METHYLGLYOXAL AND GLUCOSE METABOLISM: A HISTORICAL PERSPECTIVE AND FUTURE AVENUES FOR RESEARCH

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SUMMARY

Methylglyoxal, an α-oxoaldehyde discovered in the 1880s, has had a hectic scientific career, at times being considered of fundamental importance and at other times viewed as playing a very subordinate role. Much has been learned about methylglyoxal, but the function of its production in the metabolic machinery is still unknown. This paper gives an overview of the changing role of methylglyoxal from a historical aspect and arrives at the conclusion that methylglyoxal is tightly bound to glycolysis from an evolutionary perspective, its production therefore being inevitable. It is not situated in the main stream of the glycolytic sequence, but a role can be assigned to its production in the phosphate supply of operating glycolysis in some prokaryotes and yeast under conditions of phosphate deficiency. This function is presumed to be performed by the enzyme methylglyoxal synthase, which is specialized for the conversion of dihydroxyacetone-phosphate to methylglyoxal. However, it is still unknown whether this enzyme and this kind of regulation also exist in animals.

KEY WORDS

methylglyoxal, dihydroxyacetone-phosphate, glycolysis, methylglyoxal synthase, evolution

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INTRODUCTION

If the word methylglyoxal is typed into the search program of Medline, a clear picture is obtained of the increasing number of papers dealing with its biochemistry and toxicity, reflecting the revival of interest in this 2-oxoaldehyde.

Methylglyoxal research may be undergoing a renaissance, but the metabolic role of this compound remains unknown. This question is highly pertinent if it is considered that methylglyoxal is inevitably produced in the course of metabolism, and as an electrophile it is considered to exert toxicity (its toxic effect has recently been disputed /1/) and to play a role in various pathological events (for review see /2,3/). In this context, it is particularly interesting to pose the questions of why methylglyoxal is produced in the metabolic machinery and what is its role in metabolism.

This paper presents a historical survey of methylglyoxal metabolism, and discusses the different views concerning its metabolic place and its significance in the biochemical machinery. It is shown that methylglyoxal is situated at the core of metabolism, even if its role has changed during evolution.

THE DISCOVERY OF METHYLGLYOXAL

Methylglyoxal was first prepared in 1887 by von Pechmann, who warmed isonitrosoacetone with dilute sulfuric acid /4/. By the end of the 19th century, the work of Baumann led to the finding that methylglyoxal, similarly to other aldehydes and ketones, interacted with thiols /5/.

THE ADVENT OF METHYLGLYOXAL BIOCHEMISTRY - AN INTERMEDIATE OF GLUCOSE BREAKDOWN

By the beginning of the 20th century, energy production in living systems had become one of the most intriguing questions in biochemistry. At that time it emerged that the fermentation of glucose yielded lactate /6-8/. In 1913, glyoxalase, then believed to be only one enzyme, was discovered independently by Dakin and Dudley /9/ and Neuberg /10/. This enzyme proved capable of converting α-oxoaldehydes into their α-hydroxycarboxylic acid counterparts and methyl-

glyoxal turned out to be such a substrate /9,10/. Glutathione was identified as a cofactor for glyoxalase(s) by Lohmann /11/. This was corroborated by Jowett and Quastel /12/. It later emerged that glyoxalase was not a single enzyme, but rather two enzymes, designated glyoxalase I and II /13-15/. Glyoxalase I (*S*-lactoylglutathione methlylglyoxal lyase; E.C.4.4.1.5) acts on the adduct of methylglyoxal and glutathione, catalysing the one-substrate isomerization of the hemimercaptal to *S*-D-lactoylglutathione, while glyoxalase II (*S*-2-hydroxyacylglutathione hydrolase; E.C.3.1.2.6) splits *S*-D-lactoylglutathione into D-lactate and glutathione (Fig. 1) /15-17/.

