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Methylglyoxal, glyoxalases and the development of diabetic complications

Review Article

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Summary. The formation of the reactive α,β-dicarbonyl metabolite, methylglyoxal, is increased during hyperglycaemia associated with diabetes mellitus. Methylglyoxal is metabolised to S-D-lactoylglutathione and D-lactate by the glyoxalase system and to hydroxyacetone (95%) and D-lactaldehyde by aldose reductase. Methylglyoxal and hydroxyacetone bind and modify protein, producing fluorescent products. Red blood cell activities of glyoxalase enzymes are risk factors for the development of clinical complications of diabetes. Aldose reductase inhibitors decrease the concentration of methylglyoxal in experimental diabetic rats to normal levels, aminoguanidine and L-arginine scavenge methylglyoxal; these effects may be involved in their prospective preventive therapy of diabetic complications. Biochemical and clinical evidence suggests that the metabolism of methylglyoxal in diabetes mellitus is linked to the development of diabetic complications. A causal relationship may involve modification of protein by methylglyoxal and hydroxyacetone.

Keywords: Amino acids – Methylglyoxal – Glyoxalase – Hydroxyacetone – Aldose reductase – Aminoguanidine – Diabetic complications

Introduction

Methylglyoxal (2-oxopropanal) is a reactive α,β-dicarbonyl metabolite. It is formed from glyceraldehyde-3-phosphate and dihydroxyacetonephosphate by non-enzymatic elimination of phosphate (Phillips and Thornalley, 1993a; Richard, 1991), and enzymatically from dihydroxyacetonephosphate by methylglyoxal synthase in some mammalian tissues (Ray & Ray, 1984) and leakage of the active site-bound phospho-enediolate intermediate of triosephosphate isomerase (Pompliano et al., 1990). It is also formed from aminoacetone in the

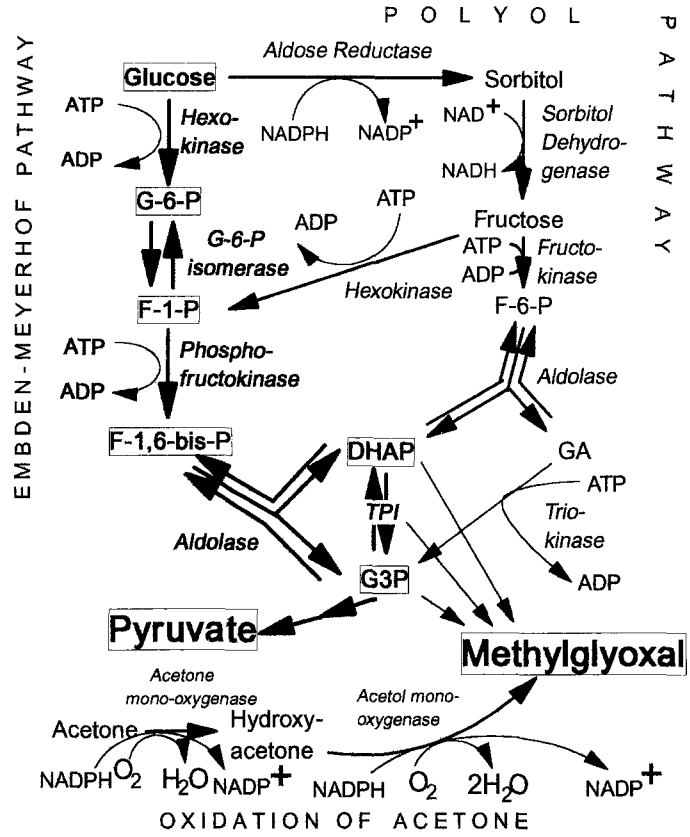

Fig. 1. Metabolic pathways for the formation of methylglyoxal. Abbreviations: *G-6-P*, glucose-6-phosphate, *F-1-P*, fructose-1-phosphate; *F-1,6-bis-P*, fructose-1,6-bis-phosphate; *F-6-P*, fructose-6-phosphate; *DHAP*, dihydroxyacetonephosphate; *G3P*, glyceraldehyde-3-phosphate; *TPI*, triosephosphate isomerase; *GA*, D-glyceraldehyde

catabolism of threonine (Ray and Ray, 1987) and from hydroxyacetone in the metabolism of acetone (Reichard et al., 1986) (Fig. 1).

