Critical Review

Cells Producing Their Own Nemesis: Understanding Methylglyoxal Metabolism

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Abstract

Methylglyoxal, which is technically known as 2-oxopropanal or pyruvaldehyde, shows typical reactions of carbonyl compounds as it has both an aldehyde and a ketone functional group. It is an extremely cytotoxic physiological metabolite, which is generated by both enzymatic and nonenzymatic reactions. The deleterious nature of the compound is due to its ability to glycate and crosslink macromolecules like protein and DNA, respectively. However, despite having toxic effects on cellular processes, methylglyoxal retains its efficacy as an anticancer drug. Indeed, methylglyoxal is one of the well-known anticancer therapeutic agents used in the treatment. Several studies on methylglyoxal biology revolve around the manifestations of its inhibitory effects and toxicity in microbial growth and diabetic complications, respectively. Here, we have revisited the chronology of methylglyoxal research with emphasis on metabolism of methylglyoxal and implications of methylglyoxal production or detoxification on bacterial pathogenesis and disease progression. © 2014 IUBMB Life, 66(10):667–678, 2014

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Beginning of the era of Methylglyoxal Research

Methylglyoxal, a small dicarbonyl compound has been the experimental muse of many researchers since its discovery in the late 19th century partly due to its involution in many of the central metabolic pathways. The research on methylglyoxal dates back to as early as 1930s, when the metabolite was thought to be an intermediate in glucose utilization pathway; however, subsequent lack of evidences regarding its role in biological systems led to the quick decline of the interest of researchers in methylglyoxal and it was rejected as artifactual (1–4). Since then and for a long period, it was believed that methylglyoxal can only be synthesized by acid catalysis of triosephosphates as was shown by Meyerhof and Lohmann (4), in line with its initial discovery in acid-treated biological extracts (1,3). Methylglyoxal's role in metabolic pathways did not pick up pace, even after determination of the complete Embden-Meyerhof pathway, as the sequential enzyme-catalyzed route of methylglyoxal synthesis from glycolytic intermediates still remained a puzzle (1,5). However, it was in 1970s, when evidence of enzymatic methylglyoxal production from dihydroxy acetone phosphate (DHAP) by methylglyoxal synthase (MgsA) activity started accumulating (5–7). Methylglyoxal was detected in the cells of *Pseudomonas sachharophila* during growth on glucose and succinate (1,6), and it was also shown to be an intermediate (although further investigations are lacking) in the pathway of glyceraldehyde 3-phosphate conversion to pyruvate in a *Pseudomonas* spp. (8). In the same era, MgsA from several organisms, including *E. coli* were extensively characterized (3,7). The 67-kDa enzyme was found to catalyze the conversion of DHAP with a K_m of 0.47 mM.

In the beginning of the 20th century, an enzyme that was named glyoxalase was discovered for its capacity to convert oxoaldehydes into their respective hydroxyl acids, and methylglyoxal was thought to be one of the substrates for this enzyme (2). The findings by Dudley and Neuberg in 1913, showing the conversion of methylglyoxal to D-lactate by the action of dog/rabbit liver glyoxalase, were the first evidences in support of

Abbreviations: *GlxI, glyoxalaseI; DHAP, dihydroxy acetone phosphate; NAD, nicotinamide adenine dinuceotide; GSH, glutathione; AGE, advanced glycation endproduct; AKR, aldo-keto reductase.*

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methylglyoxal as a component of glycolytic reactions (9,10). The glyoxalase system, which is now known to consist of two enzymes, that is, glyoxalase I (GlxI) and glyoxalase II (GlxII), is present in the cytosol and organelles like mitochondria and is found ubiquitously in all forms of life (11). Furthermore, it was shown that many bacteria and certain yeasts possess D-lactate dehydrogenase which converts D-lactate into pyruvate (12), the end product of glycolysis, thus bringing forth the methylglyoxal bypass as an alternative to glycolysis under certain conditions (1). Although conversion of methylglyoxal via D-lactate to pyruvate is a bypass of the Embden-Meyerhof pathway (glycolysis), mutants defective in glycolytic enzymes do not show detectable growth on glucose (1), indicating that the bypass is not the natural alternative to glycolysis. This phenomenon challenged the notion of methylglyoxal bypass as a favorable choice for pyruvate generation in conditions of glycolytic impediment (13). However, extensive research in the last decade regarding the importance of methylglyoxal bypass in cells indicates that this pathway is predominantly a strategy to relieve the stress from accumulated phosphorylated glycolytic (C3) intermediates while offering survival advantage (13,14). Despite methylglyoxal being a growth inhibitory metabolite, bacterial species invest in the synthetic reaction as glyoxalases rapidly and robustly detoxify the physiologically produced methylglyoxal. Thus, the survival advantage conferred by the production of this apparently toxic metabolite methylglyoxal is actually dependent on its efficient neutralization by the glyoxalase system.

Parallely, in 1959, Elliott predicted that aminoacetone, which accumulated in the cells of *Staphylococcus aureus*, may oxidatively deaminate or transaminate to give rise to methylglyoxal (15,16). In the following year, Elliott reported the formation of methylglyoxal from aminoacetone by a fraction of ox plasma, which has an amine oxidase and therefore deaminated aminoacetone into methylglyoxal (17). Since then for almost a decade, surprisingly, methylglyoxal formation pathway from aminoacetone received less attention. Later on, several reports from Turner, Neuberger, and Elliott showed the formation of aminoacetone from microbial precursors like aminopropanol, threonine, glycine, and acetaldehyde in a variety of bacteria and the possibility of aminoacetone being further catalyzed to methylglyoxal was shown to exist (18–20). This possibility remained elusive for a while as the product(s) of aminoacetone oxidation could not be identified in any bacterial extracts or animal tissues/plasma even though these preparations completely catabolized aminoacetone (21). Interestingly, in 1967, in a species of *Arthrobacter*, aminoacetone conversion to methylglyoxal was observed by involvement of a probable amine oxidase (21). Amine oxidases are a large family of enzymes with members having distinct cofactor specificities (2).

Cytotoxicity: AGEs, and Crosslinked Adducts

Cytotoxic nature of methylglyoxal, an α,β-dicarbonyl metabolite, has been extensively researched in the works of Kalapos and Thornalley (2,22–24). The multitude of processes/macromolecules that methylglyoxal inhibits or damages greatly outnumber the limited beneficial roles it possess. The deleterious nature of methylglyoxal is due to its ability to generate advanced glycation end products (AGEs) on the proteome (24). Glycation reactions of methylglyoxal with the eventual formation of complex and irreversible adducts often impairs the functionality of the target proteins (25). The reactions, collectively called as Maillard reactions, usually begin by the formation of a Schiff's base adduct by the reaction of methylglyoxal with the lysine or arginine side chain of any protein. A common AGE formed by the methylglyoxal's reaction with arginine is argpyrimidine (AG) (26,27) which has been implicated in apoptosis due partly to intracellular accumulation of AG-modified proteins (28,29). A prolonged reaction with arginine residues results in the slow formation of irreversible hydroimidazolone derivatives, of which more than 90% are N_δ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H1) adducts. Other products such as 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-1*H*-imidazol-5-ylidene]amino)hexanoate (MODIC) account for only 10% of the total arginine adducts (24,25,30). At physiological concentrations, methylglyoxal can modify arginine and lysine residues of blood serum proteins, whereas chronic exposure to higher concentration often leads to pathological changes in the protein structure (23,25,30). Dicarbonyl proteome which includes lens crystallins, transcription factors (TFs), albumin, hemoglobin, and mitochondrial proteins among several others, are especially susceptible to methylglyoxal glycation (30). Recent data has shown that 0.1–2% of the total cellular arginine is modified by methylglyoxal and that human lens protein accumulates the maximum amount of arginine adducts (31). Accumulation of AGEs on proteins generally correlates with the clinical complications associated with diabetes like retinopathy, nephropathy, and neuropathy (32). Accumulation of hydroimidazolones in the proteome is usually observed to increase with age, which results in the formation of chemically stable glycation end products resulting in a cascade of protein damage. Another inhibitory feature of methylglyoxal is its ability to induce oxidative stress. When incubated with endothelial cells, neutrophils, and vascular smooth muscle cells, methylglyoxal (30–300 μmol/L) induces the generation of superoxide, peroxide, and peroxynitrite free radicals in a dose-dependent manner. Additionally, at this concentration, methylglyoxal induces production of, proinflammatory cytokines like TNFα, IL-6, and IL-8 in neutrophils leading to apoptosis (33). At still higher concentrations (1–5 mmol/L), methylglyoxal manifests a multitude of effects on cellular macromolecules like serum albumin and antioxidant class of enzymes. The extent of methylglyoxal-induced damage is inversely related to the antioxidant capacity and the glutathione (GSH) pool of the cell (13). Methylglyoxal inhibits both GSH peroxidase and GSH reductase further increasing the time of endogenous methylglyoxal persistence (30,34). Usually, lower rate of formation/exposure of methylglyoxal coupled with

degradation by GlxI does not allow deleterious accumulation of hydroimidazolone adducts, which themselves are degraded proteasomally with time (30).

