The Role of Reactive α-Dicarbonyls in AGE/ALE Formation and Implication in Diabetic Complications

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Iacovella, Gina M., *et al.* The Role of Reactive α-Dicarbonyls in AGE/ALE Formation and Implication in Diabetic Complications. South Carolina Journal of Molecular Medicine (SCJMM) 5: 73-101; 2004.

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The manuscript has been seen and approved by the author and the editor.

Diabetes is a chronic debilitating disease characterized by hyperglycemia. The risk for development of complications is considerably reduced by rigorous blood glucose control. The duration and extent of diabetic state (hyperglycemia) is directly proportional to the increased risk of developing complications and is the leading cause of kidney failure, adult blindness, non-traumatic amputations, cardiovascular disease, and nerve damage. Persons affected by diabetes do not die from diabetes per se, but from complications that arise from the disease, so it is important to understand the origin of these complications.

The Advanced Glycation End Product (AGE) hypothesis proposes that chronically elevated levels of blood glucose and subsequent chemical modification of proteins are the major culprits in the pathogenesis of diabetic complications (Raj, *et al*, 2000; Degenhardt, *et al.*, 2002). Hyperglycemia causes reversible alterations in cellular metabolism and cumulative, irreversible changes in tissue protein (Baynes and Thorpe, 1999). The non-enzymatic reaction of glucose with protein leads to the formation of AGEs and changes in both protein structure and function (Baynes and Thorpe, 1999; Brownlee, *et al.*, 1984; 1988; Ellis, 1959). Glycation is the first step in the browning or Maillard reaction between reducing sugars and protein. (Figure 1) Glycated proteins undergo several physical and chemical changes with time. They gradually become brown, fluorescent, and cross-linked (Nagaraj, *et al*., 1996; Raj, *et al*., 2000). As we age, our skin becomes progressively less elastic, less soluble, and less digestible by proteolytic enzymes (Hayase, *et al*., 1994; Sims, *et al*., 1996). These processes occur naturally with age and are accelerated in diabetes (Hamlin, *et al*., 1975; Kohn, 1983; Schnider and Kohn, 1981). "Maillard-type fluorescence" (Ex. 370 nm and Em. 440 nm) is increased in connective tissue collagen in diabetic vs. non-diabetic patients (Monnier, *et al*., 1986; Monnier, 1989). AGEs are hypothesized to contribute to decreased

collagen turnover and vascular dysfunction including the thickening of the basement membranes in the microvasculature of the kidney and retina (Wells-Knecht, *et al*., 1996). The concentration of AGEs present in collagen correlates with the severity of retinopathy, nephropathy, and vascular disease in diabetes (McCance, *et al*., 1993). This implicates but does not prove a role for both the Maillard reaction and AGEs in the pathogenesis of diabetic complications (McCance, *et al.*, 1993; Sell, *et al.*, 1992; Suzuki and Miyata, 1999). Lens crystallin is another protein susceptible to Maillard-type modification. There is an increased accumulation of yellow-brown fluorescent pigments with age (Abraham, *et al*., 1989). This process is accelerated in diabetic vs. non-diabetic patients (Monnier and Cerami, 1981). These pigments are also capable of cross-linking proteins, which is an early indication of cataract formation.

Glycated proteins can react through oxidative and non-oxidative pathways to form reactive dicarbonyl compounds. These compounds are capable of reacting with lysine, arginine, and cysteine residues on proteins to form irreversible AGEs associated with the browning and fluorescence of proteins with age (Ahmed, *et al*., 1997; Thorpe and Baynes, 1996). (Figure 2) AGEs can also form under both oxidative and non-oxidative conditions. Glyoxal (GO), methylglyoxal (MGO), 3-deoxyglucosone (3-DG), and glucosone (GLO) are four reactive dicarbonyls that form a group of heterogeneous AGEs. The oxidation of polyunsaturated fatty acids (PUFAs) will also produce both GO and MGO. In the presence of protein and copper-catalyzed low-density lipoprotein (LDL), GO and MGO go on to form irreversible advanced lipoxidation end-products (ALEs). (Figure 2)

GO

GO is a highly reactive, short-chained dicarbonyl capable of modifying and cross-linking proteins. Wolff and Dean (1987) observed that the incubation of glucose, with or without protein, under oxidative conditions produced dicarbonyl compounds and reactive oxygen species (ROS). The addition of a transition metal chelator under the same reaction conditions reduced the yield of dicarbonyls (Hunt, *et al*., 1988; Wolff and Dean, 1987; Wolff, *et al*., 1991). Based on these findings, Wolff and Dean (1987) proposed that metal ions catalyze the oxidative formation of dicarbonyls during the Maillard reaction, most likely with the Schiff base undergoing oxidation and fragmentation. GO can also be generated by the oxidation of other reducing sugars (Glomb and Monnier, 1995) and was later identified as the major dicarbonyl formed during the autoxidation of glucose and glycolaldehyde (Okado-Matsumoto and Fridovich, 2000; Thornalley, *et al*., 1984; Wells-Knecht, *et al*., 1995). In addition to carbohydrates, GO forms during the oxidation of polyunsaturated fatty acids (PUFAs) involving formation of hydrogen peroxide and β-fragmentation (Mlakar and Spiteller, 1994). (Figure 3)

GO-modified proteins can disrupt biochemical processes leading to protein dysfunction, resistance to enzymatic digestion, and cytokine-mediated immune responses (Degenhardt, *et al*., 1998; Papoulis, *et al*., 1995; Vaca, *et al*., 1994; Westwood, *et al*., 1997). GO reacts with lysine, cysteine, and arginine residues on proteins to form irreversible AGEs. The best characterized AGE is N^{ε}-(carboxymethyl)lysine (CML) (Dunn, *et al*., 1989; Dyer, *et al*., 1991b, 1993). (Figure 4) Dyer *et al.* (1993), using isotope dilution gas chromatography-mass spectrometry, showed that CML was increased 5-7 fold with age in skin collagen. CML is formed by the

