Published in final edited form as: Drug Metabol Drug Interact. 2008 ; 23(1-2): 125–150.

Protein and nucleotide damage by glyoxal and methylglyoxal in physiological systems - role in ageing and disease

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Abstract

Glycation of proteins, nucleotides and basic phospholipids by glyoxal and methylglyoxal – physiological substrates of glyoxalase 1 - is potentially damaging to the proteome, genome and lipidome. Glyoxalase 1 suppresses glycation by these α-oxoaldehyde metabolites and thereby represents part of the enzymatic defence against glycation. Albert Szent-Gyorgyi pioneered and struggled to understand the physiological function of methylglyoxal and the glyoxalase system. We now appreciate glyoxalase 1 protects against dicarbonyl modifications of the proteome, genome and lipome. Latest research suggests there are functional modifications of this process – implying a role in cell signalling, ageing and disease.

Letter from Albert Szent-Gyorgyi

"I thank you for your kind lines from May 17th, and the reprints that reached me only now. It was very long ago that I was interested in keto-aldehydes and worked on them. Since then I forgot most of what I knew. So, to my regret I am unable to advise you. Yours truly, Albert Szent-Gyorgyi, MD, PhD, NL." From a letter to the author dated 7th June 1984.

Above are the words of Albert Szent-Gyorgyi in a letter replying to my request for advice on how to pursue a research career on studies of methylglyoxal and the glyoxalase system. The letter was sent from the National Foundation for Cancer Research, Woods Hole, Massachusetts, USA. It has been framed, along side newspaper obituary tributes to Szent-Gyorgyi when he died in October 1986, hanging in my office for over the last 20 years whilst I grappled with similar problems that once interested him. Our common interest was, for Szent-Gyorgyi, and still is for me the physiological significance of the glyoxalase system and its physiological substrates – primarily the reactive dicarbonyl compounds and physiological metabolites, glyoxal and methylglyoxal /1,2/ – Figure 1.

Methylglyoxal and glyoxal – physiological substrates of the glyoxalase system

The presence and significance of reactive acyclic α -oxoaldehydes, methylglyoxal and glyoxal, in physiological systems was the subject of research before, during and after the research career of Szent-Gyorgyi. Neuberg had discovered the glyoxalase system and metabolism of methylglyoxal in 1913 /3/. Szent-Gyorgyi advanced his hypothesis of the "retine and promine" theory of control of cell proliferation /1/, and recent research views

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glyoxalase 1 (Glo1) as part of the enzymatic defence against glycation /2/. Methylglyoxal has been considered to be an environmental and/or bacterial toxin and metabolite /4,5/. We now know methylglyoxal is formed spontaneously from triosephosphates in all organisms with anaerobic glycolysis /6/ and from other non-enzymatic and enzymatic pathways of differing significance – depending on the organism /7/. Glyoxal is formed by lipid peroxidation and the degradation of monosaccharides, saccharide derivatives and glycated proteins /8,9/. A continuing problem in the area of research is reliable estimation of the concentrations of methylglyoxal and glyoxal in physiological systems. Both of these compounds may be formed by degradation of monosaccharides, glycated proteins, glycolytic intermediates – and indeed from degradation of derivatising agents – during sample processing for estimation of their concentrations in physiological samples. As a consequence, physiological concentrations of methylglyoxal and glyoxal have often been overestimated. Chemical derivatisation of α -oxoaldehydes is essential for assay to adequate sensitivity. Some analytical methods used to estimate physiological concentrations of methylglyoxal and glyoxal (and other α -oxoaldehydes) are given in Table 1. The latest and most advanced methods employ derivatisation with 1,2-diaminobenzene and detection by gas chromatography with mass spectrometric detection (GC-MS) /10/ or liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) with stable isotopic dilution analysis /11/. Recent estimates of the concentrations of methylglyoxal and glyoxal in human blood plasma are in the range 100 - 120 nM /12,13/ and cellular concentrations of methylglyoxal $(1 - 5 \mu M)$ and glyoxal $(0.1 - 1 \mu M) / 11/$. With inadequate control of interferences during sample processing, estimates of 10 - 1000-fold higher than this may be easily recorded. Studies investigating the effects of glyoxal and methylglyoxal on cultured cells and tissues using at concentrations > 10-fold higher than this are likely to be only of relevance for acute intoxication and cytotoxicity. The use of methylglyoxal at millimolar concentrations to demonstrate impairment of insulin signalling, for example, is of unlikely physiological relevance /14/. For studies investigating the effects of increased methylglyoxal concentrations, it is more appropriate to increase endogenous methylglyoxal concentrations by inhibition of Glo1 with specific cell permeable inhibitors – such as S-pbromobenzylglutathione cyclopentyl diester (SpBrGSHCp₂)/15/ or decrease Glo1 by siRNA techniques /16/.