Crosslinked adducts by methylglyoxal with the amino group of DNA bases like adenine and guanine are also of common occurrence and is seen mostly with chronic elevation in methylglyoxal concentration (24). Deoxyguanine (dG) is most susceptible to glycation by methylglyoxal (24). Methylglyoxal-induced modifications on DNA are 3-(2′-deoxyribosyl)-6,7-dihydro-6,7-dihydroxy-6-methylimidazo-[2,3-*b*]purine-9(8)one (dG-MG) and *N*2-(1-carboxyethyl)-deoxyguanosine (CEdG). Apart from initiating nucleotide AGE formation, methylglyoxal also mediates interpolymer (DNA–DNA, DNA–protein, and protein–protein) crosslinking (35,36). Few examples of such interpolymer interactions are as follows: DNA polymerase Klenow fragment crosslinked with DNA strand on exposure to 1 mM methylglyoxal for 60 min (35), crosslinkage of calf thymus DNA (36), and DNA–protein crosslink in Chinese hamster ovary (CHO)cells on exposure to 1.5 mM methylglyoxal for 90 min (37). A recent report by Tu *et al.* (36) suggested that the process of crosslinking is independent of ROS; however, accumulation of methylglyoxal has typically been observed under oxidative stress.

Reaction of methylglyoxal with proteins and amino acids has been shown to produce biologically active free radicals and oxidative stress in the cells (2,38). The findings showed that incubation of methylglyoxal with lysine produced ~24 nmol $O_2^{\cdot-}$ superoxide anion per hour (38). Hence, the oxidative free radical-mediated DNA-strand breakage is only observed when methylglyoxal is incubated with both DNA and amino acid along with divalent metal ion (38). Methylglyoxal-induced DNA damages mainly fall under multibase deletions and base pair substitutions (GC:CG and GC:TA transversions) (39). Inhibitory effect of methylglyoxal has also been seen on histone acetylation, which may be one of the modes of action of methylglyoxal on DNA replication inhibition (22).

Metabolic Production of Methylglyoxal

Glycolytic Bypass

Methylglyoxal formation ubiquitously takes place in all cells and organisms (24). It is a product of many physiological reactions (13,14). Among the various physiological routes of methylglyoxal production, the dominant one is catalyzed by MgsA from DHAP by the removal of the phosphate group (Fig. 1). MgsA is homotropically activated by its substrate, DHAP, whereas it is inhibited allosterically by its product, phosphate (14). Enzymatic production of methylglyoxal through glycolytic intermediates is governed tightly by several factors like expression level of MgsA, rate of detoxification by glyoxalases, phosphate pool of the cell and rate of influx of carbon sources. Usually, MgsA catalyzes the bypass reaction when there is an elevation in the rate of carbon acquisition than the rate of utilization of these carbon substrates by glycolysis (13,14). Growth of *E. coli* on glucose, however, does not lead to significant methylglyoxal production, as the capacity of GSH-dependent detoxification surpasses the rate of production, which is limited by several factors (13). In ruminal bacterium *Prevotella ruminicola*, insufficient nitrogen can cause excess carbon substrates to produce methylglyoxal along with decrease in ATP production (40). Such a strategy is used by ruminal bacteria to minimize the energy spill when nitrogen sources are limiting and the bacteria inevitably acquire excessive carbon sources. Facultative anaerobic bacteria of human gut produce and secrete methylglyoxal in feces. This activity is unusually enhanced in MgsA overexpressing Proteus spp. (41). Other intestinal species like *Salmonella*, *Aeromonas*, and *E. coli* also exhibit MgsA activity; however, detectable level of secreted methylglyoxal is not observed in culture media (41). Nonenzymatic fragmentation of triosephosphates via phosphoenediolate intermediate also gives rise to methylglyoxal. Notably, methylglyoxal bypass of glycolysis is not the pathway of choice under normal physiological conditions. Rather it is most often the result of an uncontrolled or amplified uptake of sugar or sugar phosphates (14). A single report in 1971 showed that a strain of *E. coli* with mutated glycerol kinase accumulated toxic levels of methylglyoxal because of uncontrolled dissimilation of glycerol to triosephosphates (42). Accordingly, methylglyoxal accumulation in microbes has also been noticed during excess intake of non-glucose carbon sources like fructose and mannose, which can directly enter glycolysis in cells overproducing UhpT, a hexose-phosphate transport protein (Fig. 1) (43). These phosphotransferase (PTS)-dependent sugars get phosphorylated and become a part of the glycolytic cycle. Excess transport of these direct intermediates of glycolysis leads to methylglyoxal accumulation with varying degrees of growth inhibition (43). Another route by which *E. coli* cells undergo growth inhibition is when non-PTS sugars like pentoses are fed along with cAMP. Xylose or arabinose along with cAMP stimulates its own uptake by *E. coli* cells leading to growth arrest due to methylglyoxal accumulation (44). Moreover, excess intracellular pentose sugars like ribose and xylose also get converted by transketolase and transaldolase to glycolytic intermediates (45) and become a part of the glycolytic reactions (Fig. 1). Apart from excess sugar substrates, phosphate insufficiency also drives methylglyoxal favoring reaction. Limitation of phosphate alone, however, causes only limited accumulation of methylglyoxal in cells. Phosphate limitation blocks the reaction onward of triosephosphate isomerase, as the next reaction of glycolysis which is catalyzed by glyceraldehyde 3-phosphate dehydrogenase requires inorganic phosphate (Pi) for producing 1,3-bisphosphoglycerate. Glyceraldehyde 3-phosphate dehydrogenase has a low K_m for its substrate Pi (0.29 mM) (1) than MgsA has for DHAP (0.5 mM) (46). Hence, under normal physiological conditions when phosphate availability is not limiting and it is above the K_m of glyceraldehyde 3-phosphate dehydrogenase, the enzyme continues the glycolytic pathway and MgsA is practically inhibited (1,13,14).