reaction of GO and lysine via a Canizzaro rearrangement (Ahmed, *et al*., 1986) and the oxidative cleavage between C-2 and C-3 of FL (Zyzak, *et al*., 1995). GO generated from metal-catalyzed autoxidation of glucose reacts with lysine to also form CML. The oxidation of PUFAs in the presence of protein and copper catalyzed oxidation of low-density lipoproteins (LDL) both generate CML (Fu, *et al*., 1996). As a product of carbohydrate and lipid oxidation, it is classified as both an AGE and an advanced lipoxidation end- product (ALE). (Figure 5) CML is colorless, non-fluorescent, and does not cross-link proteins. Because of its side chain polarity and charge density, CML is most likely located on the surface of most AGE proteins and thought to be the predominant antigen recognized by anti-AGE antibodies (Reddy, *et al*., 1995). CML is elevated in skin collagen (Dunn *et al*., 1990; 1991) and the urine (Wells-Knecht, *et al*., 1995) of non-diabetic vs. diabetic patients. Concentrations of CML in human lens proteins increase from trace amounts to two mmol CML/mol lysine from infancy to old age (Dunn, *et al*, 1989; 1991). CML levels also correlate with the severity of diabetic complications, including retinopathy and nephropathy (Fu, *et al*., 1996; McCance, *et al*., 1993).

In addition to CML, GO can react with two lysine residues to form the AGE/ALE cross-link, glyoxal-lysine dimer (GOLD) (Brinkmann-Frye, *et al*., 1995; Thornalley, *et al*., 1999; Wells-Knecht, *et al*., 1995). Canizzaro type rearrangement and elimination reactions have been proposed for GOLD formation. GOLD is elevated in serum proteins of uremic and hemodialysis patients (Odani, *et al*., 1998) but does not correlate with increased glucose or triglyceride concentrations (Chan, *et al*., 1981). This cross-link also increases in lens protein and skin collagen with age (Brinkmann-Frye, *et al*., 1998). It is unclear if these increases can be attributed to oxidative stress. Although GOLD can serve as a biomarker of protein cross-links, it does not contribute to diabetic pathology (Odani, *et al*., 1998).

GO reacts with the guanidino group of arginine to form the AGE, N^{ε}-(carboxymethyl)arginine (CMA). CMA is unstable to acid hydrolysis and can only be analyzed after enzymatic digestion. Glomb and Lang (2001) have made an argument for the formation of CMA *in vitro* but have not yet shown its detection *in vivo*.

Recently, AGE research has expanded into characterization of sulfhydryl AGEs. It is logical to hypothesize that cysteine residues would be targets of chemical modification because of their nucleophilicity. Modified cysteine residues are more likely to disrupt protein function than modified lysine residues because free sulfhydryl groups are typically found in the active sites of numerous regulatory enzymes. Sulfhydryl AGEs are formed exclusively from dicarbonyl compounds because the thiohemiacetal adduct of a reducing sugar cannot rearrange to form a Schiff base or Amadori product. The reaction of GO with cysteine forms S-(carboxymethyl)cysteine (CMC). Thus far, CMC has been identified in rat or human plasma, skin collagen, red cells, muscle protein, and urine (Alderson, *et al*., in press).

MGO

MGO, like GO, is formed through multiple biochemical pathways (Murata, *et al*., 1989; Ohmori, *et al*., 1989; Steinberg and Kaplan, 1984; Thornalley, 1990) (Figure 6). The nonenzymatic β-elimination of phosphate from the triose phosphate intermediates, glyceraldehyde 3-phosphate and dihydroxyacetone phosphate, results in the formation of MGO (Abordo, *et al*., 1999; Oya, *et al*., 1999). Hyperglycemia increases the flux of glucose through anaerobic glycolysis, which leads to an increase in

MGO formation (Thornalley, 1996). MGO concentrations were elevated 5-6 fold in the blood of type I diabetic patients and 2-3 fold in type II diabetic patients relative to normal controls (McLellan, *et al*., 1993). Another means to generate MGO non-enzymatically is by amine-catalyzed sugar fragmentation (Hayashi, *et al*., 1986; Richard, 1991). *In vitro*, MGO quickly modifies proteins via Maillard reactions resulting in the covalent cross-linking of proteins and generation of protein-bound fluorescence (Haik, *et al*., 1994; Riley and Harding, 1995; Thornalley, 1990; Vander Jagt, *et al*., 1992). MGO is a physiological substrate of the glutathione-dependent glyoxylase pathway, which catalyzes the detoxification of MGO to its metabolite, D-lactate (Phillips and Thornalley, 1993; Ruggiero-Lopez, *et al*., 1999; Thornalley, 1990). Elevated levels of both MGO and D-lactate in the blood of type I and II diabetic patients have been detected, indicating an increased flux through the glyoxylase pathway. (McLellan, *et al.,* 1993; Thornalley, *et al.,* 1989). Beisswenger *et al*. (1996) provided evidence that decreased MGO metabolism via the glyoxylase pathway correlates with increased retinopathy in type I diabetic patients. Optimal activity of the glyoxylase system is dependent on the presence of adequate levels of reduced glutathione (GSH) (Shamsi, et al., 2000; Thornalley, 1993). Because oxidative stress depletes GSH concentrations (Baynes and Thorpe, 1999), it can cause impaired MGO detoxification. Nagaraj *et al*. (2002) reported increased MGO concentrations and decreased GSH levels in diabetic rats. Increased flux of glucose through other pathways could also increase plasma concentrations of MGO, including cleavage of Amadori compounds (Hayashi, *et al*., 1986), cyctochrome P4502E1 catalyzed oxidation of acetone from ketone bodies (Thornalley, 1996), or the catabolism of threonine via aminoacetone (Bird, *et al*., 1984). Although these alternate pathways are a minor source of MGO production, it raises the possibility they can increase with diabetes.

MGO is formed through the autoxidation of sugars and oxidation of PUFAs (Hayashi, *et al*., 1986; Keyhani and Yaylayan, 1996). It is also a minor product of acetone metabolism and catabolism of threonine (Ray and Ray, 1987; Reichard, *et al*., 1986). The principal *in vivo* pathway for MGO generation is unknown, since it is formed during both non-oxidative and oxidative chemistry. It has been detected using gas chromatography-mass spectrometry, high-performance liquid chromatography, and electro-spray ionization liquid chromatography- mass spectrometry. Proteins incubated with MGO show increased fluorescence, browning, and cross-linking. MGO is 20,000 times more reactive than glucose in irreversibly glycating proteins such as bovine serum albumin, lysozyme, and collagen (Lo, *et al*., 1994; Selwood and Thornalley, 1993; Vander Jagt, *et al*., 1992) and has the capability to disrupt metabolic functions including mitochondrial respiration and glycolysis (Bowes and Cater, 1968; Halder, *et al*., 1993).