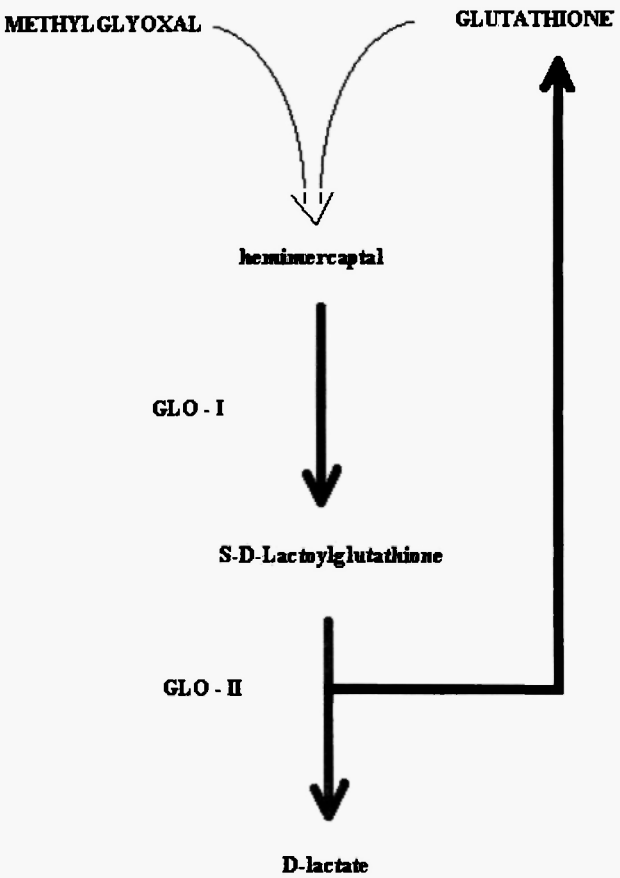

Fig. 1: The function of glyoxalases.

The discovery of the glyoxalases and the realization of their ubiquitous nature in species and tissues /8/ initiated a search for a biological function. As glycolysis results in lactate production and the glyoxalases also yielded lactate, it seemed promising to fit the glyoxalases into the biochemical machinery of glycolysis, with methylglyoxal as an intermediate in this route /18/. The idea of methylglyoxal as a glycolytic intermediate received support from the repeated identification of methylglyoxal among the products of alcoholic fermentation /19/. A metabolic sequence of alcoholic fermentation starting from glucose was postulated by Neuberg and Kerb, with methylglyoxal situated in a crucial position in the pathway (Fig. 2) /19/.

Hence, in the 1920s, the methylglyoxalase route was considered a part of glycolysis, and until the development of the Embden-Meyerhof scheme of glucose breakdown in the period 1932-1939, methylglyoxal was presumed to function in the main stream of glycolysis leading to lactate formation /20-30/. This opinion was widely held, despite the conclusion by Foster from experiments with rabbit muscle that methylglyoxal was unlikely to be the precursor for the lactate formed in the course of glycolysis /31/. This product of glycolysis was L-lactate, whereas the glyoxalases produced D-lactate /31/. Nevertheless, this was not regarded as clear-cut evidence since the conversion of D-lactate to L-lactate could not be excluded. The suspicion was strengthened, however, by two further findings: tissues readily utilized L-lactate, but not D-lactate /32/; and, although methylglyoxal was converted to lactate more rapidly than sugars, lactate formation from

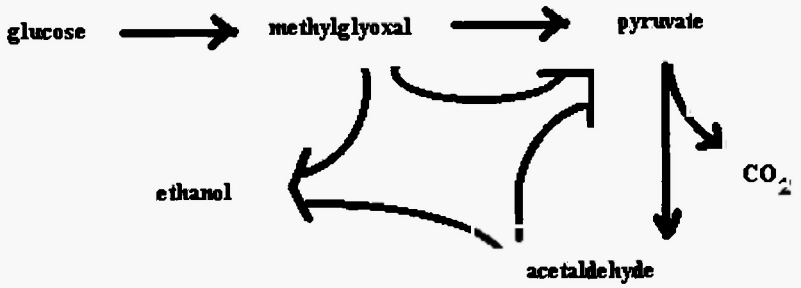

Fig. 2: Sequence for alcoholic fermentation as suggested by Neuberg and Kerb /19/.

methylglyoxal was not inhibited by respiration, whereas its formation from glucose was fairly sensitive to such inhibition /33/.

The major breakthrough came in the 1930s, with an ever greater number of publications opposing the concept that methylglyoxal was in the main stream of glycolysis. The most crucial finding that led to the rejection of the glycolytic role of the glyoxalases and methylglyoxal came from the experiments of Lohmann, who demonstrated that NAD^+ was the agent that restored the glycolytic activity of dialyzed muscle extracts, while reduced glutathione, the coenzyme for the glyoxalases, was ineffective /11/. As the enzymatic conversion of triose-phosphates to methylglyoxal was not proved, but its non-enzymatic production from triose-phosphates did occur in mixtures in which it was tested in the presence of trichloracetic acid, it was suggested that methylglyoxal was an experimental byproduct due to the assay conditions /34/. It was, therefore, concluded that the glyoxalases did not lie in the main route of metabolism and that methylglyoxal was not an intermediate of phosphorylating glycolysis /7,34/.