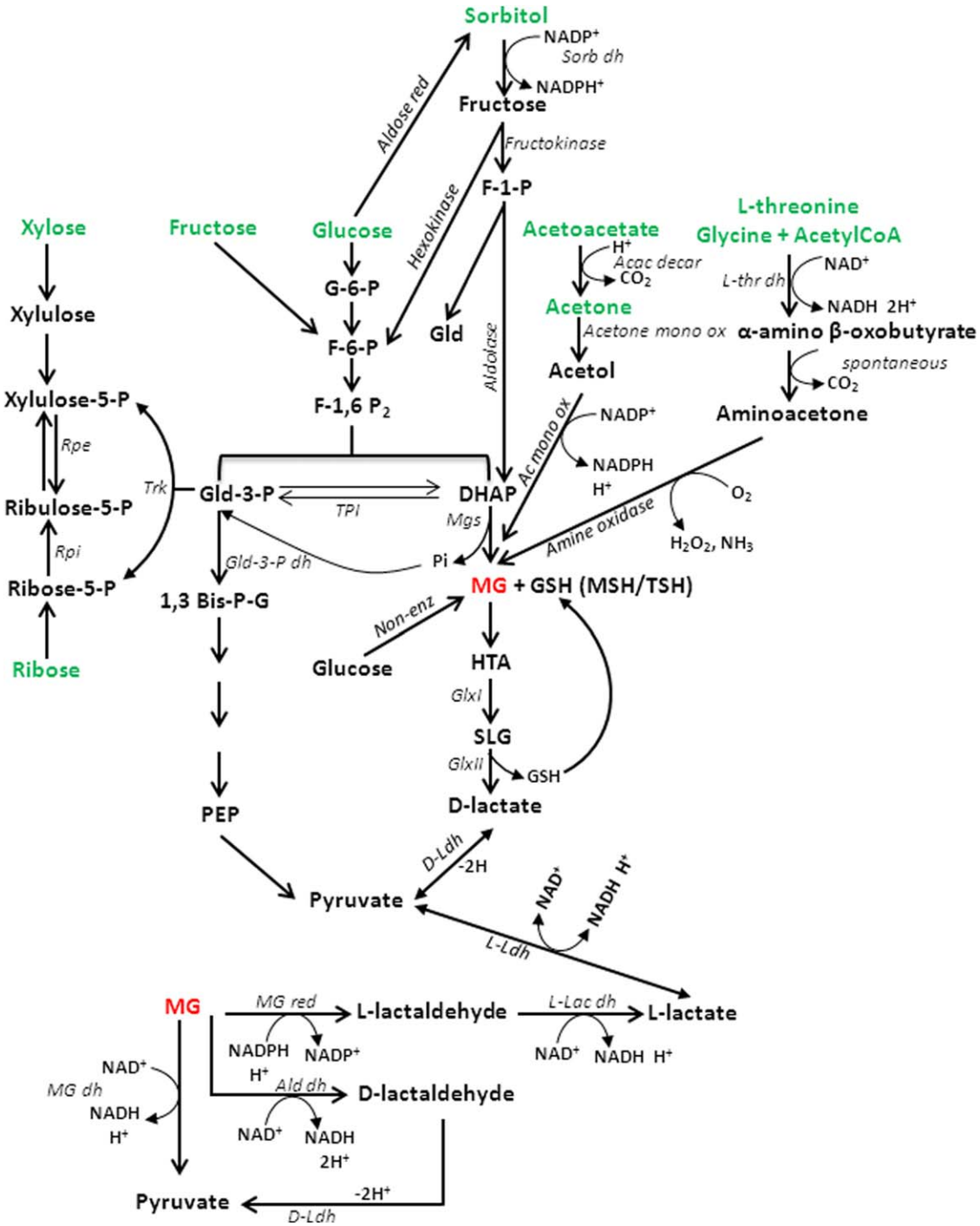

FIG 1

Overview of the methylglyoxal metabolism. Abbreviations: G-6-P, glucose-6-phosphate; F-6-P, fructose-6-phosphate; F-1,6-P_2, fructose-1,6-bisphosphate; Gld-3-P, glyceraldehyde-3-phosphate; DHAP, dihydroxyacetone phosphate; 1,3-BisPG, 1,3-bisphosphoglycerate; Gly-P, glycerolphosphate; GSH, glutathione; MSH, mycothiol; TSH, trypanothione; Gld, glyceraldehydes. The enzymes involved in the reactions: TPI, triosephosphate isomerase; Mgs, methylglyoxal synthase; GlxI, glyoxalase I; GlxII, glyoxalase II; D-Ldh, D-lactate dehydrogenase; L-Ldh, L-lactate dehydrogenase; L-thr dh, L-threonine dehydrogenase; Acac decarb, acetoacetate decarboxylase; MG red, methylglyoxalreductase; L-lac dh, L-lactaldehyde dehydrogenase; Ac mono ox, acetolmonooxygenase; Ald dh, aldehyde dehydrogenase; MG dh, methylglyoxal dehydrogenase; Sorb dh, sorbitol dehydrogenase; Rpi, ribose-5 phosphate isomerase; Rpe, ribulose-5 phosphate epimerase; Trk, transketolase; Aldose red, aldose reductase. Substrates in green are the initiators of methylglyoxal-producing reactions. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Aminoacetone

Research on methylglyoxal metabolism was in its prime during 1960s to 1980s, first due to the role of glyoxalases in methylglyoxal degradation receiving renewed interests and second for the possibility of methylglyoxal as a glycolytic intermediate (1,5,42,46). Only few reports existed at that time which demonstrated the nonglycolytic production of methylglyoxal (17,19,21,47). It was observed that bacteria/yeast grown on threonine or glycine accumulated aminoacetone (15,16,48), which eventually gave rise to the growth inhibitory component methylglyoxal (49–51). Methylglyoxal production was suggested to be a part of the oxidative deamination pathway of threonine and glycine catabolism. L-Threonine is initially oxidized by threonine dehydrogenase into oxobutyrate, which spontaneously decomposes to yield aminoacetone (Fig. 1). Glycine and acetyl CoA also condense to give aminoacetone by aminoacetone synthase. Aminoacetone thus formed can either get reduced to aminopropanol or deaminated to methylglyoxal. Although enzymatic production of aminoacetone from threonine and glycine was clearly observed in the cells of *S. aureus*, further deamination of this aminoketone to methylglyoxal was under speculation until 1960 when Elliott first showed an enzyme from ox plasma to catalyze the formation of methylglyoxal from aminoacetone by oxidative deamination (17). Later on, bacterial production of methylglyoxal from aminoacetone was also observed in other bacterial species like *Arthrobacter* and *Bacillus subtilis* which degraded threonine and NAD or glycine and acetyl CoA to aminoacetone (21,49). *Salmonella* Typhimurium is also predicted to produce methylglyoxal via aminoacetone in LB medium (52). The genome encodes a putative monoamine oxidase and a noncanonical lactoylglutathione lyase (GlxI). The lactoylglutathione lyase mutant rapidly loses viability due to uncontrolled methylglyoxal production, and this could be in part due to monoamine oxidase-mediated methylglyoxal production (52), as it has been known that bacteria grown in complex nutrient media dissimilate threonine into aminoacetone and hence to methylglyoxal (41,45,49,50).

A similar amine oxidase from goat plasma was purified by Ray and Ray after almost two decades, which also showed the capability to convert aminoacetone into methylglyoxal (53). Amine oxidase from goat liver, which is distinct from plasma amine oxidase, too showed similar enzymic activity (54). Methylglyoxal was thus demonstrated to form as a result of oxidative deamination of aminoacetone (53). Another class of amine oxidase which is semicarbazide sensitive (SSAO) and is Cu^{2+}, quinine dependent, is found in all mammalian cells either in tissue-bound form or plasma-soluble form. SSAO from human umbilical artery homogenates deaminated aminoacetone to methylglyoxal (55). An elevation in the level of methylglyoxal in patients with diabetes mellitus has been implicated as a function of increased aminoacetone concentration and the expression of plasma SSAO, which along with O_2 and Cu^{2+} converts aminoacetone into methylglyoxal (56).

Acetone

In 1984, methylglyoxal formation was suggested to take place from acetone degradation in rats. Enzymes from rat liver were shown to convert acetone into methylglyoxal via acetol formation. Acetone monooxygenase first hydroxylates acetone to acetol and then acetol is converted to methylglyoxal by acetol monooxygenase (57). Subsequently, cytochrome P450 2E1 was identified in mouse, rabbit and rat as the acetone monooxygenase and acetol monooxygenase enzyme system which catabolized acetone into methylglyoxal (Fig. 1) (58) and (59). Methylglyoxal and acetol subsequently get utilized into gluconeogenic pathway, predominantly operational during conditions of starvation and ketoacidosis (58). Monooxygenase-catalyzed methylglyoxal accounts for a significant source of the metabolite in liver during elevated acetone concentrations (22).

Other Minor Routes

Methylglyoxal is also generated through several other secondary pathways such as auto-oxidation of glucose, lipid peroxidation, and myeloperoxidase-catalyzed catabolism of amino acids (60).

Methylglyoxal Elimination Pathways

Glyoxalase System

GlxI and GlxII are the enzymes of dicarbonyl degradation system, which catalyze sequential reactions to convert α-oxoaldehydes into their corresponding 2-hydroxycarboxylic acids (61). GlxI isomerizes (intramolecular disproportionation) the spontaneously formed hemimercaptal products between cytotoxic oxoaldehydes and GSH into α-hydroxythioesters of GSH. The thioesters of GSH are further hydrolyzed into the corresponding α-hydroxyacids (D-lactate in case of methylglyoxal) by the action of GlxII, regenerating back the thiol cosubstrate (13). Considering the central importance of the Glx system in detoxification, it is not surprising to see an almost ubiquitous distribution of the enzymes across species. Apart from the typical function of oxoaldeyhyde elimination, glyoxalases are also implicated in osmotic/oxidative stress-related signaling pathways in yeast (62). Mycobacteria and protozoan parasites however, use mycothiol and trypanothione as the thiol cofactor for GlxI reaction (Fig. 1) (61). GlxI is a metalloprotein and requires divalent metal cations for its activity. The specificity of the cofactor cation for GlxI largely depends on the species; for example, human GlxI is active with Zn^{2+}, whereas the *E. coli* enzyme requires Ni^{2+} or Co^{2+} ions for its activity. It has been observed that GlxIs of Gram-negative bacteria are predominantly activated by Ni^{2+}/Co^{2+} ions with the exception of *Pseudomonas*, whereas those of eukaryotic origins are Zn^{2+} dependent. In *Pseudomonas putida*, for example, GlxI is Zn^{2+} dependent. Almost all the Zn^{2+}-dependent GlxI enzymes characterized so far are of eukaryotic origin such as those from *Homo sapiens, Plasmodium falciparum*, and *Saccharomyces cerevisiae*. GlxI of *N. meningitides*, which is classified under the non-Zn^{2+}-dependent category, shows higher relative affinity for Co^{2+} when compared with Ni^{2+}, which is

based on unique structural features of the *Neisseria* GlxI (63). *Pseudomonas aeruginosa*, however, has three GlxI enzymes (GloA1, GloA2, and GloA3) belonging to both the Zn^{2+} and non-Zn^{2+} activation classes (61,62). The preference of GlxI enzymes of different species toward a particular class of metal ion is in part due to the differences in structural motifs and length of the amino acid chain. A longer peptide chain (~180 amino acid) is present in the Zn^{2+}-activated class and shorter chains (~130 amino acid) in the Ni^{2+}/Co^{2+}-activated class. His, Glu, His, and Glu-octahedron forms the metal-binding active site residues in case of bacterial Ni^{2+}/Co^{2+}-dependent GlxIs, whereas Gln, Glu, His, and Glu forms in case of Zn^{2+}-dependent GlxIs (63). Notably, metal selectivity cannot always be ascertained by structural features as the best inference on metal specificity can only be known by the measurement of GlxI activity by reconstitution with different divalent metal ions (63). GlxII, again a metalloenzyme, however, does not strictly fall under specific metal activation classes. The enzyme from *E. coli* is activated by Zn^{2+}, whereas the enzyme from *S.* Typhimurium shows promiscuity and binds Mn^{2+}, Fe^{2+} and Zn^{2+} with decreasing affinities (64).