3-DG

The carbohydrate-derived reactive dicarbonyl, 3-DG, is formed exclusively by non-oxidative chemistry. It can be formed non-enzymatically in the Maillard reaction by the rearrangement of fructoselysine (FL) or enzymatically via the polyol pathway (Kato, *et al*., 1987). In the polyol pathway, fructose is phosphorylated by fructose 3-phosphokinase to form fructose-3-phosphate followed by the β-elimination of phosphate, which generates 3-DG (Lal, *et al*., 1995). (Figure 7) Lal *et al*. (1997) suggest that because 3-DG has been detected in diabetic rat hearts, it may contribute to glycated-cardiac proteins. Levels of 3-DG, measured

by gas chromatography–mass spectrometry, are also increased 2-3 fold in the plasma of diabetic vs. non-diabetic patients (Hamada, *et al*., 1997; Niwa, *et al*., 1993; 1995).

3-DG is detoxified to 3-deoxyfructose by a 3-DG reducing enzyme (Feather, *et al*., 1995). 3-Deoxyfructose has been identified as a major urinary metabolite in diabetic patients but is undetectable in healthy subjects (Kato, *et al*., 1990; Wells-Knecht, *et al*., 1994). The question of why 3-DG is increased in the serum of uremic patients remains unanswered. Since 3-DG is not excreted by patients with normal renal function, reduced renal clearance of 3-DG by diseased kidneys does not readily explain the increased serum levels. Therefore, the 3-DG-reducing enzymatic activity may be suppressed and compromise the efficiency of 3-DG detoxification (Knecht, *et al*., 1992).

3-DG forms non-enzymatically from fructose-3-phosphate via the polyol pathway, while fructose-3-phosphate is phosphorylated enzymatically from fructose (Kato, *et al*., 1969). Both fructose-3-phosphate and 3-DG are increased in the tissues of diabetic subjects presumably because of an increased flux of glucose through the polyol pathway (Malone, *et al*., 1980; Cohen, 1987). Fructose-3-phosphate and 3-DG have been detected in elevated concentrations in the lens and hearts of diabetic rats (Lal, S., *et al*., 1995; 1997; Petersen, et al., 1990; Szwergold, *et a.l*, 1990) and in the erythrocytes and serum of diabetic patients compared with healthy subjects (Kanaza, *et al*., 1991). It has been hypothesized that elevated serum levels of 3-DG are produced mainly from the degradation of Amadori products via the Maillard reaction, whereas elevated 3-DG levels in erythrocytes derive from increased polyol pathway activity (Tsukushi, *et al*., 1999). This hypothesis could be tested by measuring levels of FL, a compound derived exclusively from the Maillard reaction, in both serum and erythrocytes.

In addition to erythrocytes, the polyol pathway is enhanced in the retina, aorta, lens, kidneys, and peripheral nerves in diabetic patients (Hotta, 1997). All of these tissues contain the enzyme, aldose reductase, which is involved in both the synthesis of a 3-DG precursor and in the detoxification of 3-DG (Matsura, *et al*., 1995, Kato, *et al*., 1969). Aldose reductase is the rate-limiting enzyme that forms fructose in the polyol pathway and detoxifies 3-DG by reducing its highly reactive carbonyl groups (Szwergold, *et al*., 1990). Hasuike and colleagues (2002) assessed the relationship between increased concentrations of 3-DG and aldose reductase in the plasma of non-diabetic and diabetic patients with progressive renal disease. They observed a decrease in renal function, and an increase in both 3-DG and aldose reductase concentrations. There was also a positive correlation between increased plasma 3-DG and aldose reductase in all the diabetic groups (Hasuike, *et al*., 2002). This suggests that the polyol pathway plays an important role in renal function and development of diabetic nephropathy. The non-enzymatic formation of 3-DG from FL, its enzymatic formation via the polyol pathway, and catabolism via 3-DG-reducing enzymes demonstrates the complex relationship between chemical and metabolic formation of reactive dicarbonyl compounds. The reactions of 3-DG with lysine and arginine residues on protein forms the AGEs: pyrraline and imidazolone, respectively. Both of these products are formed from dehydration and fragmentation reactions under non-oxidative conditions (Thorpe, *et al*., 2000).

<u>GLO</u>

GLO is the least characterized reactive dicarbonyl compared to GO, MGO, and 3-DG in terms of AGE formation and possible

contribution to pathology in diabetes. *In vitro* studies have suggested GLO formation from both the autoxidation of glucose and rearrangement of the Amadori product (Wolff and Dean, 1987). Nagai et al. (2002) also demonstrated the formation of glucosone from peroxynitrite-treated glucose. Measurement of GLO levels *in vivo* is problematic because of its rapid degradation in the presence of transition metals (Cheng, *et al*., 1992). (Figure 8)

Glomb and Tschirnich (2001) hypothesize that 3-DG and GLO are less reactive than GO and MGO because their longer carbon chain increases the steric complexity of the hemiacetal ring opening. In addition to ring opening, 3-DG and GLO also must isomerize from the α-oxoenediol form to the α-dioxo form in order to react with dicarbonyl- trapping reagents, like 2,3-diaminonapthalene (DAN) (Glomb and Tschirnich, 2001; Thornalley, et al., 2000). Since 3-DG forms under non-oxidative conditions and GLO under oxidative conditions, it is likely that different pathways are involved in the increase of these two dicarbonyls.