Modification of proteins by glyoxal and methylglyoxal

Glyoxal and methylglyoxal are potent glycating agents. Glycation of proteins is a complex series of parallel and sequential reactions collectively called the Maillard reaction. It occurs in all tissues and body fluids. Early stage reactions in glycation of protein by glucose lead to the formation of fructosyl-lysine (FL) and N-terminal amino acid residue-derived fructosamines. Later stage reactions form stable end-stage adducts called advanced glycation endproducts (AGEs) /17/. FL degrades slowly to form AGEs - and also glyoxal and methylglyoxal /9/. In contrast, glyoxal and methylglyoxal react with proteins to form AGE residues directly and relatively rapidly. For example, addition of 1 μ M [¹⁴C]methylglyoxal to human plasma ex vivo and incubated at 37 °C produced complete and irreversible binding of methylglyoxal to plasma protein within 24 h/18/. Important AGEs quantitatively are hydroimidazolones derived from arginine residues modified by glyoxal and methylglyoxal - N_{δ} -(5-hydro-4-imidazolon-2-yl)ornithine (G-H1) and N_{δ} -(5-hydro-5-methyl-4imidazolon-2-yl)-ornithine (MG-H1) /19,20/; there are also related structural isomers /21/. There is concurrent formation of minor lysine-derived adducts – N_{e} -carboxymethyl-lysine (CML) and N_e -carboxyethyl-lysine (CEL) residues, and bis(lysyl) crosslinks – GOLD and MOLD /21,22/. For glyoxal, there is a further minor arginine-derived adduct formed – N_{ω} carboxymethylarginine (CMA) /23/; the corresponding methylglyoxal-derived adduct, $N_{\rm ex}$ carboxyethylarginine, is unstable and not detected. For methylglyoxal, there is a trace fluorescent adduct formed called argpyrimidine /24,25/. Hydroimidazolones have relatively

moderate half-lives (2 - 6 weeks) and slow dynamic reversibility; therefore, protein content of hydroimidazolones can be decreased if the concentrations of the precursor α -oxoaldehydes are decreased /19,21/. Where hydroimidazolones accumulate with donor age, the increase is related to decreased activity of Glo1 and increased methylglyoxal concentration /26,27/ – Figure 2a.

Protein glycation was viewed originally as a post-translational modification that accumulated mostly on extracellular proteins. Specifically, AGEs were thought to be formed slowly throughout life and the concentrations of AGEs found represent a life-long accumulation of the glycation adduct. This applies to chemically stable AGEs formed on long-lived proteins. For example, CML and CEL residue accumulation on skin collagen / 28,29/. Hydroimidazolones have relatively short chemical half-lives under physiological conditions (2 - 6 weeks), however, and their concentration depends on the balance of the rates of formation and decomposition. Moreover, protein glycation adduct residues are also formed on cellular and short-lived extracellular proteins. The turnover of these proteins by cellular proteolysis releases glycation free glycation adducts (glycated amino acids) /19/. Protein damage by glycation is implicated in protein misfolding. Misfolded proteins are degraded by the proteasome to ensure the high quality of intracellular proteins /30/; the median half-life of cellular proteins was 32 h/31/. Protein glycation free adducts are, therefore, the excreted debris of cellular proteolysis of damaged proteins. This appears to be an efficient process since there was little evidence of low molecular mass damaged peptides in peripheral venous plasma. Additional sources of free adducts are direct glycation of lysine and arginine. This probably plays a minor contribution because of the relatively low concentration of free amino acids compared to the concentration of corresponding amino acid residues in proteins /19/.