Methylglyoxal is the physiological substrate of the glyoxalase system, a cytosolic metabolic pathway which catalyses the conversion of methylglyoxal to D-lactate via the intermediate S-D-lactoylglutathione. The glyoxalase system comprises two enzymes, glyoxalase I and glyoxalase II, and a catalytic amount of reduced glutathione. Glyoxalase I (EC 4.4.1.5) catalyses the formation of S-D-lactoylglutathione from the hemithioacetal formed non-enzymatically from methylglyoxal and reduced glutathione.

$$MeCOCHO + GSH \rightleftharpoons MeCOCH(OH)—SG \xrightarrow{\text{Glyoxalase I}} MeCH(OH)CO—SG$$

Glyoxalase II (EC 3.1.2.6) catalyses the hydrolysis of S-D-lactoylglutathione to D-lactate, reforming the reduced glutathione consumed in the glyoxalase I-catalysed reaction.

$$MeCH(OH)CO—SG + H_2O \xrightarrow{\text{Glyoxalase II}} MeCH(OH)CO_2^- + GSH + H^+$$

D-Lactate is metabolised in mammalian tissues by 2-hydroxyacid dehydrogenase, an FAD-dependent mitochondrial enzyme, to pyruvate. The function of

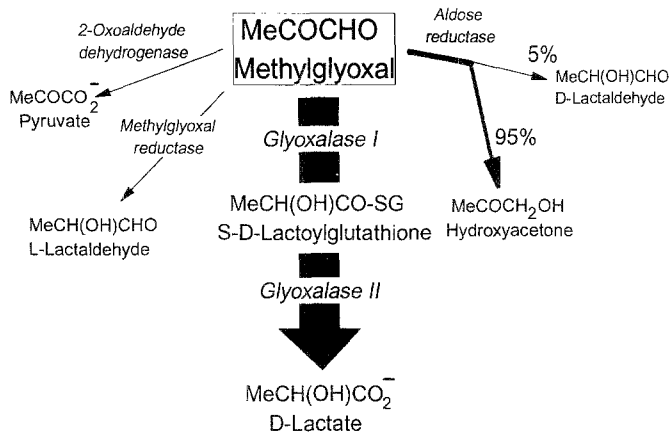

Fig. 2. The metabolism of methylglyoxal. Activities of 2-oxoaldehyde dehydrogenase and methylglyoxal reductase have also been detected in some mammalian tissues (reviewed in Thornalley, 1990)

the glyoxalase system is unknown but is related to the detoxification of methylglyoxal and/or growth control (Thornalley, 1990). Methylglyoxal is also metabolised by aldose reductase to hydroxyacetone (95%) and D-lactaldehyde (Vander Jagt et al., 1992) (Fig. 2).

Reactivity of methylglyoxal with biological molecules

Methylglyoxal reacts with lysine and arginine residues in proteins (Takahashi, 1977a, b; Cheung and Fonda, 1979). Initially, reversible Schiff's base adducts are formed but thereafter, with arginine residues only, there is slow irreversible reaction which forms an imidazolone derivative, N_δ-(3,4-dihydro-4-methylimidazol-5-on-2-yl)-2,5-diaminopentanoic acid (Selwood and Thornalley, 1993) (Fig. 3). This has a characteristic absorption and fluorescence (Selwood and Thornalley, 1993; Vander Jagt et al., 1992). Methylglyoxal reacts with bovine serum albumin (BSA) to initially modify 5 reactive arginine residues per albumin molecule. The rate of binding of methylglyoxal to BSA was first order with respect to methylglyoxal and first order with respect to albumin where the apparent bimolecular rate constant, $k_{BSA,MG} = (1.2 \pm 0.04) \times 10^{-5}\ M^{-1}s^{-1}$ ($n = 6$) at pH 7.4 and 37°C (Selwood & Thornalley, 1993). With high concentrations of methylglyoxal (100 mM), all the arginine residues in BSA may be modified (M. Reeves and P. J. Thornalley, unpublished results). The rate constant for the reaction of methylglyoxal with L-arginine was $1.1 \times 10^{-8}\ M^{-1}s^{-1}$. Arginine, N_α-acetyl-arginine and aminoguanidine compete with albumin for methylglyoxal and prevent the formation of the imidazolone derivative; lysine and N_α-acetyl-lysine did not. Methylglyoxal, at physiological concentration (1 μM), binds to proteins in blood plasma; binding is inhibited by aminoguanidine and L-arginine (Lo et al., 1993).