Reductases and Dehydrogenases

Apart from the evolutionarily conserved glyoxalases, enzymes like aldose reductase and lactaldehyde dehydrogenase also eliminate methylglyoxal toxicity (2,65). These enzymes convert methylglyoxal into acetol and lactaldehyde and have been reported to exist in *E. coli*, yeast, plants, and mammals (65). In humans, aldose reductase family is actually represented by three enzymes, namely, ALR1 (alcohol:$NADP^+$ oxidoreductase), ALR2 (alditol:$NADP^+$ oxidoreductase), and ALR3 (carbonyl reductase) (2). Among these three enzymes, ALR2 has the highest specificity toward methylglyoxal, whereas aldehyde reductases typically catalyze a range of carbonyl substrates (Fig. 1). Methylglyoxal at the aldehyde position is reduced in a NADH-dependent way by ALR2 to yield acetol (66). Disposal of methylglyoxal by either GlxI or ALR2 depends on the cellular pool of reduced GSH (67). Initial activities of both the enzymes converge at the physiological concentration of GSH (3 mM) to act on methylglyoxal/hemithioacetal substrates (67). ALR2-mediated detoxification predominates at lower GSH concentrations. These enzymes are classified as aldo-keto reductases (AKRs) and generally have broad substrate specificities. SakR1, an AKR from *Synechococcus* spp. reduces methylglyoxal with NADPH during growth in the presence of glycerol (68). YghZ, YeaE, YqhE, and YafB are *E. coli* AKRs, belonging to the AKR14 family, catalyzes NADPH-dependent reduction of methylglyoxal to acetol (65). Generally, bacteria have multiple genes belonging to the AKR family. Yeast aldose reductase, a NADPH-dependent oxidoreductase, encoded by the *GRE3* gene gets transcriptionally upregulated during various stresses (69). Stress conditions like high salt, H_2O_2, carbon starvation and so forth in *S. cerevisiae* induce elevated levels of methylglyoxal, in agreement with the fact that aldose reductase activation is also observed in these situations and constitutes the principal methylglyoxal degradative pathway in GlxI mutants (69). Another *S. cerevisiae* enzyme which is specific for methylglyoxal unlike the promiscuous aldose reductases is methylglyoxal reductase (NADPH dependent), a 43-kDa enzyme (70). Methylglyoxal reductase converts methylglyoxal into L-lactaldehyde, which is subsequently converted to L-lactate by NAD^+-dependent L-lactaldehyde dehydrogenase (Fig. 1) (70).

Methylglyoxal as Signal Transducer

Methylglyoxal has been observed to directly or indirectly induce many TFs transcription factors and proteins that lead to nuclear localization of those TFs and activation of the target genes. These signaling events are mostly components of stress tolerance pathways. In yeast, TF Yap1 induces the expression of several antioxidant genes during oxidative stress (71). The upregulation is brought about by the elevation of the redox potential of the cell, which is sensed by the cysteine residues at C-terminal of Yap1. It was, however, observed that Yap1 can function irrespective of the cellular redox potential under the influence of methylglyoxal. Methylglyoxal was proposed to reversibly modify Yap1 polypeptide at the cysteine residues by forming an adduct and bring about its activation (71). In *S. cerevisiae*, methylglyoxal has also been implicated as a signal initiator for HOG-MAP kinase pathway via Sln1 branch and intracellular Ca^{2+} uptake (72). Excessive extracellular methylglyoxal therefore is a potential inducer of many signaling pathways in yeast cells and parallels can be drawn to mammalian diabetic studies which show that the mammalian ortholog of Hog1 (p38) is increased in type 2 diabetes. Therefore, some of the typical chronic complications usually observed in diabetes may arise from aberrant signal transductions due to constitutive exposure of the cells to high levels of external methylglyoxal (72). In pancreatic β-cell lines, methylglyoxal blocks insulin function and insulin-dependent activation of PI3 kinase pathway and expression of Ins1, Gck, and Pdx1 mRNA, altogether adding to the dysfunction of β-cells (73). In fact, methylglyoxal directly inhibits insulin by covalent modifications (74). Methylglyoxal-mediated induction of ROS/RNI ($O_2^{\cdot-}$ and NO) is believed to cause insulin-resistant state in cells. A similar effect of methylglyoxal on inhibition of PI3 kinase is seen during thrombin-induced platelet aggregation and thrombus formation in diabetic conditions (75). During intestinal diseases and conditions of lactose or other food intolerances, methylglyoxal-induced activation of Ca^{2+} channels in bacteria is predicted to exacerbate the disease conditions further. In *E. coli*, methylglyoxal was observed to induce high level of Ca^{2+} transients, and the cytosolic Ca^{2+} levels reached 8–9 μM when compared with the resting free Ca^{2+} level of 0.1–0.3 μM (76). As Ca^{2+} levels are known to be parallel to their effect on cell cycle processes in eukaryotes, it was observed that in the presence of methylglyoxal, external Ca^{2+} levels in conjunction with free intracellular Ca^{2+} affects bacterial generation time (76). Considering the high output of methylglyoxal in gut from consumption of several food products and gut bacteria themselves, Campbell et al. (74) proposed that

modulation of Ca^{2+} signaling in bacteria in response to methylglyoxal can have bearings on the advancements of diseases like irritable bowel syndrome and lactose intolerance. In eukaryotic neuronal cells, the deleterious effects of methylglyoxal have been observed in the form of increased ATP depletion, ROS, and lactate production (77).

Methylglyoxal Metabolism: Role in Disease Pathogenicity, a Friend or a foe?

Bacterial Pathogenesis

Methylglyoxal has long been seen as the active antibacterial component of Manuka honey (78). It has been shown that methylglyoxal present in this honey effectively destroys biofilms of *Pseudomonas aeruginosa* and *Staphylococcus aureus* which are responsible for chronic rhinosinusitis disease (78). Although, administration of methylglyoxal containing honey does not negatively influence the digestive enzymes of the host, it rapidly carbonylates the free amino acid side chains of the proteins (79). In mammalian gut, methylglyoxal is produced by the Enterobacteriaceae species (41). Moreover, in mammals, plasma methylglyoxal levels are elevated in many of the disease pathologies (24). Therefore, infectious agents and the host itself are constantly exposed to toxic metabolites like methylglyoxal. Although literature abounds with reports on the various pathologies caused by methylglyoxal on mammalian cells, less is known about the implications of methylglyoxal metabolism in host–pathogen interactions. In patients with chronic periodontitis, the gingival crevicular fluid contains high amount of methylglyoxal, which is produced by relatively high concentration of *T. forsythia* (earlier known as *Bacteroides forsythus*) at the infection sites (Fig. 2) (80). The strategy of producing toxic amounts of methylglyoxal is seen as a virulence mechanism (80). Methylglyoxal accumulation in the periodontal pockets leads to protein and tissue damage of the host (80,81). In granulomatous Mycobacterial infections, a plethora of modulations in macrophagic responses to methylglyoxal accumulation has been observed (82). The infected sites contain elevated levels of methylglyoxal, which causes apoptosis of the macrophages via activation of the JNK signaling (82). The possibility of methylglyoxal production through ROS-mediated oxidation of polyunsaturated fatty acids of MTb cell wall during infection is considered as an important contributor of elevated levels of the metabolite. Furthermore, methylglyoxal and AGEs together elicit many immune response genes in the Mycobacteria-infected macrophages such as TNFα, members of TNFα receptor superfamily, and TNFα-related proteins like TRAF1 and TRAF2 (Fig. 2; ref. (82). Lactoylglutathione lyase, the enzyme for relieving methylglyoxal toxicity, has also been implicated to enhance virulence of certain intracellular bacterial pathogens (52,83,84). *Brucella abortus* and *Salmonella* Typhimurium induce the expression of their respective lactoylglutathione lyase genes to aid in macrophage persistence. The *glxI* gene was shown to get upregulated constitutively following 4 h of *B. abortus* and *S.* Typhimurium infection in macrophages (Fig. 2; refs. (52) and (83). As overproduction of lactoylglutathione lyase (GlxI) confers tolerance to methylglyoxal, it may be used by the intracellular bacteria to survive and proliferate in macrophages. Our studies have shown that *S.* Typhimurium lactoylglutathione lyase null mutant demonstrate low rates of detoxification and undergo rapid death in nonphagocytic cells (52). In *Salmonella* nontyphoidal serovars, the presence of this noncanonical GlxI-like enzyme (encoded by STM3117 in *S.* Typhimurium LT2 strain) has caught the attention of researchers, as the homologs of the encoding gene does not exist in typhoidal serovars or other enterobacteria species; however, surprisingly, the flanking genes are present in *Yersinia* spp., *Mycobacteria* spp., *Burkholderia* spp., and *Coxiella* spp. The selective presence of particular genes in one species but not in other related species indicate host-specific adaptation (52,85). Not only methylglyoxal-detoxifying enzymes but also the methylglyoxal-producing enzyme, MgsA, gets induced under bacterial infections like in *Actinobacillus pleuropneumoniae* acute infections in porcine lung (Fig. 2). As per the findings, the bacteria probably experience carbon excess in the early phases of infection due to excessive host cell lysis by secreted exotoxins with release of surplus carbohydrates (86). By inducing MgsA, *A. pleuropneumoniae* ensures rapid utilization of DHAP before toxic levels are reached (86). Bacteria generally adapt to the challenging and stringent host environments by either inducing or repressing certain metabolic pathways. The methylglyoxal bypass is an adaptation strategy by bacteria to alleviate the growth-limiting effect of sugar phosphates. An elevated level of lactoylglutathione lyase has been observed in *Streptococcus mutans*, an inhabitant of dental plaques, during its growth in the acidic environment of dental caries. The report highlights the involvement of the enzyme in aciduricity of *S. mutans* in host environments and how the expression of *lgl* helps to detoxify the MG produced as a result of increased glycolytic flux and acid end product metabolism in the vicinity of dental plaques (84). AGEs, which are crosslinked complex adducts irreversibly developed on lysine and arginine side chains of proteins, are one of the primary pathophysiological factors in several age-related disorders (23,87). Majority of the AGEs are contributed by reactions of methylglyoxal on macromolecules. The role of AGEs, in shifting the host immune bias toward production of proinflammatory cytokines and oxidative stress, has been known for quite some time (82,87). Actively secreted AGEs by *E. coli* induce THP-1 cells to secrete TNFα (87). Off late, the significance of RAGE, the multiligand receptors for AGEs, in transducing intracellular signaling and modulation in disease responses by the host has come into light (extensively reviewed in ref. (88).