Saccharide-rich and thermally processed foods are a good source of AGE residues /32/. There is a low bioavailability of AGE residues in proteins of ingested foods such that <10% is absorbed /33/ – attributed to resistance to proteolysis of highly glycated proteins in food / 22/ and some AGEs inhibit intestinal proteases /34/. The highest concentration of absorbed food AGEs is expected in portal venous plasma where we found the hydroimidazolone MG-H1 enriched by 10-fold in peptides /35/. AGEs are therefore probably absorbed from food as both AGE free adducts and AGE-rich peptides; the latter appear to be degraded efficiently after absorption. Hydroimidazolones G-H1 and MG-H1, CML and CEL free adducts had high renal clearances /19/. This suggests that as long as renal function is normal, protein glycation and oxidation adducts absorbed from food may pose little threat – although this remains controversial /36/ – Figure 3.

Proteins in mammalian tissues, plasma and extracellular matrix *in vivo* have relatively high MG-H1 residue content (0.1 - 15 mmol/mol amino acid modified), much lower CML and CEL residue content (0.05 - 6 mmol/mol lys) and trace amounts of GOLD and MOLD residues (0.001 - 0.002 mmol/mol lys) - depending on the protein substrate and tissue location, type of AGE. Hydroimidazolone AGEs are found in highest concentrations in lens protein of elderly human subjects; MG-H1 residue content was 1 - 2% of total arginine residues. Stable AGEs such as CML residues accumulate on lens capsule, skin and cartilage collagen with age but reached <6 mmol/mol lys in cartilage in old age /28,37/.

The physiological importance of protein glycation remains under intensive investigation. Particularly damaging effects are produced by covalent crosslinking of proteins which confers resistance to proteolysis /38/. Protein modification is also damaging when amino acid residues are located in sites of protein-protein interaction, enzyme-substrate interaction and protein-DNA interaction (for transcription factors). A bioinformatics analysis of receptor binding domains indicated that arginine residues have the highest probability of

being located in such sites (19.6%) /39/. The major modification of proteins by glyoxal and methylglyoxal is on arginine residues. Formation of hydroimidazolones causes structural distortion, loss of side chain charge and functional impairment /25/. Two examples we studied recently are methylglyoxal modification of human serum albumin and vascular type IV collagen.

Modification of human serum albumin by methylglyoxal produced hotspot modification of Arg-410 with hydroimidazolone MG-H1 residue formation. Modification of Arg-410 by methylglyoxal was found in albumin glycated *in vivo*. Arg-410 is located in drug binding site II and the active site of albumin-associated esterase activity. Hydroimidazolone formation at Arg-410 inhibited drug binding and esterase activity. Molecular dynamics and modelling studies indicated that hydroimidazolone formation caused structural distortion leading to disruption of arginine-directed hydrogen bonding and loss of electrostatic interaction /25/. MG-H1 residue formation in albumin and other plasma proteins was increased in clinical diabetes and end stage renal disease /20,40/.

Modification of vascular basement membrane type IV collagen by methylglyoxal formed MG-H1 residues at hotspot modification sites in RGD and GFOGER integrin-binding sites of collagen, causing endothelial cell detachment, anoikis and inhibition of angiogenesis. Endothelial cells incubated in model hyperglycaemia *in vitro* and experimental diabetes *in vivo* produced the same modifications of vascular collagen, inducing similar responses. Increased methylglyoxal formed from triosephosphate degradation within endothelial cell crossed the plasma membrane and modified type IV collagen /11/. This may contribute to increased shedding of vascular endothelial cells and increased numbers of circulating endothelial cells in diabetes and uraemia where plasma levels of methylglyoxal and other α -oxoaldehydes are abnormally high /41,42/. The number of circulating endothelial cells, indicative of damage to the endothelium, is prognostic for vascular disease /43/.

Glycation of proteins by glyoxal and methylglyoxal is not only common in mammalian systems. MG-H1 hydroimidazolone has been isolated from bacteria /44/ and we have recently found glyoxal and methylglyoxal-derived AGE residues in proteins of higher plants with variation in light and dark growth cycles /45/.

