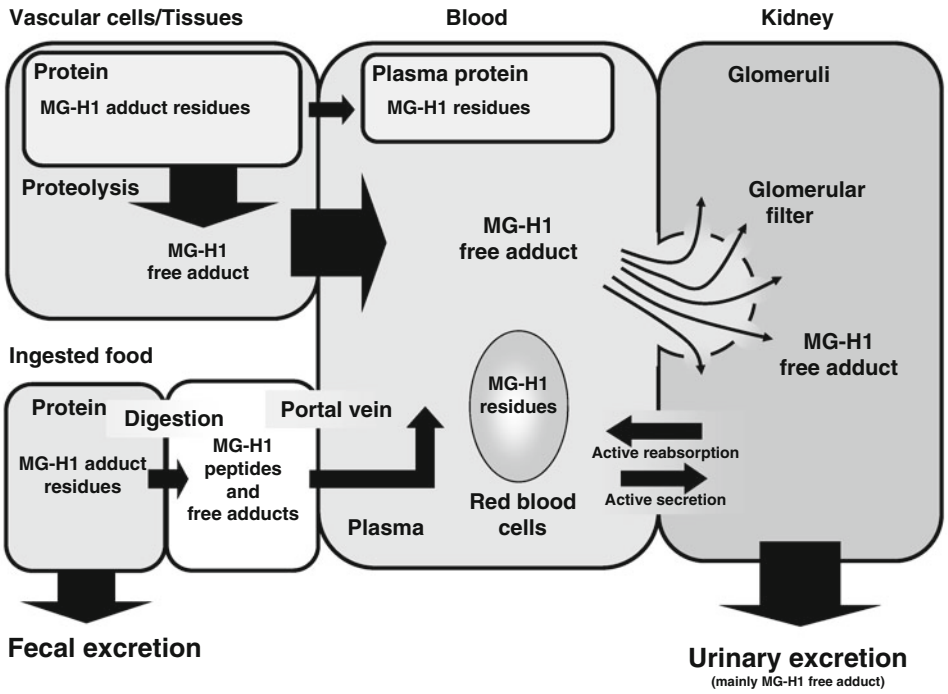

Fig. 2 Biodistribution scheme illustrating flows of formation and removal of methylglyoxal-derived hydroimidazolone MG-H1 residues with urinary excretion of free adduct

limited proteolysis (Ahmed et al. 2003). The consequences of increased MG-H1 modification are greatest where a two- to threefold change of low extent of modification can have a profound physiological effect. One such instance is MG modification of vascular type IV collagen. In healthy rats, only ca. 5% aortal collagen had an MG-H1 modification, increasing to ca. 12% in diabetic rats. For glycation of type IV collagen, MG-H1 modification occurred predominantly in integrin binding sites—α1-chain GFOGER site (sequence 395–390) and α2 chain RGD sites (sequences 889–891 and 1452–1454). The $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins recognise the GFOGER sequence found in collagens (Emsley et al. 2000; Knight et al. 2000). Several integrins recognise the RGD sequence within extracellular matrix proteins (Ruoslahti 1996; Stupack and Cheresh 2002); the RGD moiety binds astride the integrin α- and β-subunits with the arginine residue making electrostatic interaction with one or two aspartate residues of the α-subunit (Xiong et al. 2002; Gottschalk and Kessler 2002). Arginine residue modification in GFOGER and RGD sites led to endothelial cell detachment and exposure of the subendothelium (Dobler et al. 2006). This is expected to lead to blood platelet binding and activation with related thrombosis (Kim et al. 2004). In this case, therefore, increase in low level MG-H1 modification has a potentially profound physiological effect—a two- to threefold increased risk of thrombosis and vascular disease. Similar low level increase of MG-H1 modification of mitochondrial protein was linked to two- to threefold increase in reactive oxygen species formation in ageing, probably by associated protein structural distortions increasing the minor electron leakage from respiratory electron transport chains (Morcos et al. 2008). This amplification of physiological effect by MG modification fulfilling a "gate-opener" role in physiological dysfunction is where change in glycation achieves greatest response—often for harm but occasionally for good.