Hyperglycemia-Related Disorders

Direct administration of methylglyoxal in mammals results in several deleterious manifestations (2,22). The degenerative changes usually depend on the dosage of methylglyoxal. Subcutaneous injection of lethal dose of methylglyoxal in rabbits

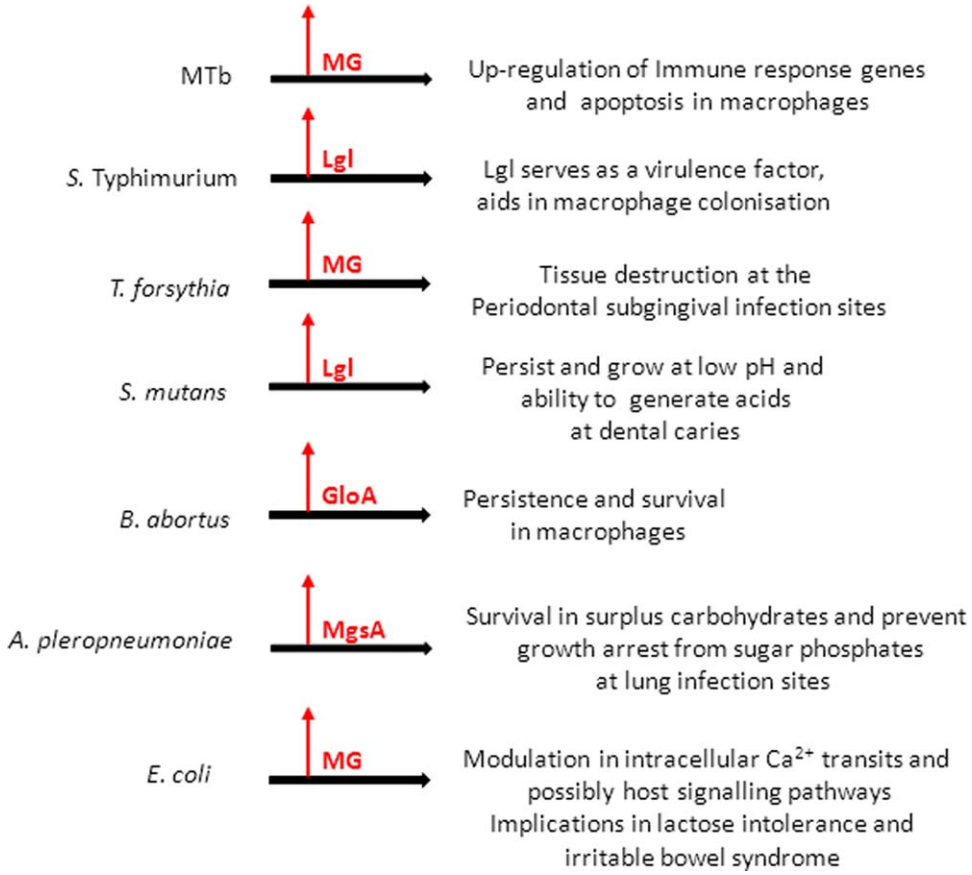

FIG 2 *Survival and persistence mechanisms of microbes in infection sites. Modulation of either the methylglyoxal production or degradation pathway is used as a virulence-enhancing strategy. The up directing arrows indicate upregulation. Abbreviations: MG, methylglyoxal; Lgl, lactoylglutathionelyase; GloA, glyoxalase I; MgsA, methylglyoxal synthase. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]*

revealed pulmonal hyperemia, edema along with degeneration of liver and kidneys (22). Toxicity of methylglyoxal is further compounded by the fact that this oxoaldehyde reduces the GSH pool in tissues, which is the cofactor for glyoxalase-dependent methylglyoxal detoxification. In mice, administration of lethal dose (800 mg/kg body wt) resulted in death within 4 h (22). A lower dose of methylglyoxal although did not result in a delayed death, the recipient mice showed extensive liver damage and an altered architecture of the cell organelles (22). Disorders of the mammalian class due to excessive plasma methylglyoxal usually lead to chronic complexities associated with diabetes: retinopathy, nephropathy, and neuropathy (22). Because of chronic hyperglycemic conditions, excess methylglyoxal glycates the repertoire of cellular proteins and forms AGEs. Long-term *in vivo* accumulation of AGE-modified proteins is often associated with aging and diabetic complications. Intracellular proteins modified by AGEs have altered function. Extracellular matrix components modified by AGE precursors interact abnormally with other matrix components and with the receptors for matrix proteins (integrins) on cells (89). In diabetic retinopathy, increased levels (279%) of MG-H1 proteins are detected by 24 weeks (90). Retinal microvascular endothelial cells accumulate hydroimidazolone and argpyrimidine (methylglyoxal-derived AGE adducts), which results in premature closure of blood capillaries (26). The hallmark of diabetic nephropathy is deposition of extracellular matrix protein in the glomerular mesangium and tubule interstitium (91). There is a pathologic accumulation of collagens, fibronectins, and laminins caused by the imbalance between synthesis and degradation of matrix components (91). Matrix proteins are prone to AGE modifications, resulting in altered structure and functions (92). The formation of crosslinks in matrix by the glycation of collagen results in change in packing density, which further lead to stiffness and resistance to hydrolysis by proteolytic enzymes (92). It is known that the diabetic condition can lead to osteoporosis (93). It is characterized by a severe decrease in the rate of bone mineral apposition and reduced bone strength, also called as diabetic osteopathy.

Role as a Cancerostatic Metabolite

During 1960s, few studies demonstrated that infusion of methylglyoxal into animals bearing tumor cells in their abdominal

cavity completely inhibited the progression and size of those tumors (22). The correlation between methylglyoxal and cancer cells can be drawn by considering the earliest evidence, first put forth by Otto Warburg, that cancer cells have distinctly high glycolytic rates under aerobic conditions (94). Accumulation of methylglyoxal in cancer cells is thus a normal consequence of their high glycolytic rates; however, the actual concentration dynamics are complex due partly to the differential activity of glyoxalase in different tissues (2,22). Various emerging studies have suggested the therapeutic effect of methylglyoxal to be mediated by cell cycle arrests and apoptosis in the tumor cells (29,95,96). A high glycolytic activity of tumor cells predisposes them to elevated intracellular methylglyoxal concentrations. Surprisingly, however, similar high levels of methylglyoxal-derived AGE-modified proteins are often not found in those tumor cells (27). Although *GlxI* expression and methylglyoxal accumulation rate differ greatly in malignant and normal tissues, still a clear action of methylglyoxal's cytotoxicity exclusively toward cancerous tissues is often not observed (2). High *GlxI* expression has been particularly found in apoptosis-resistant malignant carcinoma of ovaries, leukemia cells, skin neoplasms and pancreatic cancer cells (97–99). Inhibition of GlxI enhances the level of intracellular methylglyoxal, which has been found to evoke apoptosis in the tumor cells (97). Methylglyoxal or GlxI inhibition-mediated apoptosis of cancer cells (colon and lung carcinoma) occurs via suppression of the NK-κB-dependent antiapoptotic genes (97). Inhibitors of GlxI are thus a favored choice for cancer therapeutics. Methylglyoxal-induced apoptosis in macrophage cell lines mostly occur due to the deleterious reactions of methylglyoxal with DNA bases coupled with increased intracellular oxidative stress (100). Another report has shown that in human prostate and MCF-7 breast cancer cells, a mitochondrial apoptotic pathway is induced by dysregulation of the NF-κB-dependent pathways due to accumulation of methylglyoxal-derived arg pyrimidine residues (28,29). A simultaneous inhibition of GlxI by ROS and accumulation of argpyrimidine-derived heat-shock protein-27 (Hsp27) induced the mitochondrial apoptotic pathway via desensitization of p53 and suppression of NF-κB pathway (29). Hepatocarcinoma solid tumors rapidly proliferate due to upregulation of *GlxI*, which is essential to the survival of these cells (96). This study and similar studies have convincingly reinforced the idea of targeting GlxI as an antiproliferative and proapoptotic therapeutic for inhibition of tumor growth (95,96,101). In prostate carcinoma cell line PC-3, apoptosis ensued after an exposure to $\geq$3 mM methylglyoxal primarily due to cell cycle arrest at G1 along with lowering of glycolytic rates (102). Similar G1 cell cycle arrests have been observed in human leukemia-60 cells. Here, the inhibition was mediated through formation of DNA crosslinks and replication inhibition by methylglyoxal (101). High methylglyoxal-mediated cell cycle arrests mostly at G2/M checkpoint phase occurred due to Chk1 and Chk2 (checkpoint kinase 1 and 2) activation. Dicarbonyl metabolite-induced cell cycle arrests are speculated to be due to inhibition of cytokinesis by cytoskeletal protein damage (103). Contrary to the above findings, methylglyoxal-modified Hsp27 protein was also found to suppress cytochrome *c*-mediated caspase activation and apoptosis (27).