Modification of nucleotides by glyoxal and methylglyoxal

DNA is susceptible to glycation by glyoxal and methylglyoxal - the nucleotide most reactive under physiological conditions is deoxyguanosine (dG). Under conditions of limiting aoxoaldehyde, as applies in vivo, the major nucleotide AGEs are the imidazopurinone derivatives 3-(2'-deoxyribosyl)-6,7-dihydro-6,7-dihydroxyimidazo[2,3-b]purin-9(8)one (dG-G) derived from glyoxal /46/ and 3-(2'-deoxyribosyl)-6,7-dihydro-6,7-dihydroxy-6methylimidazo-[2,3-b]purine-9(8)one (dG-MG) derived from methylglyoxal /47,48/. Other adducts, N₂-carboxymethyl-deoxyguanosine (CMdG) and 5-glycolyldeoxycytidine (gdC) derived from glyoxal /46/, and N_2 -(1-carboxyethyl)-deoxyguanosine (CEdG) derived from methylglyoxal /47,48/ have been reported - Figure 2b. These are called nucleotide advanced glycation endproducts (nucleotide AGEs). There have been few quantitative estimates of nucleotide AGEs in mammalian cells. dG-G and dG-MG are unstable at high pH and temperatures and hence DNA extraction and hydrolysis for analysis of these DNA adducts must avoid such conditions. In recent studies, we developed an LC-MS/MS assay methodology using acid nuclease digestion and isotopic dilution analysis. Estimates of imidazopurinone adducts and the major oxidative adduct of DNA, 8hydroxydeoxyguanosine (8-HO-dG), in human mononuclear leukocytes were (adducts per 10^{6} nucleotide; n = 3): dG-G 1.59 ± 0.08; dG-MG 15.7± 0.38 and 8-HO-dG 3.33 ± 0.13 / 49/. This suggests that glycation by glyoxal and methylglyoxal is an important type of

damage quantitatively of DNA *in vivo*. DNA suffers continuous damage from glycation by methylglyoxal and glyoxal. Imidazopurinones have only moderate stability under physiological conditions but are stabilized by formation in single- and double-stranded DNA: half-lives for dG-G and residues in single- and double-stranded DNA were 14.8 h, 285 h and 595 h at pH 7.4 and 37°C, respectively. The formation of CEdG was associated with depurination of DNA /50/.

The presence of nucleotide dG-G and dG-MG in DNA is associated with increased mutation frequency, DNA strand breaks and cytotoxicity. Glyoxal (9-26 mM) induced increased mutations and decreased DNA replication. Most mutations were single-base substitutions (48%) with 83% of these occurring at C:G sites. G:C \rightarrow T:A transversions were predominant, with also some G:C \rightarrow C:G transversions, G:C \rightarrow A:T transitions and A:T \rightarrow T:A transversions. For methylglyoxal (1-10 mM), cytotoxicity and mutation frequency increased with increasing methylglyoxal concentration. Multi-base deletions were predominant (50%), with some base-pair substitutions (35%) where 89% were at C:G sites. Most mutations were G:C \rightarrow C:G and G:C \rightarrow T:A transversions. Both glyoxal and methylglyoxal were associated with non-random or hotspot mutation sites /51,52/. The mutagenicity of methylglyoxal was improved markedly in the presence of hydrogen peroxide. This was associated with the formation of N_2 -acetyl-deoxyguanosine in DNA / 53-55/.

The prevention of mutation induced by glyoxal and methylglyoxal by nucleotide excision repair (NER) was investigated by incubation of wild-type and NER-deficient *E. Coli* with glyoxal and methylglyoxal and the *lacI* gene analysed for mutations. Increasing concentration of α -oxoaldehydes led to increased mutations, decreased DNA replication and cell death. Base-pair mutations were higher in the NER-deficient strain relative to the wild-type strain. Most base-pair substitutions were at C:G sites where G:C \rightarrow T:A transversions were predominant. There were also G:C \rightarrow A:T transitions and A:T \rightarrow T:A transversions with glyoxal and TGGC frameshift mutations for methylglyoxal. These studies indicate that nucleotide glycation and its effects are suppressed by NER /56,57/. Introduction of the specific nucleotide AGE CEdG into DNA was also associated with increased mutation frequency and increased DNA strand breaks in *E. coli* /58/.