When the central position was established for the Embden-Meyerhof pathway in the metabolism of glucose, a merely subordinate role was assigned to methylglyoxal in living cells /7/, and interest in the glyoxalases and methylglyoxal faded away for several decades.

THE FORGOTTEN MATERIAL WITH A SUBORDINATE ROLE

The view that methylglyoxal played only a relatively minor role in metabolism persisted, despite the fact that methylglyoxal re-emerged in relation to carbohydrate metabolism in the 1950s. In particular, Entner and Doudoroff tried to revive the relationship between methylglyoxal and glucose catabolism, for methylglyoxal appeared as a product of glyceraldehyde 3-phosphate degradation in the course of the oxidative assimilation of glucose under experimental conditions in *Pseudomonas saccharophilia* /35/. However, at that time, this attempt to link methylglyoxal to carbohydrate metabolism was again unsuccessful. Nevertheless, it is now widely accepted that there are three major pathways of sugar catabolism: the Embden-Meyerhof scheme, the Entner-Doudoroff pathway, and the hexose-monophosphate route (Fig. 3) (for a review, see /36/).

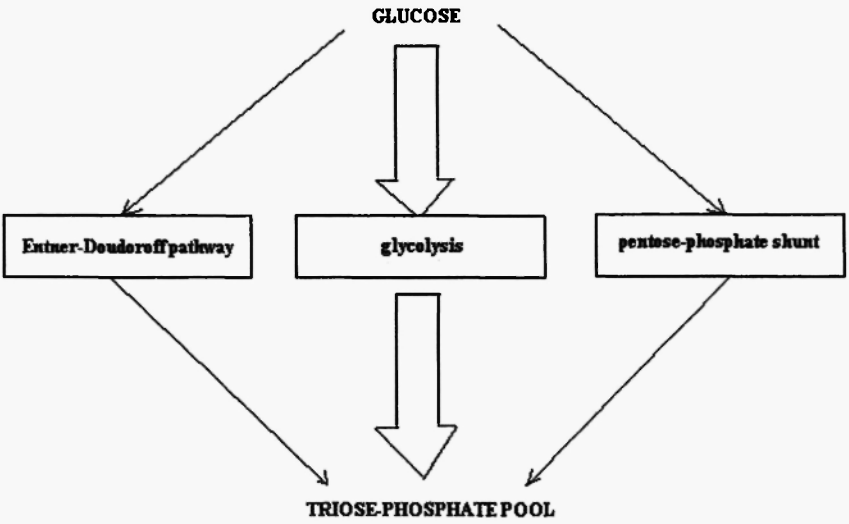

Fig. 3: The major pathways for glucose breakdown. It should be noted that approximately 98% of glucose is metabolized via the glycolytic sequence in the liver.

In the 1950s, some additional issues were addressed and papers appeared on methyglyoxal and beriberi /37-40/, and the activity of the glyoxalases and the presence of methylglyoxal in normal and in tumour tissues /41-43/. The antiviral activity of methylglyoxal was also tested /44,45/. Tests began on the antitumour activity of methylglyoxal in cultured tumor cell lines and in animals inoculated intraperitoneally with cancer cells, and by the mid-1960s the picture had changed.

METHYLGLYOXAL AND CANCER THERAPY

The idea that methylglyoxal can be used in cancer therapy was probably put forward first by French and Freedlander in 1958 /46/. This assumption attracted the attention of others /47,48/, and methylglyoxal research received considerable impetus from the work of Szent-Györgyi, who not only performed experiments to test the antitumour activity of methylglyoxal, but also developed a theory, the promine/retine theory, concerning cell division /49/. In 1965, Szent-

Györgyi first stressed that retine was a natural glyoxal derivative, promoting a hope for a solution to the old biochemical puzzle, the glyoxalase enigma, with a substrate and a function for this powerful enzyme system /50/. The major features of the concept are: (i) there are growth-inhibiting and -promoting factors in tissues; (ii) methylglyoxal or a derivative is the off-switch, retine; (iii) glyoxalase acts as an on-switch, promine; (iv) cancer cells are unable to bind the glyoxalases; (v) oxygen plays a role in carbonyl production and in this way in cell growth regulation; and (vi) the semiconductivity of proteins is a fundamental phenomenon in living organisms (for a review, see /51/).