Pharmaceutical Agents

Researchers and pharmaceutical companies are trying to find compounds aimed at preventing AGE/ALE formation by targeting different points in glycoxidation/lipoxidation pathways (Khalifah, *et al*., 1999). AGE/ALE inhibitors must be capable of trapping low molecular mass, soluble, reactive intermediates in AGE/ALE formation. These inhibitors have to intercept AGE/ALE formation without compromising the intermediary metabolism of aldehydes or ketones, trap coenzymes or their precursors. Compounds, like aspirin and pyridoxal-5'-phosphate (Khatami, *et al*., 1988), compete with glucose for free amino groups. Anti-oxidants and metal chelators, such as vitamin C, vitamin E, and ethylenediaminetetraacetic acid (EDTA), can inhibit the oxidation of carbohydrates, lipids, and the Amadori product (Hunt, *et al*., 1988; 1990; Wolff, *et al*., 1991). The formation of early Maillard products is hindered by reactions of carbonyl groups of glucose with hydrazine compounds, like gentamycine and aminoguanidine (AG). (Figure 9)

AG has been one of the most effective inhibitors of diabetic complications in animal models of diabetes. It is a dinucleophile that reacts with dicarbonyl compounds to form stable triazine adducts, preventing further reactions including the formation of cross-link (Chen, *et al*., 1993; Hirsch, *et al*., 1992; Lo, *et al*., 1994). Recent *in vivo* studies, have reported decreases of AGE/ALEs, "Maillard-type" fluorescence, and AGE cross-links in diabetic rat plasma upon treatment with AG (Degenhardt, *et al.*, 2002). It is important to point out that AG does not interfere with the formation of Amadori compounds but does inhibit the subsequent rearrangements that form AGE/ALEs. AG did not have an affect on blood glucose or glycated hemoglobin levels but successfully inhibited increases in the cross-linking and Maillard-type fluorescence of aortic collagen in diabetic rats (Brownlee, *et al*., 1986). Although this compound showed pharmaceutical promise as an AGE inhibitor, clinical trials showed high levels of toxicity.

Given the harmful side effects of AG, the search resumed for other compounds that might inhibit protein modifications and cross-links. Pyridoxamine (PM) is a vitamin B_6 derivative, which is water-soluble and non-toxic in rats and humans. It inhibits the formation of AGEs from Amadori proteins and is classified as a post-Amadori inhibitor (Khalifah, *et al*., 1999). Studies in streptozotocin (STZ)-induced diabetic rats revealed that AG and PM were comparable in their effects on AGEs. In addition to

lowering AGE levels, PM also improved renal function, hypercholesterolemia and hypertriglyceridemia, and retarded the development of retinopathy (Degenhardt, *et al*., 2002; Stitt, *et al*., 2002). Despite the physiological improvements, PM has had on diabetic complications, its biochemical mechanism of action *in vivo* remains under investigation.

PM, originally described as a post-Amadori inhibitor, is hypothesized to trap reactive dicarbonyl intermediates in AGE/ALE formation (Metz, *et al*., in press). Onorato *et al*. (2000) further demonstrated that PM inhibited ALE formation from lipoproteins and peroxidizing lipids *in vitro*. PM may also decrease oxidative stress, which subsequently decreases AGE/ALE formation from reactive oxygen species. The role of oxidative stress in diabetic pathology is controversial. There is uncertainty whether oxidative stress causes diabetic complications, or whether the presence of complications such as tissue ischemia resulting from vascular disease, causes an increase in oxidative stress (Thorpe, *et al.*, 2000). From our *in vivo* findings, we speculate that PM may protect against kidney disease by lowering plasma triglyceride levels thus inhibiting the formation of lipid peroxidation products, and thereby decreasing oxidative stress.

AGE Breakers

Other compounds, termed "AGE-breakers", have been reported to reverse AGE cross-links. Phenylacylthiazolium bromide (PTB) is an AGE breaker that destroyed cross-links between AGE-BSA and collagen (Vasan, *et al*., 1996). OPB-9195, structurally similar to PTB, not only disrupts existing AGE cross-links but prevents the formation of additional AGEs (Nakamura, *et al*., 1997). Both of these compounds were useful *in vitro* but their application *in vivo* is questionable because of their instability (Thornalley, *et al*., 1999).

By developing a sensitive and reproducible assay for plasma dicarbonyls, this should provide a new avenue for assessing the relationship between increased plasma dicarbonyls, hyperlipidemia, and diabetic nephropathy. Dicarbonyls are an appropriate area to focus on because they form from both carbohydrates and lipids. Future work built on a method of dicarbonyl detection will provide an important means for detecting those diabetic subjects at risk for the development of diabetic complications.

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Figure 1: Early steps of the Maillard reaction. The open chain form of a reducing sugar reacts with an amino group on protein to form a reversible Schiff base. The Schiff base can form a cyclic glycosylamine or can rearrange to an enaminal and then to a ketoamine (Amadori compound). The Amadori compound is also stabilized by its cyclization to a furanose or pyranose ring.