The formation of crosslinks of protein to DNA by glycation with methylglyoxal has been known for many years /59/. An explanation for this may be crosslinking of the DNA template to the Klenow fragment of DNA polymerase. Methylglyoxal (1 mM) reacted with dG in the DNA template /60/. Glyoxal was much less effective than methylglyoxal in this crosslinking. This may be a very damaging effect of nucleotide glycation although the physiological significance is not yet clear.

Glyoxalase 1 – a critical role in the enzymatic defence against glycation

Accumulation of protein glycation adducts is associated with enzyme inactivation, protein denaturation and a cellular-mediated immune response. Excessive nucleotide glycation is associated with mutagenesis and apoptosis, and excessive lipid glycation with membrane lipid bilayer disruption /17/. In 2003, I developed the concept of an enzymatic defence against glycation protecting against glycation-mediated cell damage. It involves the enzymatic activities that suppress the formation of glycation adducts and repair sites of early glycation: Glo1 and certain aldehyde reductase and dehydrogenase isozymes detoxify reactive carbonyl and α -oxoaldehyde glycating agents (glyoxal, methylglyoxal, 3-deoxyglucosone and others) /61,62/, and amadoriase and fructosamine 3-phosphokinase catalyse the removal of fructosamine glycation adducts formed by glycation of proteins by glucose /63-65/.

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 but at a low level. Steady state levels of glycation adducts are ca. 0.1-1% lysine and arginine residues in proteins, 1 in 10⁵ nucleotides in DNA, and 0.1% basic phospholipids. Protein glycation adducts are removed by proteasomal and lysosomal proteolysis /66,67/. Nucleotide glycation adducts are cleared by nucleotide excision repair /52/, and phospholipid AGEs are removed by lipid turnover / 68/. The glycation adducts thereby liberated are eliminated as free glycation adducts (glycated amino acids) in the urine, along with glycation adducts absorbed from food. The enzymatic defence against glycation is overwhelmed in some disease states and glycation adduct concentrations increase - particularly in diabetes and chronic and acute renal failure. There is also a decline of the enzymatic defence against glycation in ageing – particularly expression of Glo1, which may contribute to the pathobiology of ageing /69/. The major mechanism for clearing glycation adducts from the body is by urinary excretion and hence glycation free adducts accumulate profoundly in experimental and clinical uraemia /40,70/. Renal replacement therapy of patients with chronic renal failure with haemodialysis and peritoneal dialysis provides clearance of glycation free adducts in the dialysate /40/.

Oxidative stress is inextricably linked to glycation because the depletion of GSH and NADPH in oxidative stress also decreases the *in situ* activities of Glo1 /71/ and thereby increases the concentrations of glyoxal and methylglyoxal and associated glycation reactions. Glycation of proteins by Glo1 substrates may also increase reactive oxygen species (ROS) – superoxide, hydrogen peroxide and hydroxyl radical, and thereby be a cause or contributory factor to oxidative stress. This is best illustrated by the increased ROS formation associated with mitochondrial dysfunction and methylglyoxal modification of mitochondrial proteins in diabetic nephropathy /72/ and ageing in *Caenorhabditis elegans* / 69/.

Glycation contributes to morbidity and mortality of diseases of major social impact (diabetes, heart disease and endstage renal disease) and is suspected to contribute to others (Alzheimer's disease, arthritis and ageing) /17/. Novel therapeutic approaches to prevent the chronic complications of diabetes and improve dialysis therapy in endstage renal disease strive to minimize vascular dysfunction linked to excessive glycation and increase clearance of glycation adducts from the body, respectively /73-75/. Nucleotide glycation is implicated in saccharide-linked mutagenesis and the pharmacological mechanism of cytotoxic antitumour drugs modifying DNA and DNA metabolism.