This theory led to the postulation of differences in glyoxalase activity between neoplastic and normal tissues, tumours containing higher glyoxalase activities (at least for glyoxalase I). Moreover, the level of methylglyoxal in neoplastic tissues should be lower than in normal tissues, and elevation of the methylglyoxal concentration in cancerous cells by the addition of methylglyoxal or its derivatives or the inhibition of the action of glyoxalases should exert a therapeutic antiproliferative effect. However, the data furnished by experiments with tumour cells and tissues did not support the view of Szent-Györgyi (for a review, see /51/).

More data tend to disprove the promine/retine theory rather than to support it, and neither promine nor retine has ever been purified and identified, but this theory had the scientific merit that it focused attention on methylglyoxal and the glyoxalases. Thus, the theory of Szent-Györgyi gave a great impulse to methylglyoxal research and accordingly to investigations of the antitumour activity of methylglyoxal.

The effective antitumour activity of methylglyoxal has been documented both in cultured tumour cell lines /52-58/ and in animals (mainly in rodents) inoculated intraperitoneally with cancer cells in most of the studies /59-63/.

Contemporary research offers two alternative modes for the application of methylglyoxal in tumour treatment. One direction is to synthesize selective glyoxalase inhibitors, thereby elevating the intracellular levels of cytotoxic methylglyoxal and killing cancerous cells /64-69/. As expected, the glyoxalase I inhibitors exhibit antitumour activity both *in vitro* and *in vivo* /58,70-72/. Immunodominant regions of glyoxalase I have recently been recognized as possible antitumour immunomodulation targets /73/. Another strategy focuses on the

addition of methylglyoxal in either encapsulated or bound form, which is then liberated inside the body, to exert antitumour activity /74,75/. Such a methylglyoxal-based formulation has recently been tried in the treatment of human cancer patients /76/. It may also be noted that methylglyoxal *bis*(guanylhydrazone) is used in several combination chemotherapeutic protocols for patients with Hodgkin's and non-Hodgkin's lymphomas (for a review, see /77/).

HOPE CONCERNING AN OLD COMPOUND - AN INTERMEDIATE IN ACETONE METABOLISM

Since 1798, acetone has been known to be present in human breath, its occurrence in the human body mainly being regarded as a feature of diabetic coma /78,79/. However, it was thought to be a metabolic end-product, as it is metabolized poorly, if at all /80,81/. The participation of cytochrome P450-type enzymes in the breakdown of acetone was not recognized until 1980 /82/. Later, in 1984, Casazza and associates published a paper in which the pathways of acetone metabolism in the rat liver were described (Fig. 4) /83/. This put methylglyoxal in a new light as it was presumed to be an intermediate in acetone metabolism (for reviews, see /84,85/).

An important role is ascribed to the cytochrome P450 isozymes in acetone degradation as both the conversion of acetone to acetol, and its further conversion to methylglyoxal, are primarily catalysed by a cytochrome P450 isozyme, CYP2E1 (cytochrome P450 2E1) (for a review, see /86/). In rodents, Cyp2E1 (CYP2E1 in humans /87/) is responsible for about 90-95% of acetone monooxygenase activity /88/. It should be noted that this isozyme is induced by the treatment of animals with acetone and a diverse range of exogenous compounds, and also by fasting and chemically induced diabetes mellitus /86/. Moreover, acetone is considered to be not only an endogenous substrate for CYP2E1 gene products, but also their physiological inducer /86/. Since CYP2E1 is expressed in a wide variety of tissues, including the liver, kidney, testis, lung, spleen, pancreas, intestines, brain and vascular endothelial cells, the production of methylglyoxal from acetone can be expected in all of these tissues in acetonaemia, as acetone freely crosses biological membranes /89-96/. Ketosis involving the elevation of acetone levels occurs in various disorders (for reviews, see /97,98/), and diet-induced ketosis has been known as a

