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Review

Glyoxalase in ageing

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ABSTRACT

The glyoxalase system has been studied since 1913. The biochemical function of this enzymatic system is the metabolism of reactive dicarbonyl metabolites, glyoxal and methylglyoxal, to less reactive products. In the last decade research has shown that methylglyoxal is the precursor of quantitatively important damage to the proteome and genome, forming mainly hydroimidazolone and imidazopurinone adducts in protein and DNA respectively. The aim of this article is to review the evidence of the involvement of the glyoxalase system in ageing and role of glyoxalase in future research into healthy ageing—mainly in mammalian systems for insights into consequences and interventions in human health.

Protein and DNA damage by glyoxalase system substrates is linked to dysfunction of proteins susceptible to dicarbonyl modification—the dicarbonyl proteome, and DNA instability and mutation. A component of the glyoxalase system, glyoxalase 1, is a gene with expression influential on lifespan—increasing longevity being associated with increased expression of glyoxalase 1. The glyoxalase 1 gene is also a site of copy number variation in both transcribed and non-transcribed regions giving rise to population variation of expression. The glyoxalase system and Glo1 expression particularly is therefore likely linked to healthy ageing.

Contents

Abbreviations: AGE, advanced glycation endproduct; CEdG, N_2-(1-carboxyethyl)-deoxyguanosine; CEL, N_ε-carboxyethyl-lysine; CMA, N_ω-carboxymethylarginine; CMdG, N_2-carboxymethyl-deoxyguanosine; CML, N_ε-carboxymethyl-lysine; CMhL, N_ε-carboxymethyl-hydroxylysine; CNV, copy number variation; GCL, γ-glutamylcysteine synthase; GCL_c, GCL catalytic subunit; GCL_m, GCL modulatory subunit; GdG, 3-(2′-deoxyribosyl)-6,7-dihydro-6,7-dihydroxyimidazo[2,3-b]purin-9(8)one; G-H1, N_δ-(5-hydro-4-imidazolon-2-yl)ornithine; GLO, glyoxalase 1 gene; Glo1, glyoxalase 1; Glo2, glyoxalase 2; GOLD, glyoxal-derived bis(lysyl) crosslink; GSH, reduced glutathione; HAGH, hydroxyacylglutathione hydrolase; MGdG, 3-(2′-deoxyribosyl)-6,7-dihydro-6,7-dihydroxy-6-methylimidazo-[2,3-b]purine-9(8)one; MG-H1, N_δ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine; MOLD, methylglyoxal-derived bis(lysyl) crosslink; NO, nitric oxide; ROS, reactive oxygen species.

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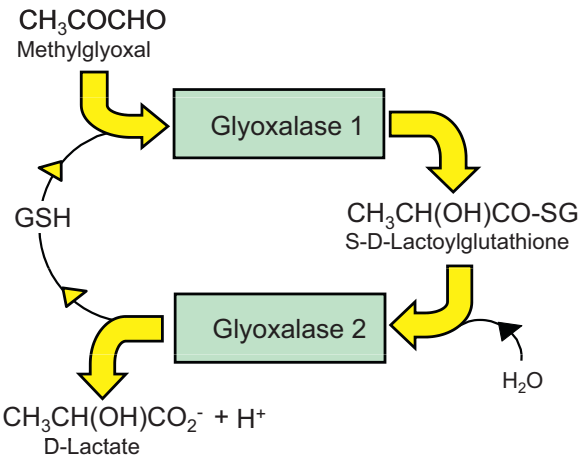

Fig. 1. The glyoxalase system and metabolism of methylglyoxal.

1. Introduction: the Glyoxalase system

The glyoxalase system catalyses the conversion of methylglyoxal to D-lactate via the intermediate S-D-lactoylglutathione. It is comprised of two enzymes, glyoxalase 1 (Glo1) and glyoxalase 2 (Glo2) and a catalytic amount of reduced glutathione (GSH). The glyoxalase system is in the cytosol of all mammalian cells—Fig. 1. It is present in animals, plants, bacteria, fungi and protoctista. The aim of this article is to review the evidence of the involvement of the glyoxalase system in ageing and role of glyoxalase in future research into healthy ageing—mainly in mammalian systems for insights into consequences for and interventions in human health.

Glyoxalase 1 (EC 4.4.1.5; Glo1) catalyses the isomerisation of the hemithioacetal, formed spontaneously from methylglyoxal CH_3COCHO and GSH to S-D-lactoylglutathione $CH_3CH(OH)CO$-SG: $CH_3COCHO + GSH \rightleftharpoons CH_3COCH(OH)$-$SG \rightarrow CH_3CH(OH)CO$-SG. For the methylglyoxal-glutathione hemithioacetal and human Glo1, the K_M is 71–130 μM and the k_{cat} is $7–11 \times 10^4\ min^{-1}$. Glyoxalase 2 (Glo2) catalyses the conversion of S-D-lactoylglutathione to D-lactate and reforms GSH consumed in the Glo1-catalysed reaction step. The major physiological substrate for Glo1 is methylglyoxal and this accumulates markedly when Glo1 is inhibited *in situ* by cell permeable Glo1 inhibitors and by depletion of GSH [1–3]. Methylglyoxal is formed mainly by the degradation of triosephosphates, and also by the metabolism of ketone bodies, threonine degradation and the fragmentation of glycated proteins. Other substrates are glyoxal—formed by lipid peroxidation and the fragmentation of glycated proteins, hydroxypyruvaldehyde $HOCH_2COCHO$ and 4,5-doxovalerate $H–COCOCH_2CH_2CO_2H$ [1,4]. Glo1 and the glyoxalase system prevent the accumulation of these reactive α-oxoaldehydes in cells and body fluids *in vivo* and thereby suppresses α-oxoaldehyde-mediated glycation reactions [5]. It is a key enzymatic system of the enzymatic defence against glycation [6,7].