Overexpression of glyoxalase 1 and multidrug resistance in cancer chemotherapy

The GSH dependent enzyme Glo1 in the major factor involved in the detoxification of glyoxal and methylglyoxal in physiological systems /76/. We developed the first effective inhibitors of Glo1 that had antitumour activity *in vitro* and *in vivo*, *S*-p-bromobenzylglutathione diesters. Addition of these inhibitors to cultured cells led to the cellular accumulation of methylglyoxal, increased nucleotide glycation and induction of apoptosis /15,77-79/. We supplied the diethyl and dicycopentyl inhibitor derivatives for evaluation against fibroblasts overexpressing Glo1. Evaluation showed that the fibroblasts overexpressing Glo1 were resistant to the Glo1 inhibitor diesters. Remarkably, the cells were also resistant to the anticancer drugs mitomycin C and doxorubicin /80/. This was the first time observation that overexpression of Glo1 may confer multidrug resistance (MDR) to tumours.

Tsuruo and colleagues made a critical observation to link Glo1 with multidrug resistance in cancer chemotherapy. Studies on MDR human monocytic leukaemia UK711 cells and erythroleukaemia K562/ADM cells, using a subtractive hybridisation mRNA approach, showed Glo1 was overexpressed in both cell lines. Moreover, a stable transfectant of Jurkat cells acquired MDR when overexpression of Glo1 was produced. The Glo1 inhibitor SpBrGSHCp₂/15/ lifted MDR and the cells regained sensitivity to antitumour agents /81/. Human lung cancer NCI-H522 and DMS114 cells with high expression and activity of Glo1 underwent apoptosis when treated with SpBrGSHCp₂. The sensitivity to SpBrGSHCp₂ correlated to the level of Glo1 expression. SpBrGSHCp₂ induced the activation of the stressactivated protein kinases c-Jun NH₂-terminal kinase 1 (JNK1) and p38 mitogen-activated protein kinase (MAPK), which led to caspase activation in Glo1-overexpressing tumour cells. SpBrGSHCp₂ significantly inhibited the growth of xenografted DMS114 and human prostate cancer DU-145 and was the most potent antitumour agent against human tumours overexpression Glo1 – particularly refractory lung and prostate carcinomas /82/. These studies suggest: (i) accumulation of Glo1 substrates are involved in the antitumour activity of important clinical antitumour agents (for example, doxorubicin), and (ii) further development of Glo1 inhibitors may provide antitumour agents active against some of the clinical tumours associated with high incidence and mortality rates (lung and prostate carcinomas). Further support for increased activity in this area of research comes form the link of increased Glo1 expression with invasive ovarian cancer /83/, prostate carcinoma /84/ renal adenocarcinoma /85/ and high expression of Glo1 in human breast cancers /86/ and precancerous lesions /87/.

The link of overexpression of Glo1 with MDR in cancer chemotherapy may involve increased formation of methylglyoxal in response to DNA repair. The cellular response to antitumour drugs that modified DNA or disrupt DNA metabolism is to activate processes of DNA repair, including poly(ADP-ribose) polymerase /88/. This depletes cells of NAD⁺ such that glyceraldehyde-3-phosphate dehydrogenase activity is depleted and triosephosphates, glyceraldehyde-3-phosphate and dihydroxyacetonephosphate, increase markedly /89/. Methylglyoxal is formed mainly by triosephosphate degradation and, hence, a consequent dramatic increase in methylglyoxal formation is expected. The increased methylglyoxal concentrations may modify DNA and protein involved in activation of apoptosis, thereby potentiating the cytotoxic effect of the antitumour agent. This potentiatory effect of methylglyoxal will be blocked by over expression of Glo1. The mechanisms of induction of apoptosis by methylglyoxal is not known although activation of JNK1 and p38 MAPK are involved /82/. Activation of protein kinase C, δ -isoform (PKC $_{\delta}$), was involved in the potentiation of the antitumour effect of cisplatin by methylglyoxal /90/; PKC_{δ} is involved in coordinating cell signalling in response to DNA damage to activate apoptosis, including interaction with hRAD9 and Bcl-2/91/. Whilst there may be key proteins modified by methylglyoxal that promote apoptosis, the increased DNA damage associated with nucleotide glycation probably also features in apoptotic mechanisms. Deployment of this effectively could provide valuable new antitumour agents.