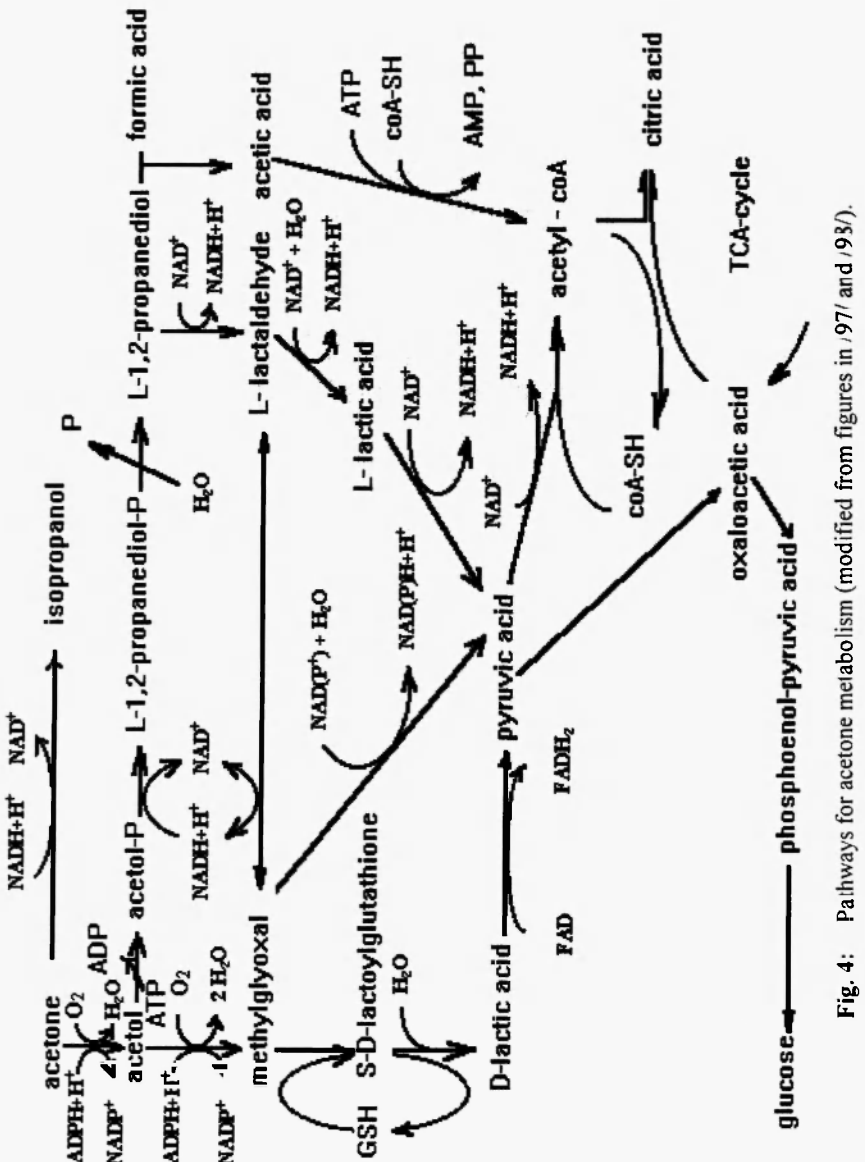

Fig. 4: Pathways for acetone metabolism (modified from figures in /97/ and /98/).

possible alimentary means of treatment of intractable seizures since 1921 (for a review, see /99/). Hence, it is only natural to presume a role for methylglyoxal in ketosis-related human pathology. Methylglyoxal was recently suggested to be implicated in the pathological events of diabetes mellitus, Alzheimer's disease and hypertension /100-102/. Interestingly, the antiseizure activity of a ketogenic diet has recently been hypothesized to be attributable to a methylglyoxal derivative, *S*-D-lactoylglutathione /103/.

PATHWAYS FOR METHYLGLYOXAL PRODUCTION AND DEGRADATION

A wide variety of reactions and pathways are currently known to be involved in the production or degradation of methylglyoxal (Fig. 5). Methylglyoxal is produced in the course of carbohydrate, lipid and amino acid metabolism, and both enzyme-catalysed steps and non-enzymatic reactions take part in this network (Fig. 5) (for reviews, see 2,3/). Carbohydrate metabolism-linked production depends strongly on the generation of triose-phosphates, which are converted to methylglyoxal either enzymatically or non-enzymatically. However, the extent of the triose-phosphate pool is not dependent exclusively on the rate of glycolysis, as the Entner-Doudoroff pathway and the hexose-monophosphate route also contribute to triose-phosphate generation (*vide supra*). The changes in the triose-phosphate pool are also linked to other factors, such as xylitol metabolism or the activity of α-glycerophosphate dehydrogenase (E.C.1.1.1.8.), by which glycerol breakdown is associated with methylglyoxal production. The non-enzymatic production of methylglyoxal through the glycation of macromolecules and the autoxidation of carbohydrates is likewise possible (for a review, see /104/). The sources of methylglyoxal production related to lipid metabolism are either enzymatic or non-enzymatic reactions in which glycerol (mentioned above), acetoacetate or acetone is converted to 1,2-dicarbonyl (Fig. 5). The metabolism of amino acids (mainly threonine and glycine, and to a certain extent tyrosine) is also connected with methylglyoxal formation, particularly under pathological conditions, but to an as yet unknown degree (for reviews, see /2,3,105/).