1.1. Molecular properties, genetics and polymorphism of human glyoxalase 1

Glo1 activity is present in all human tissues. Specific activities of Glo1 in fetal tissues are *ca.* 3 times higher than corresponding adult tissues. There is *ca.* 0.2 μg Glo1 per gram of protein in human tissues and blood cells. Human Glo1 is a dimer, expressed at a diallelic genetic locus which encodes for two similar subunits in heterozygotes. There are consequently three allozymes in heterozygotes, designated GLO 1-1, GLO 1-2 and GLO 2-2. All allozymes have molecular mass of 46 kDa (gel filtration) or 42 kDa (sequence) and isoelectric point pI values of 4.8–5.1; however, they have distinctive charge densities and/or molecular shapes and are resolved by ion exchange chromatography and non-denaturing gel electrophoresis. Each subunit contains one zinc ion, Zn^{2+} [1]. Post-translational modification gives rise to multiple forms of differing pI [8].

The translation product of human GLO1 contains 184 amino acids. The N-terminal Met is removed in post-translational processing and the N-terminal Ala acetylated. There is a vicinal disulfide bridge between cysteine residues 19 and 20 and a mixed disulfide with glutathione on cysteine-139. Cysteine-139 may also form an intra-molecular disulfide with cysteine-61. *N*-Acetylation and the oxidation state of C19/C20 did not affect Glo1 activity whereas glutathionylation strongly inhibited Glo1 activity *in vitro* [9]. Glo1 is modified by S-nitrosylation by reaction with nitric oxide (NO) on cysteine-139. The presence of both C19 and C20 were influential on S-nitrosylation which occurred preferentially on the acidic, α-form of Glo1 [10]. The NO-responsive form of Glo1 is the basic, reduced form of Glo1 without intramolecular disulfide bonding [10]. Glo1 is a substrate for calcium, calmodulin-dependent protein kinase II and is phosphorylated at Thr-107 preferentially but not exclusively on the basic, reduced and NO-responsive form [8,10].

The structure of human Glo1 in complex with *S*-benzylglutathione was determined to 2.2 Å resolution [11]—Fig. 2a. Each monomer consists of two, structurally equivalent domains. The active site is situated in the dimer interface, with the inhibitor and essential Zn^{2+} ion interacting with side chains from both subunits. The zinc binding site is two structurally equivalent residues from each domain—Gln33A, Glu99A, His126B, Glu172B and two water molecules in octahedral coordination [11,12].

The mechanism proposed for the Glo1 reaction involves base-catalysed shielded-proton transfer from C1 to C2 of the hemithioacetal, bound in the active site, to form an ene-diol intermediate and rapid ketonisation to the thioester product—Fig. 2b. Both *R*- and *S*-forms of the hemithioacetal are bound in the active site of Glo1 and are therein deprotonated; the subsequent reprotonation of the putative ene-diol intermediate occurs stereospecifically to form the *R*-2-hydroxyacylglutathione derivative. It has been proposed that Glu172 is the catalytic base for the *S*-substrate enantiomer and Glu99 the catalytic base for the *R*-substrate enantiomer. Both reaction mechanisms form a *cis*-ene-diol intermediate coordinated directly to the Zn^{2+} ion: this is deprotonated to a *cis*-ene-diolate by Glu-172 which then reprotonates C2 stereospecifically to form the *R*-2-hydroxyacylglutathione product [13]—Fig. 2b. S-Glycolylglutathione, S-D-lactoylglutathione and S-L-glyceroylglutathione are formed from glyoxal, methylglyoxal and hydroxypyruvaldehyde by Glo1 and hydrolysed to glycolate, D-lactate and L-glycerate by Glo2, respectively [14].

The gene for glyoxalase 1 is GLO1 (or GLO in older literature). There are 3 phenotypes of human GLO1, GLO 1-1, GLO 1-2 and GLO 2-2, representing the homozygous and heterozygous expression of the diallelic gene, GLO^1 and GLO^2 at an autosomal locus; GLO^2 allele is the ancestral allele with the GLO^1 allele arising by mutation. GLO1 alleles are inherited in a simple co-dominant manner, with characteristic phenotypic expression present in all tissues. The GLO1 locus is on chromosome 6, between the centromere and HLA–DR [15]. The allele expression products differ in amino acid sequence only at position 111: in subunit Glo1-A there is an alanine residue, and in subunit Glo1-E there is a glutamic acid residue [16]. The gene promoter contains insulin response and metal response elements [17]. Other functional promoter elements known are: E2F-binding to transcription factor E2F4 [18], and activating enhancer binding protein 2 alpha (AP-2 alpha—also known as TFAP2A) [19]. Glo1 in the nematode *Onchocerca volvulus* was induced by oxidative stress but the mechanism is unknown [20].

Glo1 of other mammalian species are similar to the human enzyme. Mammalian, bacterial and plant Glo1 enzymes are usually dimeric. The yeast enzymes of *Saccharomyces cerevisae* and