Binding and modification of protein by methylglyoxal may normally be associated with protein turnover and age-associated changes in extracellular matrix proteins. However, chronic exposure to abnormally high concentrations of methylglyoxal may induce pathological changes in protein structure and cell

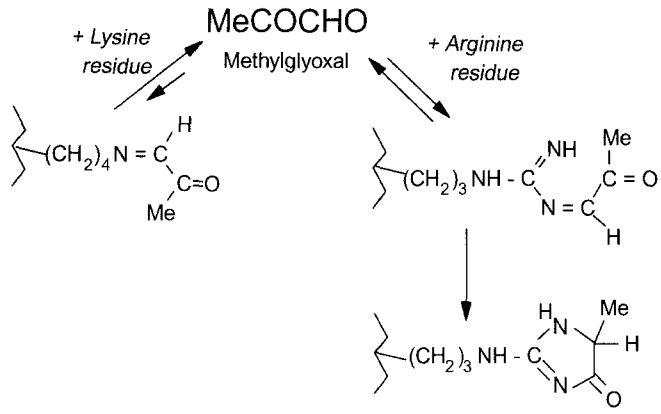

Fig. 3. The reaction of methylglyoxal with arginine and lysine residues in proteins

function. This appears to be increasingly found in clinical diabetes mellitus where a link of the metabolism of methylglyoxal by the glyoxalase system with specific associated complications (retinopathy, neuropathy, nephropathy) has emerged.

Hyperglycaemia, diabetes mellitus and diabetic complications

When human red blood cells were incubated in short-term culture (2 h) with 5 mM glucose (model of euglycaemia) and 50 mM glucose (model of hyperglycaemia in diabetes mellitus), there were increased cellular concentrations of methylglyoxal, S-D-lactoylglutathione and D-lactate, attributed to an increase in flux of methylglyoxal metabolised to D-lactate via the glyoxalase system in hyperglycaemia. It was suggested that if this increase in methylglyoxal concentration was found in red blood cells and elsewhere (lens fibre cells, capillary endothelial cells, peripheral neurones) in clinical diabetic patients, the modification of the glyoxalase system in hyperglycaemia may be linked to the development of diabetic complications (Thornalley, 1988). Methylglyoxal formation is increased by increased flux through the Embden-Meyerhof pathway, supplementation of the triose pool from metabolic flux through the polyol pathway with fructose converted to fructose-1-phosphate (and incorporated into the Embden-Meyerhof pathway) or to fructose-1-phosphate and cleaved by aldolase to dihydroxyacetonephosphate and glyceraldehyde, and by the oxidation of acetone (Hallfrisch, 1990; Reichard et al., 1986). Fructose formation is part of the polyol pathway through which there is also increased metabolic flux in diabetes mellitus (Gabbay, 1973) (Fig. 1).