In whatever way methylglyoxal is generated, the main detoxifying route is the ubiquitous glyoxalase system (*vide supra*). Besides the

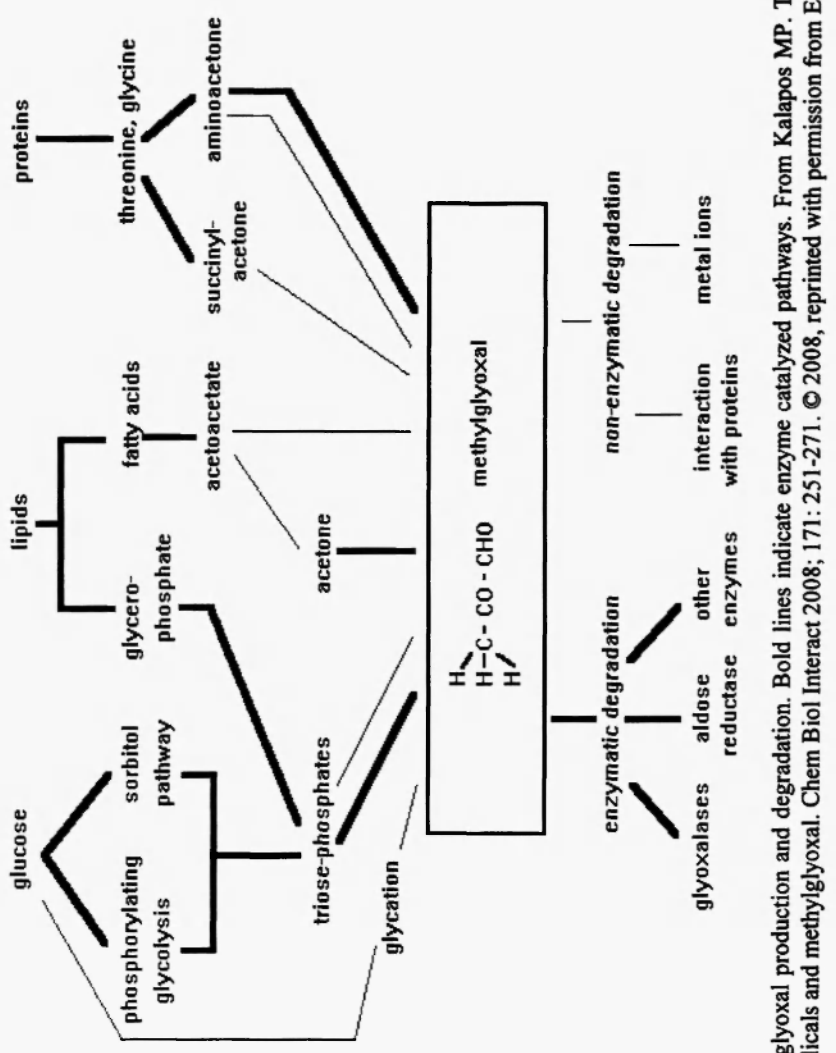

Fig. 5: Methylglyoxal production and degradation. Bold lines indicate enzyme catalyzed pathways. From Kalapos MP. The tandem of free radicals and methylglyoxal. Chem Biol Interact 2008; 171: 251-271. © 2008, reprinted with permission from Elsevier.

glyoxalase route, other enzymes also participate in the detoxification (Fig. 5) (for reviews, see /3,105/). Particular importance may be attached to aldose reductase (E.C.1.1.1.21), which functions as a ketone reductase in the presence of glutathione, in this way reducing methylglyoxal to lactaldehyde /106/. However, when the intracellular glutathione concentration is subnormal, the metabolic importance of aldose reductase in the disposal of 1,2-dicarbonyl exceeds that of the glyoxalase route and it catalyses the formation of acetol /106/. If this is so, then acetol can be converted back to methylglyoxal either by oxidation governed by CYP2E1, or by undergoing disproportionation in the presence of copper ions without the involvement of any enzyme /106/.

METHYLGLYOXAL PRODUCTION - EVOLUTIONARY ATAVISM OR AN OPPORTUNITY TO SUPPLY GLYCOLYSIS WITH PHOSPHATE LIBERATED FROM TRIOSE-PHOSPHATES

Over the years, though many hypotheses have been formulated to explain the ubiquitous nature of glyoxalases and to offer a role for methylglyoxal in metabolism (for a review, see /3/), none of them has provided a generally acceptable theory as to the part played by glyoxalases. A link between methylglyoxal production and glucose turnover appears most plausible, despite the possibility of its production by several other routes (Fig. 5). To support this proposal, two aspects are considered here, both associated to some degree with evolution.