Glyoxalase 1 and the enzymatic defence against glycation

Glo1 is part of the glyoxalase system present in the cytosol of most cells. The glyoxalase system catalyses the conversion of MG to D-lactate via the intermediate *S*-D-lactoylglutathione. It comprised two enzymes, Glo1 and glyoxalase 2 (Glo2) and a catalytic amount of glutathione GSH. Glo1 catalyses the isomerisation of the hemithioacetal, formed spontaneously from MG and GSH, to *S*-D-lactoylglutathione. Glo2 is a thiolesterase and catalyses the hydrolysis of *S*-D-lactoylglutathione to D-lactate and reforms GSH consumed in the Glo1-catalysed reaction step (Fig. 3). MG is the major physiological substrate for Glo1 and this accumulates markedly when Glo1 is inhibited in situ by cell permeable Glo1 inhibitors, siRNA silencing of Glo1 and depletion of GSH (Thornalley 1993; Thornalley et al. 1996; Abordo et al. 1999; Santarius et al. 2010). Other substrates of Glo1 are: glyoxal—formed by lipid peroxidation and the fragmentation of glycated proteins (Thornalley et al. 1999), hydroxypyruvaldehyde $HOCH_2COCHO$, and 4,5-doxovalerate $H\text{-}COCOCH_2CH_2CO_2H$ (Thornalley 1993, 1998). Glo1 activity prevents the accumulation of MG and these other reactive dicarbonyls and thereby suppresses dicarbonyl-mediated glycation reactions (Shinohara et al. 1998). It is, therefore, a key enzyme of the anti-glycation defence.

Molecular and genetic characteristics of Glo1 were reviewed previously (Thornalley 2003a). Glo1 is a GSH-dependent enzyme. Under physiological conditions in situ, the rate of fragmentation of hemithioacetal to GSH and MG is of the order of 10^3 times faster than the rate of isomerisation of hemithioacetal catalysed by Glo1. This has the consequence that the activity of Glo1 in situ is proportional to the cellular concentration of GSH. MG accumulates in oxidative stress and may contribute to oxidant-induced cytotoxicity (Abordo et al. 1999). Increased MG modification of proteins is a likely consequence of oxidative stress and increased MG modification of mitochondrial proteins may also induce oxidative stress (Morcos et al. 2008; Miyazawa et al. 2010).

Glo1 suppresses the formation of MG-derived AGEs. Overexpression of Glo1 prevented the increase in MG, increased the formation of D-lactate and prevented accumulation of cellular AGEs in endothelial cells in vitro (Shinohara et al. 1998). Overexpression of Glo1 also prevented: (a) dysfunction of co-repressor mSin3A in renal endothelial cells leading to increased angiopoietin-2 and pro-inflammatory signalling in progression of nephropathy

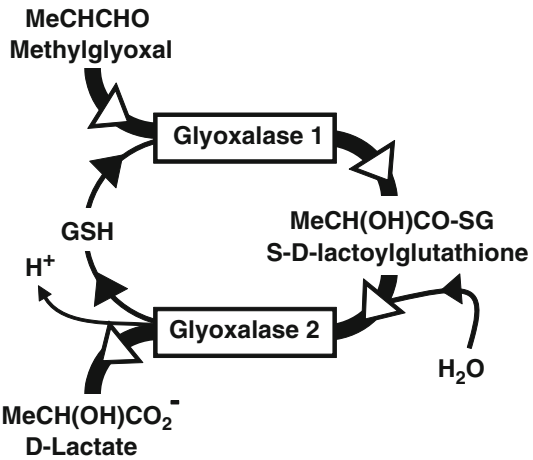

Fig. 3 The glyoxalase system

(Yao et al. 2007), (b) dysfunction of hypoxia-inducible factor 1α (HIF1α) leading to impaired endothelial progenitor cell and endothelial nitric oxide synthase response and failure to correct tissue hypoxia (Ceradini et al. 2008), (c) sustained release of p65 and prolonged activation of the NF-κB system amplifying inflammation (Yao and Brownlee 2010), and (d) impairment of the proteasome system (Queisser et al. 2010b). In vascular systems, overexpression of Glo1 prevented impairment of angiogenesis in hyperglycaemia (Ahmed et al. 2008), impairment of NO-mediated vascular dilatation in diabetes (Brouwers et al. 2010) and renal ischaemia-reperfusion injury (Kumagai et al. 2009). In whole organism health, overexpression of Glo1 in *Caenorhabditis elegans* produced increases in median and maximum lifespan (Morcos et al. 2008). This implicates MG-modified proteins in cellular dysfunction and the ageing process.