The modification of the glyoxalase system in tissues and red blood cells was investigated in streptozotocin-induced diabetic rats, and of diabetic rats given the aldose reductase inhibitor Statil (Phillips et al., 1993b). Glyoxalase activities were decreased in the liver and increased in skeletal muscle of diabetic rats; the significance and mechanism of these changes in glyoxalase activities are unknown. The concentrations of methylglyoxal in the kidney cortex and medulla, lens and blood were increased in diabetic rats, relative to normal controls. These increases were prevented by Statil except in the kidney cortex. The concentration

of D-lactate was increased in the lens and blood of diabetic rats, relative to normal controls. This effect was partially prevented in blood but not in the lens of Statil-treated diabetic rats. The increase in glyoxalase metabolite levels in the lens, kidney and blood is consistent with an increased metabolic flux through the glyoxalase pathway in hyperglycaemia in lens and kidney tissue and red blood cells (although methylglyoxal and D-lactate readily cross cell plasma membranes, hence, it is not possible to define the precise origin of the pools of these metabolites). The increased concentrations of methylglyoxal in the kidney, lens and blood, and the decreased concentration of non-protein sulphydryl groups in the lens may be related to the development of diabetic complications (Phillips et al., 1993b).

An initial clinical survey revealed that the concentrations of methylglyoxal, S-D-lactoylglutathione and D-lactate were increased in blood samples from patients with insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus patients (NIDDM), relative to normal controls (Thornalley et al., 1989; McLellan et al., 1992). These data are consistent with an increased flux through the glyoxalase pathway during hyperglycaemia associated with clinical diabetes mellitus. IDDM patients with retinopathy had a significantly higher activity of glyoxalase I and a significantly lower activity of glyoxalase II in red blood cells than patients without retinopathy (Thornalley et al., 1989), and IDDM patients without complications (retinopathy, neuropathy) had a higher frequency of the glyoxalase phenotype GLO 1-1 than patients with complications (McCann et al., 1981). A further survey was required to confirm or deny the modification of the glyoxalase system in blood samples from diabetic patients and the link with the development of diabetic complications.

In our latest study, the glyoxalase system was characterized in blood samples from IDDM patients ($n = 43$), NIDDM ($n = 102$), and in normal controls ($n = 23$) (McLellan et al., 1993). The concentrations of glyoxalase metabolites were increased in diabetic patients. The median concentration of methylglyoxal in whole blood samples from IDDM patients was increased approximately 6 fold ($P < 0.001$), and in NIDDM patients approximately 2 fold ($P < 0.001$), relative to normal controls. This is consistent with the effect of hyperglycaemia on the glyoxalase system (Thornalley, 1988; Phillips and Thornalley, 1993b). However, the activities of glyoxalase I were also significantly increased in IDDM and NIDDM patients ($P < 0.001$), and the activity of glyoxalase II increased in NIDDM patients ($P < 0.05$), relative to normal controls. Regulatory mechanisms for the modification of glyoxalase activities in red blood cells and their precursors are unknown. Glyoxalase activities did not correlate with glucose concentration or percentage of glycated haemoglobin, therefore non-enzymic glycation of glyoxalases probably does not mediate the observed increase in glyoxalase activities. Chronic exposure to increased systemic methylglyoxal concentration in diabetic patients may lead to induction of increased glyoxalase synthesis in red blood cell precursors.

Simple and multiple correlation analysis showed that the concentration of methylglyoxal correlated positively with the duration of diabetes in IDDM and the combined diabetic patient (IDDM & NIDDM) groups, suggesting that the

concentration methylglyoxal concentration progressively increased with duration of diabetes. The concentration of D-lactate correlated positively with the concentrations of glucose and HbA_{1c} in the combined diabetic patient group (McLellan et al., 1993), suggesting a link with glucose metabolism and glycaemic control. Logistical regression showed as expected that duration of disease was a risk factor for development of complications. Patients with and without complications were paired by duration of diabetes and biochemical variables in complicated and uncomplicated groups compared. For neuropathy, complicated patients had a higher mean activity of glyoxalase II than uncomplicated patients; and for any of the 3 complications studied (retinopathy, neuropathy, nephropathy), complicated patients had a higher mean activity of glyoxalase I than uncomplicated patients. Future studies on the risk factors for the development of diabetic complications should consider glyoxalase characteristics for inclusion in the logistical model. This link between glyoxalase variables and microvascular diabetic complications may be indicative of similar underlying modifications in tissues where complications develop. A possible causal link with protein modification by methylglyoxal remains to be investigated.