The first is an early evolutionary aspect, discussed in a series of papers in the last decade /107-110/. The function of the methylglyoxal pathway is traced back to the era of prebiotic evolution by supposing that it might have served as an anaplerotic route for the reductive citric acid cycle /107/. Methylglyoxal would have been generated from trioses, glyceraldehyde and dihydroxyacetone, presumed to be intermediates in the formose cycle, with formaldehyde as a starting molecule /107/. An advantage of this hypothesis is that the network already fits carbohydrate metabolism in the early stage of evolution and hence it is not surprising that methylglyoxal is still ultimately bound to glycolysis in extant metabolism /107/. Furthermore, the emergence of energy-rich bonds arises from the concept in a plausible manner /107/. Of course, the metabolic significance of this route

changed during the evolutionary process, and this directs attention to the second aspect, the formation of methylglyoxal as a byproduct of extant glycolysis.

Considerable evidence has accumulated in support of the enzymatic conversion of glycolytic intermediates to methylglyoxal in cells. There are at least two enzymes that can catalyse the formation of methylglyoxal from triose-phosphates, and in both reactions triose-phosphates are hydrolysed to remove phosphate and yield methylglyoxal. In the triose-phosphate isomerase (E.C.5.3.1.1)-catalysed reaction, the enediolate intermediate of the isomerization releases phosphate and methylglyoxal is produced, while in the methylglyoxal synthase (E.C.4.2.99.11)-catalysed reaction dihydroxyacetone-phosphate is converted to methylglyoxal /111,112/. In the aldolase (E.C.4.1.2.13.) reaction a pyruvaldehyde-aldolase-orthophosphate complex is formed, which slowly breaks to release methylglyoxal and inorganic phosphate /113/. Two enzymes, triose-phosphate isomerase and aldolase, are well-known members of the glycolytic sequence, while methylglyoxal synthase does not participate in glycolysis. It now seems that methylglyoxal synthase alone is of biological significance in governing the conversion of dihydroxyacetone-phosphate to methylglyoxal; the other two enzymes produce methylglyoxal as a byproduct of catalysis /111-113/.

It may be recalled that, in the 1930s, one of the reasons for rejection of the idea of the central position of methylglyoxal in glucose breakdown was the failure to demonstrate the enzymatic conversion of triose-phosphates to methylglyoxal /34/. This view was held up to 1970, when an enzyme catalysing methylglyoxal formation from dihydroxyacetone-phosphate (an intermediate of glycolysis), methylglyoxal synthase, was identified in *E. coli* /114/. Shortly thereafter, the presence of this enzyme was also detected in Enterobacteriaceae and Enterobacteriaceae-like organisms, and additionally in aerobes and anaerobes (for a review, see /115/). Since then, this enzyme has also been found in halophilic Archaea, *Saccharomyces cerevisiae* and methylotrophic yeast /116-118/. Methylglyoxal synthase has been purified, characterized, sequenced and crystallized, and the gene encoding the enzyme has been identified, cloned and overexpressed /119-123/. The methylglyoxal synthase reaction liberates inorganic phosphate and leads to methylglyoxal formation:

$$\text{dihydroxyacetone-phosphate} \Rightarrow \text{methylglyoxal} + P_i \qquad (1)$$

The glyceraldehyde 3-phosphate dehydrogenase (E.C.1.2.1.12)-catalysed step of glycolysis,

$$\text{glyceraldehyde 3-phosphate} + NAD^+ + P_i \Rightarrow 1,3\text{-diphosphoglycerate} + NADH + H^+ \quad (2)$$

needs inorganic phosphate to proceed, and therefore the methylglyoxal synthase reaction has been suggested as a control site for glycolysis /124/. The physiological role assigned to the methylglyoxal synthase reaction would be to provide inorganic phosphate for the second stage of glycolysis when the cells are deficient in P_i /124/. The function governed by methylglyoxal synthase is shown in Figure 6.

Only one paper in the literature reports the presence of methylglyoxal synthase in animal tissues /125/. Since that enzyme was not purified to homogeneity, it is not absolutely certain that the conversion of dihydroxyacetone-phosphate to methylglyoxal in that extract was catalysed by methyglyoxal synthase and not by triose-phosphate isomerase contaminating the sample.