Apart from controlling tumor progression, GlxI pathway also seems to present a viable drug target in parasites. The emergence of multidrug resistance in parasites has put a constant need to develop newer drugs that target unique and exclusive parasite pathways which are different from host pathways. In protozoans such as Trypanosomatids and *Leishmania*, Glx I uses trypanothione as the thiol cofactor for methylglyoxal detoxification instead of GSH and thus presents an exclusive target for inhibition. Furthermore, with abnormally high glycolytic rates, Trypanosomatids and *Leishmania* favor the idea of methylglyoxal-induced growth inhibition brought about by selective GlxI inhibition (reviewed extensively by Wyllie and Fairlamb; see ref. (104). Malarial parasite *Plasmodium falciparum* trophozoite stage undergoes extensive glycolysis, and it was reported that infected erythrocytes secrete 30 times higher D-lactate (detoxified product of methylglyoxal) than uninfected RBCs (105). The high level of methylglyoxal turnover in the infected cells makes this pathway distinct and targetable. Apart from this classical pathway, there have been speculations regarding methylglyoxal reductase of *Plasmodium*, also befitting as a drug target (105).

Conclusion

Investigations on the bearings of methylglyoxal pathway in microbial infections of host cells are rare and limited. As far as methylglyoxal metabolism in prokaryotic cells goes, literature mainly discusses the various synthetic and degradative pathways of methylglyoxal metabolism under particular culture conditions, meanwhile focusing less on the events of the same during host pathogen interactions or in infection scenarios. Some of the vital questions which still remain mostly unanswered are whether a host cell environment is capable of inducing methylglyoxal generation in intracellular pathogens, whether host cells themselves produce methylglyoxal to curb microbial activities or their proliferation, whether methylglyoxal production by bacterial pathogens has the ability to modulate host signaling pathways to the pathogen's advantage, and so forth. As of date, very limited amount of data exist to answer these queries; however, understanding methylglyoxal metabolism in microbial pathogenesis and the related signaling events would open up new horizons of therapeutic developments.

References

[1] Cooper, R. A. (1984) Metabolism of methylglyoxal in microorganisms. Annu. Rev. Microbiol. 38, 49–68.

[2] Kalapos, M. P. (1999) Methylglyoxal in living organisms: chemistry, biochemistry, toxicology and biological implications. Toxicol. Lett. 110, 145–175.

[3] Hopper, D. J., and Cooper, R. A. (1972) The purification and properties of *Escherichia coli* methylglyoxal synthase. Biochem. J. 128, 321–329.

[4] Meyerhof, O., and Lohmann, K. (1934) Enzymic equilibrium reaction between hexosediphosphate and dihydroxyacetone-phosphate. Biochem. Z. 271, 89–110.
[5] Cooper, R. A., and Anderson, A. (1970) The formation and catabolism of methylglyoxal during glycolysis in Escherichia coli. FEBS Lett. 11, 273–276.
[6] Cooper, R. A. (1974) Methylglyoxal formation during glucose catabolism by Pseudomonas saccharophila. Eur. J. Biochem. 44, 81–86.
[7] Tsai, P. K., and Gracy, R. W. (1976) Isolation and characterization of crystalline methylglyoxal synthetase from Proteus vulgaris. J. Biol. Chem. 251, 364–367.
[8] Rizza, V., and Hu, A. S. L. (1973) Gluconate metabolism in pseudomonas: a novel pathway of glyceraldehyde-3-phosphate metabolism. Biochem. Biophys. Res. Commun. 54, 168–175.
[9] Neuberg, C. (1931) The destruction of lactic aldehyde and methylglyoxal by animal organisms. Biochem. Z. 49, 502–506.
[10] Dakin, H. D., and Dudley, H. W. (1913) An enzyme concerned with the formation of hydroxy acids from ketonic aldehydes. J. Biol. Chem. 14, 155–157.
[11] Thornalley, P. J. (1990) The glyoxalase system: new developments towards functional characterization of a metabolic pathway fundamental to biological life. Biochem. J. 269, 1–11.
[12] Kline, E. S., and Mahler, H. R. (1965) The lactic dehydrogenases of E. coli. Ann. NY Acad. Sci. 119, 905–919.
[13] Ferguson, G. P., Totemeyer, S., MacLean, M. J., and Booth, I. R. (1998) Methylglyoxal production in bacteria: suicide or survival? Arch. Microbiol. 170, 209–218.
[14] Booth, I. R., Ferguson, G. P., Miller, S., Li, C., Gunasekera, B., et al. (2003) Bacterial production of methylglyoxal: a survival strategy or death by misadventure? Biochem. Soc. Trans. 31, 1406–1408.
[15] Elliott, W. H. (1959) Amino-acetone: its isolation and role in metabolism. Nature 183, 1051–1052.
[16] Elliott, W. H. (1958) A new threonine metabolite. Biochim. Biophys. Acta 29, 446–447.
[17] Elliott, W. H. (1960) Methylglyoxal formation from aminoacetone by ox plasma. Nature 185, 467–468.
[18] Lowe, D. A., and Turner, D. J. M. (1970) Microbial metabolism of amino ketones: D-1-aminopropan-2-ol and aminoacetone metabolism in *Escherichia coli*. J. Gen. Microbiol. 63, 49–61.
[19] Neuberger, A., and Tait, G. H. (1962) Production of aminoacetone by Rhodopseudomonas spheroides. Biochem. J. 84, 317–328.
[20] Green, M. L., and Elliott, W. H. (1964) The enzymic formation of aminoacetone from threonine and its further metabolism. Biochem. J. 92, 537–549.
[21] Green, M. L., and Lewis, J. B. (1968) The oxidation of aminoacetone by a species of Arthrobacter. Biochem. J. 106, 267–270.
[22] Kalapos, M. P. (1994) Methylglyoxal toxicity in mammals. Toxicol. Lett. 73, 3–24.
[23] Thornalley, P. J. (1994) Methylglyoxal, glyoxalases and the development of diabetic complications. Amino Acids 6, 15–23.
[24] Thornalley, P. J. (1996) Pharmacology of methylglyoxal: formation, modification of proteins and nucleic acids, and enzymatic detoxification—a role in pathogenesis and antiproliferative chemotherapy. Gen. Pharmacol. 27, 565–573.
[25] Lo, T. W. C., Selwood, T., and Thornalley, P. J. (1994) The reaction of methylglyoxal with aminoguanidine under physiological conditions and prevention of methylglyoxal binding to plasma proteins. Biochem. Pharmacol. 48, 1865–1870.
[26] Padayatti, P. S., Jiang, C., Glomb, M. A., Uchida, K., and Nagaraj, R. H. (2001) High concentrations of glucose induce synthesis of argpyrimidine in retinal endothelial cells. Curr. Eye Res. 23, 106–115.
[27] Sakamoto, H., Mashima, T., Yamamoto, K., and Tsuruo, T. (2002) Modulation of heat-shock protein 27 (Hsp27) anti-apoptotic activity by methylglyoxal modification. J. Biol. Chem. 277, 45770–45775.
[28] Antognelli, C., Mezzasoma, L., Fettucciari, K., and Talesa, V. N. (2013) A novel mechanism of methylglyoxal cytotoxicity in prostate cancer cells. Int. J. Biochem. Cell Biol. 45, 836–844.
[29] Antognelli, C., Palumbo, I., Aristei, C., and Talesa, V. N. (2014) Glyoxalase I inhibition induces apoptosis in irradiated MCF-7 cells via a novel mechanism involving Hsp27, p53 and NF-κB. Br. J. Cancer 111, 395–406.
[30] Rabbani, N., and Thornalley, P. J. (2012) Methylglyoxal, glyoxalase 1 and the dicarbonyl proteome. Amino Acids 42, 1133–1142.
[31] Thornalley, P. J. (2003) Glyoxalase I—structure, function and a critical role in the enzymatic defence against glycation. Biochem. Soc. Trans. 31, 1343–1348.
[32] Thornalley, P. J., Langborg, A., and Minhas, H. S. (1999) Formation of glyoxal, methylglyoxal and 3-deoxyglucosone in the glycation of proteins by glucose. Biochem. J. 344, 109–116.
[33] Desai, K. M., Chang, T., Wang, H., Banigesh, A., Dhar, A., et al. (2010) Oxidative stress and aging: is methylglyoxal the hidden enemy? Can. J. Physiol. Pharmacol. 88, 273–284.
[34] Park, Y. S., Koh, Y. H., Takahashi, M., Miyamoto, Y., Suzuki, K., et al. (2003) Identification of the binding site of methylglyoxal on glutathione peroxidase: methylglyoxal inhibits glutathione peroxidase activity via binding to glutathione binding sites Arg 184 and 185. Free Radic. Res. 37, 205–211.
[35] Murata-Kamiya, N., and Kamiya, H. (2001) Methylglyoxal, an endogenous aldehyde, crosslinks DNA polymerase and the substrate DNA. Nucleic Acids Res. 29, 3433–3438.
[36] Tu, C.-Y., Chen, Y.-F., Lii, C.-K., and Wang, T.-S. (2013) Methylglyoxal induces DNA crosslinks in ECV304 cells via a reactive oxygen species-independent protein carbonylation pathway. Toxicol. Vitr. 27, 1211–1219.
[37] Brambilla, G., Sciabà, L., Faggin, P., Finollo, R., Bassi, A.M., et al. (1985) Methylglyoxal-induced DNA–protein cross-links and cytotoxicity in Chinese hamster ovary cells. Carcinogenesis 6, 683–686.
[38] Kang, J. H. (2003) Oxidative damage of DNA induced by methylglyoxal in vitro. Toxicol. Lett. 145, 181–187.
[39] Murata-Kamiya, N., Kamiya, H., Kaji, H., and Kasai, H. (2000) Methylglyoxal induces G:C to C:G and G:C to T:A transversions in the supF gene on a shuttle vector plasmid replicated in mammalian cells. Mutat. Res. 468, 173–182.
[40] Russell, J. B. (1998) Strategies that ruminal bacteria use to handle excess carbohydrate. J. Anim. Sci. 76, 1955–1963.
[41] Baskaran, S., Rajan, D. P., and Balasubramanian, K. A. (1989) Formation of methylglyoxal by bacteria isolated from human faeces. J. Med. Microbiol. 28, 211–215.
[42] Freedberg, W. B., Kistler, W. S., and Lin, E. C. (1971) Lethal synthesis of methylglyoxal by *Escherichia coli* during unregulated glycerol metabolism. J. Bacteriol. 108, 137–144.
[43] Kadner, R. J., Murphy, G. P., and Stephens, C. M. (1992) Two mechanisms for growth inhibition by elevated transport of sugar phosphates in Escherichia coli. J. Gen. Microbiol. 138, 2007–2014.
[44] Ackerman, R. S., Cozzarelli, N. R., and Epstein, W. (1974) Accumulation of toxic concentrations of methylglyoxal by wild-type *Escherichia coli* K-12. J. Bacteriol. 119, 357–362.
[45] Kim, I., Kim, E., Yoo, S., Shin, D., Min, B., et al. (2004) Ribose utilization with an excess of mutarotase causes cell death due to accumulation of methylglyoxal. J. Bacteriol. 186, 7229–7235.
[46] Hopper, D. J., and Cooper, R. A. (1971) The regulation of *Escherichia coli* methylglyoxal synthase; a new control site in glycolysis? FEBS Lett. 13, 213–216.
[47] Neuberger, A., and Tait, G. H. (1960) The enzymic conversion of threonine to aminoacetone. Biochim. Biophys. Acta 41, 164–165.
[48] Rahhal, D. A., Turner, J. M., and Willetts, A. J. (1967) The role of aminoacetone in L-threonine metabolism by *Bacillus subtilis*. Biochem. J. 103, 73P.
[49] Willetts, A. J., and Turner, J. M. (1970) Threonine metabolism in a strain of *Bacillus subtilis*: enzymes acting on methylglyoxal. Biochim. Biophys. Acta 222, 668–670.
[50] Willetts, A. J., and Turner, J. M. (1970) Threonine metabolism in a strain of Bacillus subtilis. Biochem. J. 117, 27P–28P.
[51] Murata, K., Saikusa, T., Fukuda, Y., Watanabe, K., Inoue, Y., et al. (1986) Metabolism of 2-oxoaldehydes in yeasts. Possible role of glycolytic bypath as a detoxification system in L-threonine catabolism by *Saccharomyces cerevisiae. Eur.* J. Biochem. 157, 297–301.