The induction of apoptosis and cytotoxicity associated with the accumulation of MG (Kang et al. 1996) is avoided under normal physiological states. Hence, a key physiological function of Glo1 is detoxification of MG and other dicarbonyls as part of the enzymatic defence against glycation (Thornalley 2003b). In certain disease states—such as cancer and microbial infections—induction of apoptosis of tumour cells and biocidal activity against microbial organisms is desirable. In such circumstances, accumulation of MG induced by cell permeable Glo1 inhibitor achieves this, exhibiting antitumour activity (Thornalley et al. 1996) and anti-microbial activity (Thornalley et al. 1994). This now appears to be of future vital importance in cancer chemotherapy as overexpression of Glo1 is linked to multidrug resistance in human tumours. In this case, inhibition of Glo1 is the only currently effective countermeasure (Santarius et al. 2010).

AKRd isozymes also metabolise MG leading to the formation of mainly hydroxyacetone. They become particularly important when Glo1 activity is impaired or Glo1 expression is low. Recent research has indicated that AKRd isozymes of classes 1A and 1B metabolised MG where isozyme 1B1, aldose reductase, was most active (Baba et al. 2009)—as found previously (Vander Jagt et al. 1992). This contributed to the anti-glycation defence in human umbilical vein endothelial cells in vitro and rat heart in vivo (Baba et al. 2009).

Methylglyoxal-modified proteins: the dicarbonyl proteome

We defined collectively proteins susceptible to dicarbonyl modification with related functional impairment as the "dicarbonyl proteome" (DCP) (Rabbani and Thornalley 2008b) and led proteomic application to identify them (Ahmed et al. 2005b; Dobler et al. 2006). Susceptible proteins with identified hotspot sites of MG modification within them include albumin (Ahmed et al. 2005b), haemoglobin (Chen et al. 2005), co-repressor protein sina3A (Yao et al. 2007), type IV collagen (Dobler et al. 2006), αA lens crystallin (Gangadhariah et al. 2010), HIF1α co-activator protein p300 (Thangarajah et al. 2009) and 20S proteasome subunits (Queisser et al. 2010a). Other proteins known to be modified by MG at sites unknown are: mitochondrial proteins—linked to mitochondrial dysfunction in diabetes (Rosca et al. 2005), ageing (Morcos et al. 2008), oxidative stress (Rabbani and Thornalley 2008a) and apoptosis (Chan et al. 2007), lipoproteins—linked to dyslipidaemia (Rabbani et al. 2009), extracellular matrix proteins—linked to cell detachment and anoikis (Pedchenko et al. 2005; Duran-Jimenez et al. 2009), and other lens crystallins (Ahmed et al. 2003).

Proteomics analysis of subcellular fractions will be required to identify comprehensively proteins susceptible to MG modification and hotspot sites for MG-H1 formation. Care is required, however, as trypsinisation protocols using high temperatures and high pH for long periods compromise the adduct detection: MG-H1 (mass increment +54) hydrates to the dihydroxyimidazolidine (mass increment +72) which fragments to MG and unmodified protein—a process which under physiological conditions has a half-life of 12 days (Ahmed et al. 2002) but can be markedly accelerated in some conventional, often-used trypsinisation protocols using high temperatures and high pH. Physiological temperature, short proteolysis periods with compensating increased trypsin/protein ratio—or different proteases functioning under acidic conditions—is required to avoid this.

"Hotspot" arginine residues have extraordinary high reactivity for MG modification being activated by neighbouring group interactions to decrease the microscopic pK_a and provide a proximate conjugate base to catalyse the dehydration involved in MG-H1 formation, cf. formation of glycation of lysine residues (Venkatraman et al. 2001; Blom et al. 2004; Fig. 4).

Assessing susceptibility to MG modification and loss of biochemical and physiological function

The definition of DCP requires demonstration of both susceptibility to modification by MG and consequent functional impairment. We may assess biochemical susceptibility of a protein to modification by MG by determining the rate constant for the reaction of MG with the protein of interest—mostly occurring at a hotspot site. In this regard, normalising rate constants to that of a reference protein such as serum albumin may be beneficial for