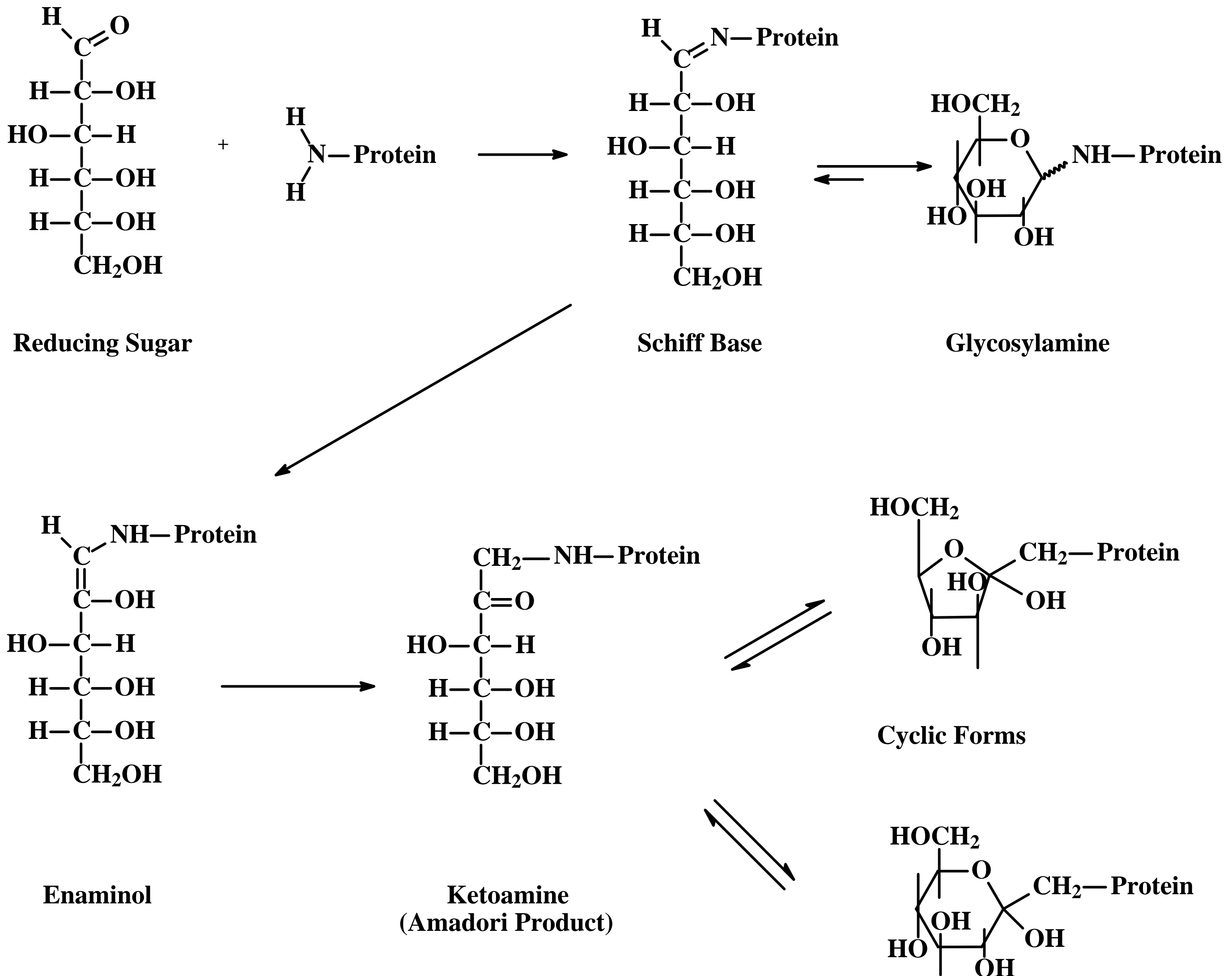
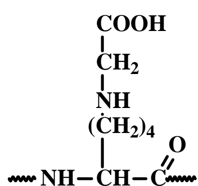
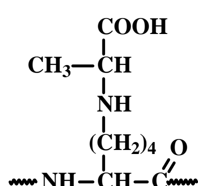

Reducing Sugar
Protein
Schiff Base
Glycosylamine
Enaminol
Ketoamine
(Amadori Product)
Cyclic Forms

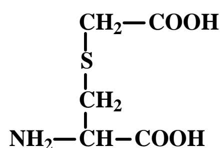
Figure 2: Heterogenous structures of AGEs. CML, CEL, MOLD, GOLD, pentosidine are formed under both non-oxidative and oxidative chemistry. Arg-pyrimidine, pyrraline, and imidazolone are formed exclusively from non-oxidative reactions. CMC and CEC are sulfhydryl-AGEs formed from the reaction of reducing sugars with cysteine residues.



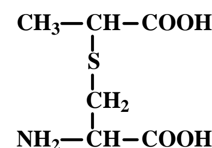
N$^{\varepsilon}$-(carboxymethyl)-lysine
CML



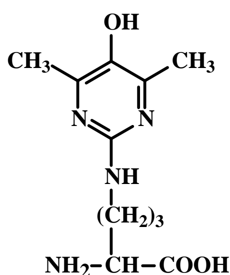
N$^{\varepsilon}$-(carboxyethyl)-lysine
CEL



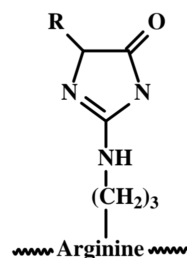
S-(carboxymethyl)-cysteine
CMC



S-(1-carboxyethyl)-cysteine
CEC

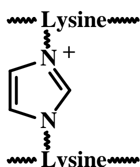


Argpyrimidine

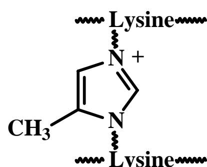


R = 3-DG

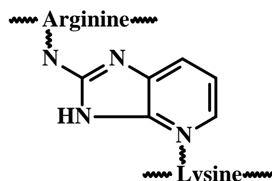
Imidazalone



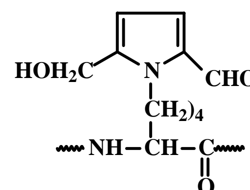
Glyoxal-lysine dimer
GOLD



Methylglyoxal-lysine dimer
MOLD



Pentosidine



Pyrraline

Figure 3: Pathways of GO formation. GO is formed from metal-catalyzed oxidation during the Maillard reaction, autoxidation of reducing sugars, and the oxidation PUFAs. GO reacts with lysine, arginine, and cysteine residues to form the irreversible compounds CML, GOLD, CMA, and CMC.