[52] Chakraborty, S., Chaudhuri, D., Balakrishnan, A., and Chakravortty, D. (2014) Salmonella methylglyoxal detoxification by STM3117 encoded lactoylglutathione lyase affects virulence in coordination with SPI-2 and phagosomal acidification. Microbiology 160, 1999–2017.
[53] Ray, S., and Ray, M. (1983) Formation of methylglyoxal from aminoacetone by amine oxidase from goat plasma. J. Biol. Chem. 258, 3461–3462.
[54] Ray, M., and Ray, S. (1987) Aminoacetone oxidase from goat liver. Formation of methylglyoxal from aminoacetone. J. Biol. Chem. 262, 5974–5977.
[55] Lyles, G. A., and Chalmers, J. (1992) The metabolism of aminoacetone to methylglyoxal by semicarbazide-sensitive amine oxidase in human umbilical artery. Biochem. Pharmacol. 43, 1409–1414.
[56] Sartori, A., Garay-Malpartida, H. M., Forni, M. F., Schumacher, R. I., Dutra, F., et al. (2008) Aminoacetone, a putative endogenous source of methylglyoxal, causes oxidative stress and death to insulin-producing RINm5f cells. Chem. Res. Toxicol. 21, 1841–1850.
[57] Casazza, J. P., Felver, M. E., and Veech, R. L. (1984) The metabolism of acetone in rat. J. Biol. Chem. 259, 231–236.
[58] Bondoc, F. Y., Bao, Z., Hu, W. Y., Gonzalez, F. J., Wang, Y., et al. (1999) Acetone catabolism by cytochrome P450 2E1: studies with CYP2E1-null mice. Biochem. Pharmacol. 58, 461–463.
[59] Johansson, I., Eliasson, E., Norsten, C., and Ingelman-Sundberg, M. (1986) Hydroxylation of acetone by ethanol- and acetone-inducible cytochrome P-450 in liver microsomes and reconstituted membranes. FEBS Lett. 196, 59–64.
[60] Negre-Salvayre, A., Coatrieux, C., Ingueneau, C., and Salvayre, R. (2008) Advanced lipid peroxidation end products in oxidative damage to proteins. Potential role in diseases and therapeutic prospects for the inhibitors. Br. J. Pharmacol. 153, 6–20.
[61] Suttisansanee, U., and Honek, J. F. (2011) Bacterial glyoxalase enzymes. Semin. Cell Dev. Biol. 22, 285–292.
[62] Sukdeo, N., and Honek, J. F. (2007) *Pseudomonas aeruginosa* contains multiple glyoxalase I-encoding genes from both metal activation classes. Biochim. Biophys. Acta 1774, 756–763.
[63] Sukdeo, N., Clugston, S. L., Daub, E., and Honek, J. F. (2004) Distinct classes of glyoxalase I: metal specificity of the *Yersinia pestis, Pseudomonas aeruginosa* and *Neisseria meningitidis* enzymes. Biochem. J. 384, 111–117.
[64] Stamp, A. L., Owen, P., El Omari, K., Nichols, C. E., Lockyer, M., et al. (2010) Structural and functional characterization of *Salmonella enterica serovar Typhimurium* YcbL: an unusual type II glyoxalase. Protein Sci. 19, 1897–1905.
[65] Ko, J., Kim, I., Yoo, S., Min, B., Kim, K., et al. (2005) Conversion of methylglyoxal to acetol by *Escherichia coli* aldo-keto reductases. J. Bacteriol. 187, 5782–5789.
[66] Anil Kumar, P., and Bhanuprakash Reddy, G. (2007) Focus on molecules: aldose reductase. Exp. Eye Res. 85, 739–740.
[67] Vander Jagt, D. L., Hassebrook, R. K., Hunsaker, L. A., Brown, W. M., and Royer, R. E. (2001) Metabolism of the 2-oxoaldehyde methylglyoxal by aldose reductase and by glyoxalase-I: roles for glutathione in both enzymes and implications for diabetic complications. Chem. Biol. Interact. 130–132, 549–562.
[68] Xu, D., Liu, X., Guo, C., and Zhao, J. (2006) Methylglyoxal detoxification by an aldo-keto reductase in the cyanobacterium Synechococcus sp. PCC 7002. Microbiology 152, 2013–2021.
[69] Aguilera, J., and Prieto, J. A. (2001) The *Saccharomyces cerevisiae* aldose reductase is implied in the metabolism of methylglyoxal in response to stress conditions. Curr. Genet. 39, 273–283.
[70] Murata, K., Inoue, Y., Rhee, H., and Kimura, A. (1989) 2-Oxoaldehyde metabolism in microorganisms. Can. J. Microbiol. 35, 423–431.
[71] Maeta, K., Izawa, S., Okazaki, S., Kuge, S., and Inoue, Y. (2004) Activity of the Yap1 transcription factor in *Saccharomyces cerevisiae* is modulated by methylglyoxal, a metabolite derived from glycolysis. Mol. Cell. Biol. 24, 8753–8764.
[72] Maeta, K., Izawa, S., and Inoue, Y. (2005) Methylglyoxal, a metabolite derived from glycolysis, functions as a signal initiator of the high osmolarity glycerol-mitogen-activated protein kinase cascade and calcineurin/Crz1-mediated pathway in Saccharomyces cerevisiae. J. Biol. Chem. 280, 253–260.
[73] Fiory, F., Lombardi, A., Miele, C., Giudicelli, J., Beguinot, F., et al. (2011) Methylglyoxal impairs insulin signalling and insulin action on glucose-induced insulin secretion in the pancreatic β-cell line INS-1E. Diabetologia 54, 2941–2952.
[74] Campbell, A. K., Matthews, S. B., Vassel, N., Cox, C. D., Naseem, R., et al. (2010) Bacterial metabolic 'toxins': a new mechanism for lactose and food intolerance, and irritable bowel syndrome. Toxicology 278, 268–276.
[75] Hadas, K., Randriamboavonjy, V., Elgheznawy, A., Mann, A., and Fleming, I. (2013) Methylglyoxal induces platelet hyperaggregation and reduces thrombus stability by activating PKC and inhibiting PI3K/Akt pathway. PLoS One 8, e74401.
[76] Campbell, A. K., Naseem, R., Holland, I. B., Matthews, S. B., and Wann, K. T. (2007) Methylglyoxal and other carbohydrate metabolites induce lanthanum-sensitive Ca^{2+} transients and inhibit growth in *E. coli*. *Arch*. Biochem. Biophys. 468, 107–113.
[77] de Arriba, S. G., Stuchbury, G., Yarin, J., Burnell, J., Loske, C., et al. (2007) Methylglyoxal impairs glucose metabolism and leads to energy depletion in neuronal cells—protection by carbonyl scavengers. Neurobiol. Aging 28, 1044–1050.
[78] Kilty, S. J., Duval, M., Chan, F. T., Ferris, W., and Slinger, R. (2011) Methylglyoxal: (active agent of Manuka honey) in vitro activity against bacterial biofilms. Int. Forum Allergy Rhinol. 1, 348–350.
[79] Daglia, M., Ferrari, D., Collina, S., and Curti, V. (2013) Influence of in vitro simulated gastroduodenal digestion on methylglyoxal concentration of Manuka (*Lectospermum scoparium*) honey. J. Agric. Food Chem. 61, 2140–2145.
[80] Sharma, A. (2010) Virulence mechanisms of Tannerella forsythia. Periodontology 2000 54, 106–116.
[81] Kashket, S., Maiden, M. F., Haffajee, A. D., and Kashket, E. R. (2003) Accumulation of methylglyoxal in the gingival crevicular fluid of chronic periodontitis patients. J. Clin. Periodontol. 30, 364–367.
[82] Rachman, H., Kim, N., Ulrichs, T., Baumann, S., Pradl, L., et al. (2006) Critical role of methylglyoxal and AGE in mycobacteria-induced macrophage apoptosis and activation. PLoS One 1, e29.
[83] Eskra, L., Canavessi, A., Carey, M., and Splitter, G. (2001) *Brucella abortus* genes identified following constitutive growth and macrophage infection. Infect. Immun. 69, 7736–7742.
[84] Korithoski, B., Levesque, C. M., and Cvitkovitch, D. G. (2007) Involvement of the detoxifying enzyme lactoylglutathione lyase in *Streptococcus mutans* aciduricity. J. Bacteriol. 189, 7586–7592.
[85] Periaswamy, B., Maier, L., Vishwakarma, V., Slack, E., Kremer, M., et al. (2012) Live attenuated *S.* Typhimurium vaccine with improved safety in immuno-compromised mice. PLoS One 7, e45433.
[86] Klitgaard, K., Friis, C., Jensen, T. K., Angen, O., and Boye, M. (2012) Transcriptional portrait of *Actinobacillus pleuropneumoniae* during acute disease—potential strategies for survival and persistence in the host. PLoS One 7, e35549.
[87] Cohen-Or, I., Katz, C., and Ron, E. Z. (2011) AGEs secreted by bacteria are involved in the inflammatory response. PLoS One 6, e17974.
[88] Ott, C., Jacobs, K., Haucke, E., Navarrete Santos, A., Grune, T., et al. (2014) Role of advanced glycation end products in cellular signaling. Redox Biol. 2, 411–429.
[89] Brownlee, M. (2001) Biochemistry and molecular cell biology of diabetic complications. Nature 414, 813–820.
[90] Berner, A. K., Brouwers, O., Pringle, R., Klaassen, I., Colhoun, L., et al. (2012) Protection against methylglyoxal-derived AGEs by regulation of glyoxalase 1 prevents retinal neuroglial and vasodegenerative pathology. Diabetologia 55, 845–854.
[91] Mason, R. M., and Wahab, N. A. (2003) Extracellular matrix metabolism in diabetic nephropathy. J. Am. Soc. Nephrol. 14, 1358–1373.
[92] Forbes, J. M., Cooper, M. E., Oldfield, M. D., and Thomas, M. C. (2003) Role of advanced glycation end products in diabetic nephropathy. J. Am. Soc. Nephrol. 14, S254–S258.