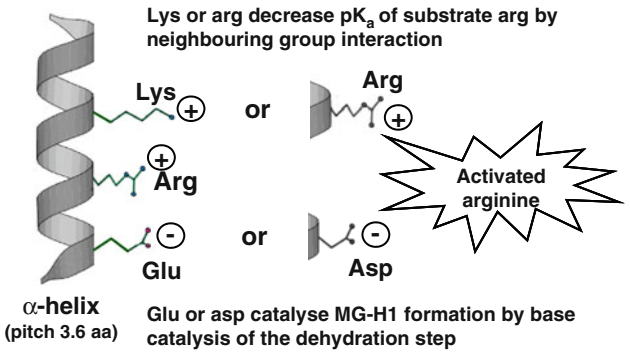

Fig. 4 Activation of arginine residues in alpha-helix domains of proteins by neighbouring group interactions with basic and acidic amino acid residues

comparison purposes, being always mindful that in situ concentrations of protein substrates determine actual rates of MG modification in physiological systems. We may also assess physiological susceptibility of a protein to MG modification by measuring the proportion of the protein modified by MG in the steady state and tendency for this to increase in periods of increased exposure to MG. Steady-state levels of MG modification depend on the balance between rates of formation and rates of clearance of MG-modified protein. These may be measured by applying the techniques of study of proteome dynamics—see Pratt et al. (2002). For example, in recent studies, we deduced that the ratio of the rate constants for reaction of MG to form MG-H1 residues in human apolipoprotein B100 of low density lipoprotein (LDL) relative to serum albumin, $k_{LDL}/k_{HSA} = 20$. Taking into account the steady-state concentrations of LDL and albumin in plasma, the ratio of the in situ rates of MG glycation of LDL and HSA, $r_{LDL}/r_{HSA} = 0.038$. The steady-state levels of MG-modified LDL and albumin are also influenced by the half-lives of these proteins, ca. 3 and 19 days, respectively (Langer et al. 1972; Peters 1996), with the final observation that for healthy human subjects 2.4% of LDL is modified by MG and only 0.9% albumin is modified by MG (Rabbani et al. 2010).

Functional impairment linked to MG modification requires experimental demonstration and it will therefore likely take much effort to address comprehensively. Prediction of modification linked to biochemical functional impairment may be made by bioinformatics computation of hotspot arginine residue location in a site of protein–protein, enzyme–substrate or protein–nucleotide interaction—see Gallet et al. (2000). The link of the modification to

Table 2 Components of the dicarbonyl proteome

Species	Protein	Arginine hotspot sites	Extent of modification	Functional impairment	References
Human	Serum albumin	410 (others 114, 186, 218 and 428)	ca. 1%	Inhibition of esterase activity and decreased drug binding	Ahmed et al. (2005b)
	Haemoglobin	α-Chain: 31, 92 and 141; β-chain: 30, 40 and 104	ca. 2.6%	Increased oxygen binding	Chen et al. (2005), Ahmed et al. (2005a), Gao and Wang (2006)
	Collagen type IV	α1-Chain: 390; α2 chain: 889 and 1452	ca. 5%	Decreased integrin binding	Dobler et al. (2006)
	αA-Crystallin	12, 65, 157 and 163	Unknown	Increased chaperone activity	Gangadhariah et al. (2010)
	HIF1α co-activator protein p300	R354	Unknown	Decreased HIF1α and responsive to hypoxia	Thangarajah et al. (2009)
	20S proteasome subunits[a]	β2: 85; β4: 224 and 231; β5: 123 and 128	Unknown	Decreased proteasome activity	Queisser et al. (2010a)
Rat	Enoyl-CoA hydratase, mitochondrial; complex III, core protein I; NADH-ubiquinone oxidoreductase 30 kDa subunit, complex I; F1-ATPase, chain G; Electron flavoprotein β-subunit; Cytochrome c1, complex III	Unknown	Unknown	Linked to increased formation of reactive oxygen species in experimental diabetes	Rosca et al. (2005)
Mouse	mSin3a co-repressor	925	Unknown	Increases angiopoietin-2 transcription	Yao et al. (2007)

[a] Found in exposure to high supraphysiological concentrations of methylglyoxal

impairment of physiological functional may be assessed from sensitivity to steady-state level of MG modification by exposure to increased MG. In this regard, inhibition or silencing of Glo1 may provide an optimum experimental approach as it is expected to produce the required increased exposure to endogenous MG—see Dobler et al. (2006) and Santarius et al. (2010). Increasing exposure of the proteome to MG by addition of extremely high supraphysiological levels of exogenous MG—such as millimolar concentrations normally experienced only in acute toxicity (Jerzykowski et al. 1975; Kang et al. 1996)—is likely to modify and impair function of many proteins that are not susceptible to this effect under physiological conditions, even in disease states. This may misguide insight into the consequences of the DCP under physiological conditions. This would appear to be the case of the claimed effect of supraphysiological concentrations of MG on insulin signalling (Tsikas et al. 2005). Some examples of the DCP are given in Table 2.

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