Physiological regulation of glyoxalase 1 and glycation by glyoxal and methylglyoxal

The modifications of proteins and nucleotides by Glo1 substrates, glyoxal and methylglyoxal, may be viewed as damage to physiological systems. This is suppressed by metabolism of glyoxal and methylglyoxal to glycolate and D-lactate catalysed by the glyoxalase system /2/. There examples emerging, however, of regulation of Glo1 activity in physiological systems that suggests Glo1 substrates and glycation may have a functional role in cell signalling.

Firstly, the high expression and activity of Glo1 in many tumour cells and some microorganisms /92-94/. This is likely to be linked to the high glycolytic activity of many tumours and some micro-organisms /95/. A high Glo1 activity is required, concomitant with a high flux of methylglyoxal associated with high flux of triosephosphates and anaerobic glycolytic activity, to protect the proteome and genome from functional impairment and mutation, respectively.

Secondly, activation of the receptor for advanced glycation endproducts (RAGE) by S100 proteins found in increased plasma concentration in inflammatory disorders was associated with decreased expression of Glo1. Induction of diabetes in wild type mice decreased the expression of Glo1 whereas induction of diabetes in RAGE (-/-) mice did not /96/. Decreased Glo1 expression leads to increased protein glycation /2/. AGE residues in tissue protein were found co-localized with RAGE. This may be due to RAGE activation by S100 proteins, decreasing the local expression of Glo1 and thereby increasing the local formation of AGE residues. This may be part of an inflammatory response leading to labeling proteins with dicarbonyl-derived hydroimidazolone AGE residues and directing these proteins to the proteasome for destruction. Methylglyoxal modification of proteins is thought to target proteins for proteasomal destruction /97/. If correct, this will link the formation of glyoxal and methylglyoxal-derived AGEs to removal and destruction of proteins, at least for intracellular proteins – a re-interpretation of the role originally attributed to AGEs by Cerami as "signals" on extracellular proteins to target them for cellular uptake and proteolysis /98/.

Thirdly, Glo1 was shown to be phosphorylated and glycation of proteins increased in apoptosis induced by tumour necrosis factor /99/. This, taken together with the overexpression of Glo1 in multidrug resistance in cancer chemotherapy, suggests glyoxal and methylglyoxal may have a role in apoptosis.

These examples of regulation of the Glo1 and cellular concentrations of methylglyoxalderived glycation is suggestive of a signalling role for methylglyoxal glycation of proteins and nucleotides in physiological systems. The role appears to include influences in malignant transformation /100/, cell death – apoptosis, anoikis (detachment stimulated apoptosis) and eryptosis (erythrocyte programmed cell death) /11,13,79/ and inflammation / 101/.

Concluding remarks: emerging evidence that glyoxalase 1 and glycation by glyoxal and methylglyoxal play a critical role in disease and ageing – the tribute to Szent-Gyorgyi

Glycation by glyoxal and methylglyoxal, and the related influence of Glo1, are now emerging as playing a critical role in ageing /69/ and disease processes – vascular complications associated with diabetes /20,41/, renal failure /40,70/, Alzheimer's disease / 102,103/ and tumourigenesis and multidrug resistance in cancer chemotherapy /82/. They may also have roles in pathologic anxiety /16,104/, autism /105/, obesity /106/ and other disorders. The abiding tribute to Albert Szent-Gyorgyi is that to the end of his scientific career he studied biological problems of fundamental importance. It is no disrespect to him that the physiological function of methylglyoxal and glyoxalase was left unresolved at the end of his career since over 20 years later we are still struggling to resolve it.

Acknowledgments

I would to thank the Wellcome Trust (U.K.), Cancer Research UK, Diabetes UK and the Juvenile Diabetes Research Foundation for support for my research, and past and present members of my research team for their contributions to glyoxalase research.