[93] Chau, D. L., and Edelman, S. V. (2002) Osteoporosis and diabetes. Clin. Diabetes 20, 153–157.
[94] Koppenol, W. H., Bounds, P. L., and Dang, C. V. (2011) Otto Warburg's contributions to current concepts of cancer metabolism. Nat. Rev. Cancer. 11, 325–337.
[95] Figarola, J. L., Singhal, J., Rahbar, S., Awasthi, S., and Singhal, S. S. (2014) LR-90 prevents methylglyoxal-induced oxidative stress and apoptosis in human endothelial cells. Apoptosis 19, 776–788.
[96] Hu, X., Yang, X., He, Q., Chen, Q., and Yu, L. (2014) Glyoxalase 1 is up-regulated in hepatocellular carcinoma and is essential for HCC cell proliferation. Biotechnol. Lett. 36, 257–263.
[97] Taniguchi, H., Horinaka, M., Yoshida, T., Yano, K., Goda, A. E., et al. (2012) Targeting the glyoxalase pathway enhances TRAIL efficacy in cancer cells by downregulating the expression of antiapoptotic molecules. Mol. Cancer Ther. 11, 2294–2300.
[98] Zou, X. Y., Ding, D., Zhan, N., Liu, X. M., Pan, C., et al. Glyoxalase I is differentially expressed in cutaneous neoplasms and contributes to the progression of squamous cell carcinoma. J. Invest. Dermatol. doi:10.1038/jid.2014.377 [Epub ahead of print].
[99] Wang, Y., Kuramitsu, Y., Ueno, T., Suzuki, N., Yoshino, S., et al. (2012) Glyoxalase I (GLO1) is up-regulated in pancreatic cancerous tissues compared with related non-cancerous tissues. Anticancer Res. 32, 3219–3222.
[100] Okado, A., Kawasaki, Y., Hasuike, Y., Takahashi, M., Teshima, T., et al. (1996) Induction of apoptotic cell death by methylglyoxal and 3-deoxyglucosone in macrophage-derived cell lines. Biochem. Biophys. Res. Commun. 225, 219–224.
[101] Kang, Y., Edwards, L. G., and Thornalley, P. J. (1995) Effect of methylglyoxal on human leukaemia 60 cell growth: modification of DNA, G1 growth arrest and induction of apoptosis. Leuk. Res. 20, 397–405.
[102] Milanesa, D. M., Choudhury, M. S., Mallouh, C., Tazaki, H., and Konno, S. (2000) Methylglyoxal-induced apoptosis in human prostate carcinoma: potential modality for prostate cancer treatment. Eur. Urol. 37, 728–734.
[103] Sejersen, H., and Rattan, S. I. (2009) Dicarbonyl-induced accelerated aging in vitro in human skin fibroblasts. Biogerontology 10, 203–211.
[104] Wyllie, S., and Fairlamb, A. H. (2011) Methylglyoxal metabolism in Trypanosomes and Leishmania. Semin. Cell Dev. Biol. 22, 271–277.
[105] Urscher, M., Alisch, R., and Deponte, M. (2011) The glyoxalase system of malaria parasites—implications for cell biology and general glyoxalase research. Semin. Cell Dev. Biol. 22, 262–270.