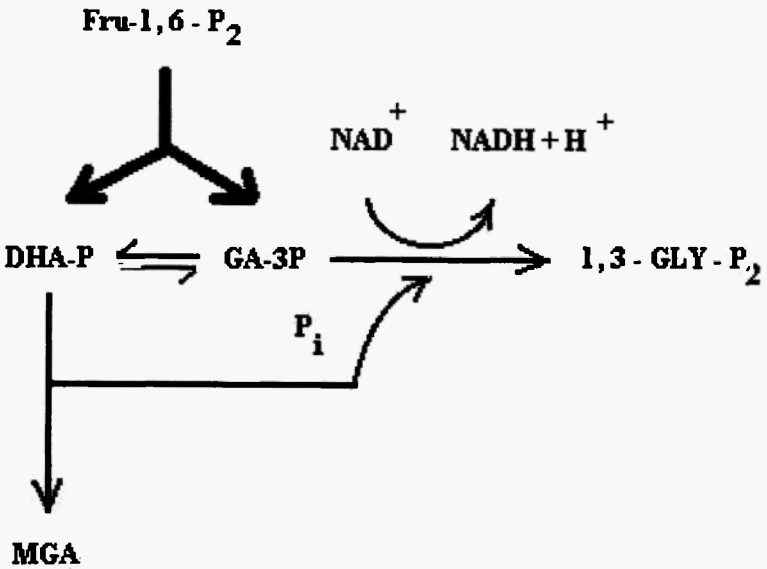

Fig. 6: Dihydroxyacetone-phosphate degradation as a source of inorganic phosphate. Fru-1,6-P_2 = fructose 1,6-bisphosphate; DHA-P = dihydroxyacetone-phosphate; GA-3P = glyceraldehyde 3-phosphate; MGA = methylglyoxal; 1,3-GLY-P_2 = 1,3-diphosphoglycerate.

PERSPECTIVES AND FUTURE DEVELOPMENTS

The studies already published have clearly demonstrated a close connection between carbohydrate metabolism and methylglyoxal. Accordingly, two main areas remain to be clarified.

One is the role of methylglyoxal in diseases, the study of which seems to be encouraging. The major issue in current research in this regard is the role of methylglyoxal in the development of diabetic complications. Since the 1990s, various aspects of this problem have been investigated (for reviews, see /100,126-129/). This avenue of research, and particularly the modification of macromolecules and methylglyoxal-provoked free radical generation, is reviewed in other papers in this issue and is not discussed here. However, it may be noted that interest in two directions of research is growing: molecular mechanisms of the interactions between methylglyoxal and macromolecules /130/, and the search for therapeutic options, including both pharmacological and genetic approaches /131,132/. From a clinical aspect, disorders involving ketosis, a threonine metabolism disturbance and an inborn error of metabolism (as in tyrosinaemia type I /133/) should be considered.

The second main area to be explored is the evolutionary aspect of methylglyoxal metabolism. It appears that the metabolic production of methylglyoxal is inevitable, as an unavoidable feature of glucose breakdown: 0.1-0.4% of glycolytic intermediates are directed to the methylglyoxalase pathway /134-137/, this being considered to be the maximum flux through the glyoxalase route /105/.

If methylglyoxal formation were already bound to energy production in the early stages of evolution, the question arises as to how its role has changed over the millions of years. The initial reactivity of the compound may have been advantageous, but this may have turned into a disadvantage in a cellular system. This might explain why cells have developed enzymatic and non-enzymatic elements to protect themselves against reactive compounds, such as aldehydes and free radicals. The presence or not of methylglyoxal synthase in animal tissues is of particular interest. This enzyme has been demonstrated in prokaryotes and yeast, but there is as yet no evidence for its presence in animal tissues. It would be an important issue to focus on the identification of methylglyoxal synthase and the gene responsible for its coding in animals. If it were also present in animals, then the

strategy for phosphate generation might well be general in all living organisms furnished with glycolysis. If methylglyoxal synthase were to prove absent from animal tissues, then some different modes of phosphate salvage should be available in animals (in comparison to prokaryotes and yeast) in order to liberate phosphate for the second stage of glycolysis.

To summarize, living organisms have the ability to form methylglyoxal and it is beyond doubt that its formation is linked to some degree to operative glycolysis. Its production is therefore unavoidable. Since glycolysis is an essential route for energy production in cells, it remains to be clarified how methylglyoxal production is minimized so as to protect cells from its deleterious effects, and its metabolic role must be fully elucidated. As mentioned above, there are opportunities for the attainment of both goals, and it is certain that methylglyoxal research faces a fruitful future.

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