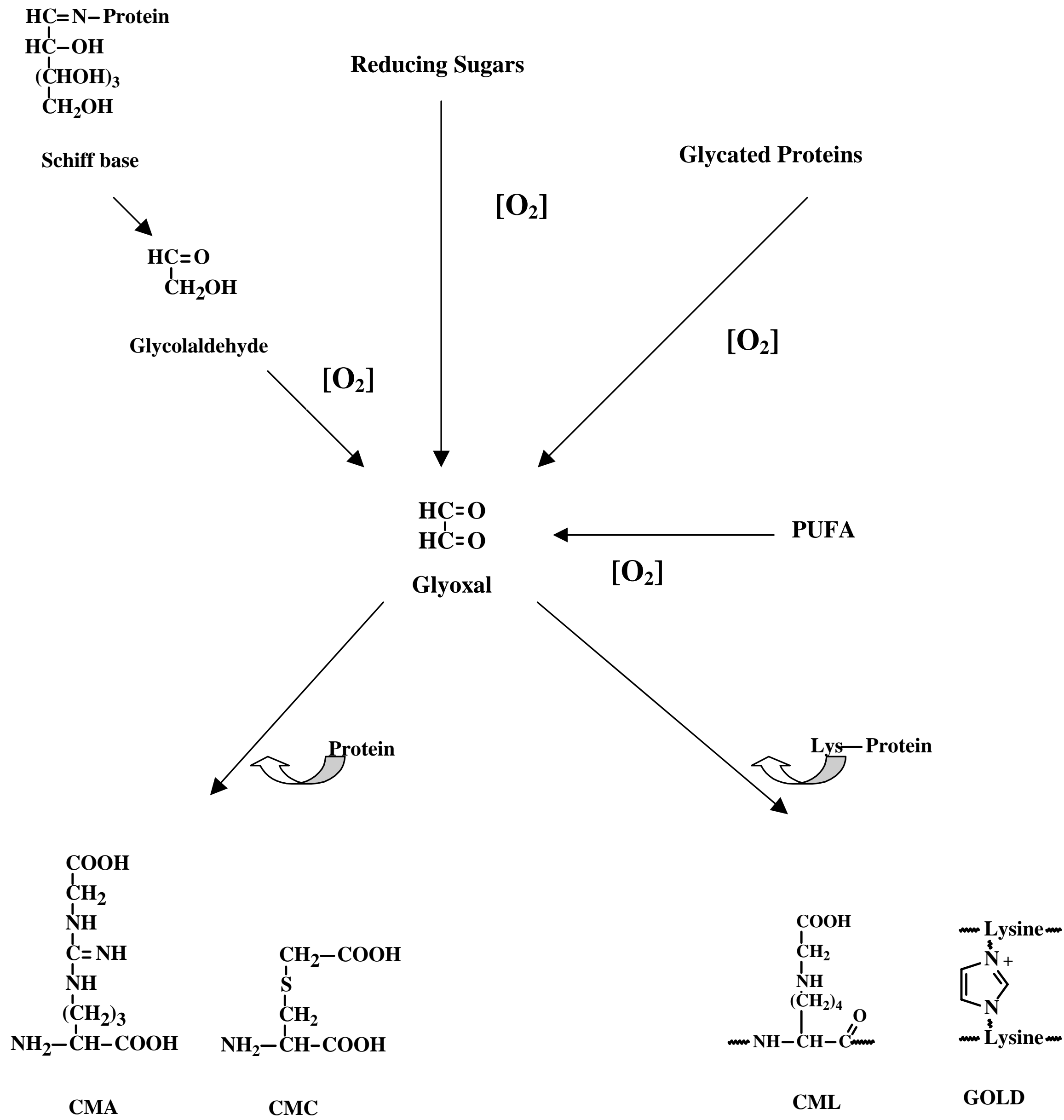

HC=N−Protein
HC−OH
(CHOH)3
CH2OH
Schiff base
Reducing Sugars
Glycated Proteins
[O2]
HC=O
CH2OH
Glycolaldehyde
[O2]
[O2]
HC=O
HC=O
Glyoxal
PUFA
[O2]
Protein
Lys—Protein
COOH
CH2
NH
C=NH
NH
(CH2)3
NH2−CH−COOH
CH2−COOH
S
CH2
NH2−CH−COOH
COOH
CH2
NH
(CH2)4
O
NH−CH−C
Lysine
N+
N
Lysine
CMA
CMC
CML
GOLD

Figure 4: Pathways to formation of CML. Under oxidative conditions, glucose reacts with an amine on a protein to form the Amadori compound, FL. CML forms from the oxidative cleavage of FL between C2 and C3, releasing the by-product erythronic acid (pathway A). Alternatively, the autoxidation of glucose forms glyoxal which can react with lysine to form a cyanohydrin intermediate followed by a Canizzaro rearrangement, yielding CML (pathway B).

NH$_2$— Protein

Lysine
NH
CH$_2$
C=O
(CHOH)$_3$
CH$_2$OH
Fructoselysine

A

[O$_2$]

COOH
HO—C—H
HO—C—H
CH$_2$OH
Erythronic Acid

GLUCOSE

COOH
CH$_2$
NH
(CH$_2$)$_4$
O
NH—CH—C
N$^{\varepsilon}$-(carboxymethyl)-lysine
CML

B

[O$_2$]

Canizzaro
Rearrangement

NH$_2$— Protein

O O
HC—CH
Glyoxal

OH O
Protein—NH$_2$—CH—CH
Cyanohydrin

H$_2$O

OH$^-$

O
Protein—$\overset{+}{\text{N}}$H=CH—C—H

Figure 5: Chemical modifications of lysine from carbohydrates and lipids. The AGEs, CML and CEL, can be formed from oxidized carbohydrates or lipids in the presence of protein, and are therefore considered both glycoxidation and lipoxidation products.