Abbreviations

AGEs	advanced glycation endproducts		
Bcl-2	B cell lymphoma/leukemia-2 protein		
CEdG	N_2 -(1-carboxyethyl)-deoxyguanosine		
CML	N_{ϵ} -carboxymethyl-lysine		
CEL	N_{e} -carboxyethyl-lysine		
СМА	N_{ω} -carboxymethylarginine		
CMdG	N_2 -carboxymethyl-deoxyguanosine		
dG	deoxyguanosine		
dG-G	3-(2'-deoxyribosyl)-6,7-dihydro-6,7-dihydroxyimidazo[2,3- b]purin-9(8)one		
dG-MG	3-(2'-deoxyribosyl)-6,7-dihydro-6,7-dihydroxy-6-methylimidazo-[2,3 b]purine-9(8)one		
FL	fructosyl-lysine		
GC-MS	gas chromatography with mass spectrometric detection		
gdC	5-glycolyldeoxycytidine		
G-H1	N_{δ} -(5-hydro-4-imidazolon-2-yl)ornithine		
GOLD	bis(lysyl) crosslink derived from glyoxal		
Glo1	glyoxalase 1		
JNK1	c-Jun NH ₂ -terminal kinase 1		
LC-MS/MS	liquid chromatography with tandem mass spectrometric detection		
МАРК	mitogen-activated protein kinase		
MDR	multidrug resistance		
MG-H1	N_{δ} -(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine		
MOLD	bis(lysyl) crosslink derived from methylglyoxal		
NER	nucleotide excision repair		
PKC _δ	protein kinase C, δ-isoform		
RAD9	radiation sensitive checkpoint protein and exonuclease		
RAGE	receptor for advanced glycation endproducts		
ROS	reactive oxygen species		
SpBrGSHCp ₂	S-p-bromobenzylglutathione cyclopentyl diester		

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Figure 1. The glyoxalase system and metabolism of methylglyoxal.

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Figure 2.

a. Advanced glycation endproducts residues formed from glyoxal and methylglyoxal with (a) proteins and (b) nucleotides. In (a), protein AGEs are shown as AGE residues with the peptide backbone –CO-CHR-NH- shown on the left. In (b), nucleotide AGEs are shown as nucleic acid base modifications with the 3-(2'-deoxyribosyl) moiety omitted for clarity and linkage indicated by the dashed link.



Figure 3.

Biodistribution scheme illustrating flows of formation and removal of protein glycation free adducts.

Table 1

Methods for quantifying glyoxal, methylglyoxal and other α , β -dicarbonyls in physiological systems

Derivatising agent	Advantages	Disadvantages	Reference
1,2-Diaminobenzene (HPLC with spectrophotometric detection)	High reactivity at low pH (also at neutral and high pH). Available commercially in high purity (also some analyte standard adducts). Good adduct stability.	Poor sensitivity – for HPLC with spectrophotometric detection. 1,2-Diaminobenzene degrades oxidatively to form glyoxal and methylglyoxal. Interferences.	/107/
1,2-Diaminobenzene (GC-MS)	As above. High sensitivity and specific detection.		/108/
1,2-Diaminobenzene (LC-MS/MS)	As above. High sensitivity and specific detection.	Interferences controlled. Limits of detection: glyoxal 0.21 pmol and methylglyoxal 0.25 pmol.	/11/
1,2-Diamino-4,5 -dimethoxybenzene	High sensitivity. High reactivity at low pH. Good adduct stability.	Highly unstable derivatising agent - degrades oxidatively to form glyoxal and methylglyoxal. Not available commercially in high purity.	/109/
6-Hydroxy-2,4,5- triaminopyrimidine	Available commercially in high purity (also some analyte standard adducts). Derivatisation at low – neutral pH.	High temperature required for derivatisation (60 °C, 45 min). Interferences.	/110/
Aminoguanidine	High reactivity at neutral and high pH. Available commercially in high purity (also some analyte standard adducts). Good analyte stability.	Difficult to detect adducts. Two isomeric adducts (except glyoxal) Interference: triosephosphates and degrading sugars.	/111/
Glutathione/ glyoxalase 1	Enzymatic reaction.	Poor sensitivity. Poor adduct stability. Inappropriate boiling of samples.	/112/