Suppression of increased methylglyoxal concentration in diabetes mellitus: a role for aldose reductase inhibitors, aminoguanidine and L-arginine

Aldose reductase activity may influence the concentration of methylglyoxal in tissues: decreasing it by conversion of methylglyoxal to hydroxyacetone (Vander Jagt et al., 1992), and increasing it by supplementation of the triose phosphate pool via metabolic flux through the polyol pathway (Gabbay, 1973) and consumption of NADPH leading to decreased reduced glutathione levels (Gonzalez et al., 1983) and decreased *in situ* glyoxalase activity (Vander Jagt, 1993). Inhibition of aldose reductase activity with Statil decreased methylglyoxal concentration in diabetic rats to control levels (except in the kidney cortex), suggesting that inhibition of polyol pathway-dependent supplementation of the triose phosphate was the most important factor.

Aminoguanidine is a efficient scavenger of methylglyoxal. Methylglyoxal reacts with aminoguanidine under physiological conditions to form two major products, isomeric triazines 3-amino-5-methyl-1,2,4-triazine and 3-amino-6-methyl-1,2,4-triazine (Smith et al., 1992; Selwood and Thornalley, 1993), and a minor product from the 1:2 stoichiometric reaction methylglyoxal bisguanylhydrazone – a clinical anti-tumour agent (Janne et al., 1985). Providing the toxicity of aminoguanidine is not prohibitive, aminoguanidine is expected to decrease the concentration of methylglyoxal and hydroxyacetone *in vivo*. Aminoguanidine prevents the development of albuminuria, decreased sciatic nerve conduction velocity and protein crosslinking in diabetic rats (Soulis-Liparota et al., 1991; Cameron et al., 1992; Brownlee et al., 1985). High concentrations of arginine also scavenge methylglyoxal and inhibit collagen crosslinking in diabetes rats and clinical diabetic patients (Menzel and Reihser, 1991; Lubec et al., 1991).

These effects may be related to the proposed potential efficacy of these agents in prevention and therapy of diabetic complications.

Pathophysiological consequences of increased systemic concentrations of methylglyoxal

Methylglyoxal binds and irreversibly modifies proteins under physiological conditions. Hydroxyacetone also binds and modify proteins to produce apparently the same fluorescent product (Vander Jagt et al., 1992) – hydroxyacetone may slowly spontaneously oxidise to form methylglyoxal (Thornalley, 1985); hydroxyacetone also accumulates to high concentrations in diabetes mellitus (Reichard et al., 1986). Modification of proteins by methylglyoxal and hydroxyacetone in diabetes may contribute to the formation of advanced glycation endproducts (Brownlee et al., 1988), leading to deterioration in protein structure and function (Baynes, 1991), and removal of modified proteins by receptor-mediated endocytosis by monocytes, macrophages and Kuppfer cells (Vlassara et al., 1988; Horiuchi et al., 1986). Other effects may arise from the inactivation of insulin, a possible contributory mechanism of insulin resistance (Bunzli and Bosshard, 1971), and inactivation of antithrombin III and C1 inhibitor (Aleksandrovskii, 1992), which may contribute to thromboembolic complications in diabetes mellitus (Fuller et al., 1979). The formation of methylglyoxal- and hydroxyacetone-protein adducts in diabetes mellitus now deserves investigation.

The reactions discussed herein for methylglyoxal with proteins, aminoguanidine and L-arginine are also available to other α-oxoaldehydes – for example, 3-deoxyglucosone and 2-glucosulose formed from the degradation of glycated proteins (Smith and Thornalley, 1992; Hirsch et al., 1992). The reactivity of glucosone derivatives with the glyoxalase system is unknown (Bayne and Fewster, 1956) but is expected to be low because of the predominance of cyclic hemiacetal and hemiketal forms in solution.

The chronic exposure to high methylglyoxal concentrations and metabolism by the glyoxalase system are factors to be considered in the development of diabetic complications.

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