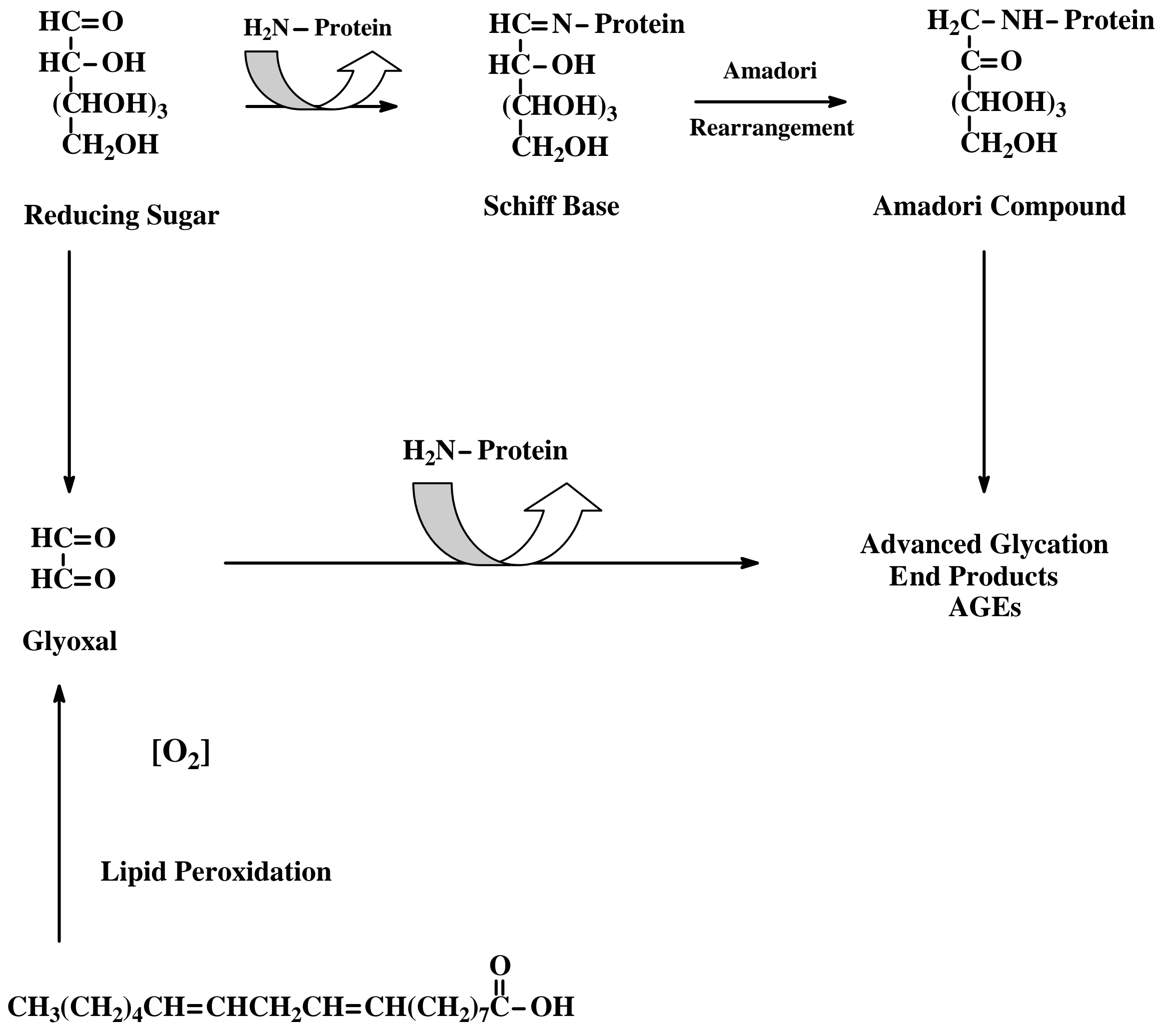

HC=O
HC–OH
(CHOH)3
CH2OH
Reducing Sugar
H2N–Protein
HC=N–Protein
HC–OH
(CHOH)3
CH2OH
Schiff Base
Amadori
Rearrangement
H2C–NH–Protein
C=O
(CHOH)3
CH2OH
Amadori Compound
H2N–Protein
HC=O
HC=O
Glyoxal
Advanced Glycation
End Products
AGEs
[O2]
Lipid Peroxidation
O
CH3(CH2)4CH=CHCH2CH=CH(CH2)7C–OH

Figure 6: Pathways of MGO formation. MGO is formed nonenzymatically by the dephosphorylation of triose phosphates and amine-catalyzed sugar fragmentation via the Maillard reaction. MGO is also formed through oxidative reactions of both carbohydrates and PUFAs. It can also form from threonine and acetone. MGO can react with lysine, arginine, and cysteine residues on a protein to form the irreversible compounds CEL, MOLD, argpyrimidine, and CEC.

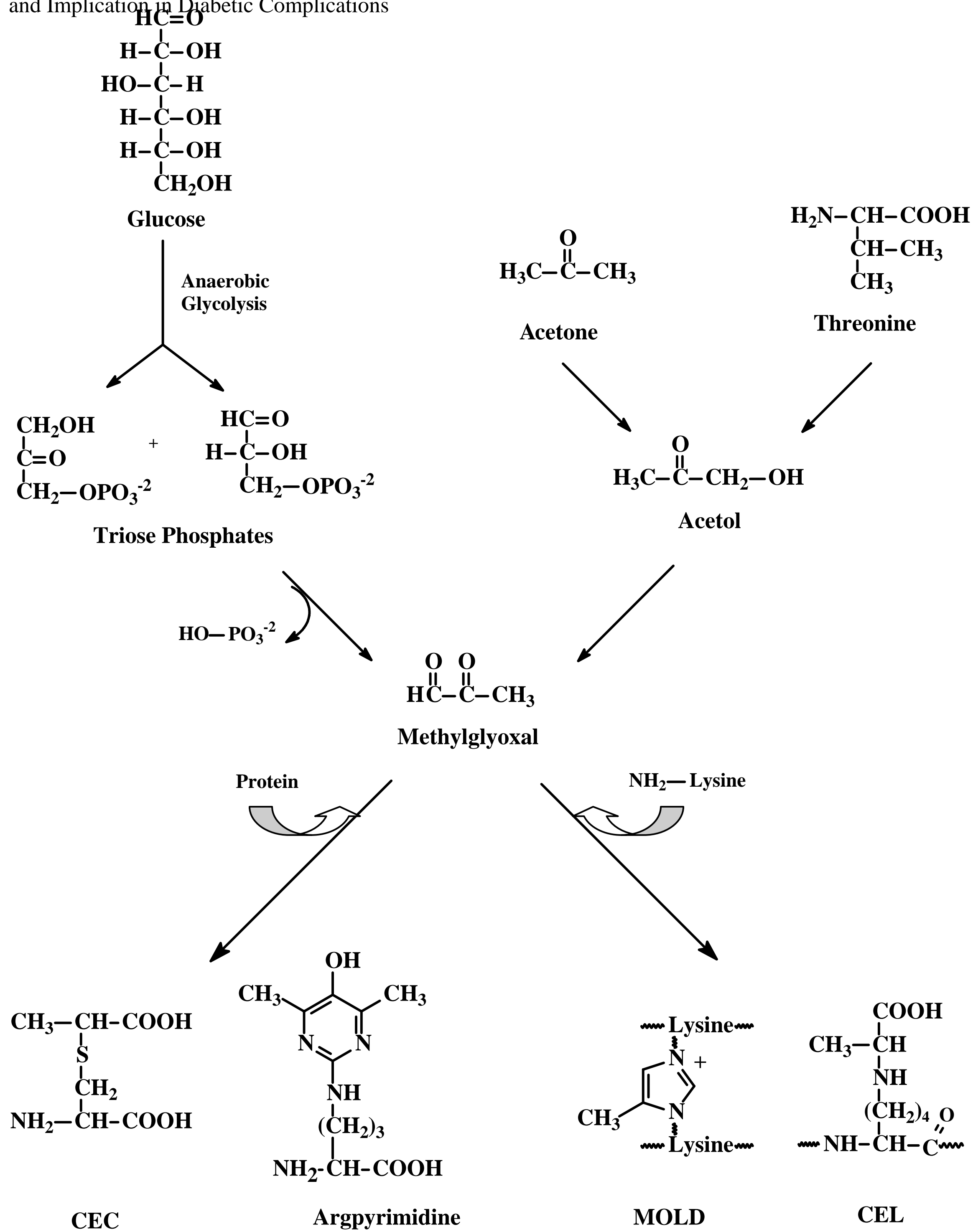
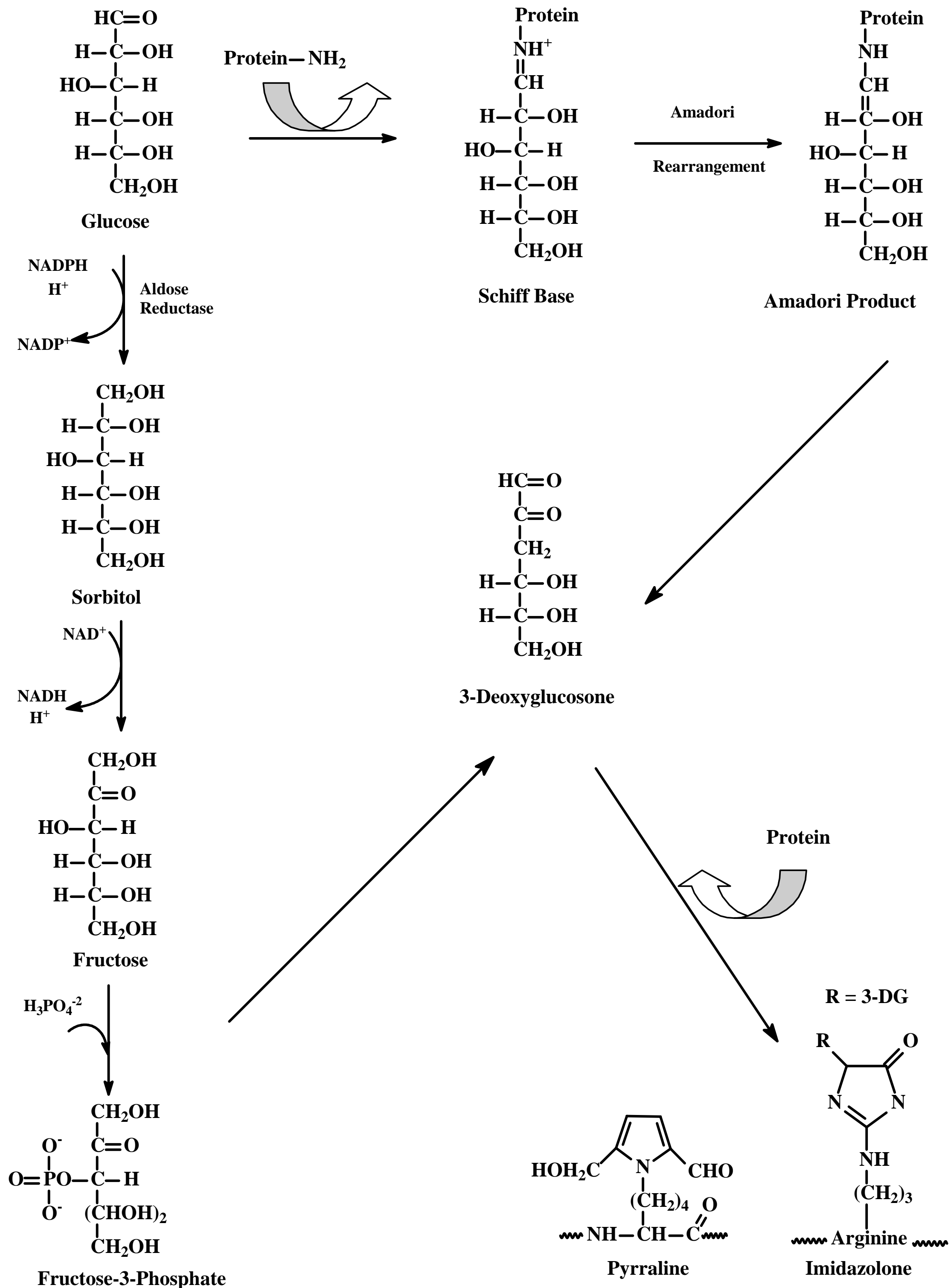

HC=O
H–C–OH
HO–C–H
H–C–OH
H–C–OH
CH2OH
Glucose
Anaerobic Glycolysis
CH2OH
C=O
CH2–OPO3-2
+
HC=O
H–C–OH
CH2–OPO3-2
Triose Phosphates
O
H3C–C–CH3
Acetone
H2N–CH–COOH
CH–CH3
CH3
Threonine
O
H3C–C–CH2–OH
Acetol
HO–PO3-2
O O
HC–C–CH3
Methylglyoxal
Protein
NH2–Lysine
CH3–CH-COOH
S
CH2
NH2–CH-COOH
CEC
OH
CH3
CH3
N
N
NH
(CH2)3
NH2-CH-COOH
Argpyrimidine
Lysine
N +
N
CH3
Lysine
MOLD
COOH
CH3–CH
NH
(CH2)4
O
NH–CH–C
CEL

Figure 7: Pathways of 3-DG formation. 3-DG is a carbohydrate-derived reactive dicarbonyl formed from non-oxidative chemistry through the Maillard reaction by the rearrangement of FL (pathway A) and enzymatically via the polyol pathway (pathway B). The AGEs formed from the reaction of 3-DG with proteins are pyrraline and imidazolone.



Glucose
Protein—NH2
Schiff Base
Amadori
Rearrangement
Amadori Product
NADPH
H+
Aldose
Reductase
NADP+
Sorbitol
NAD+
NADH
H+
Fructose
H3PO4-2
Fructose-3-Phosphate
3-Deoxyglucosone
Protein
R = 3-DG
Pyrraline
Imidazolone
Arginine

Figure 8: Pathways of GLO formation. GLO forms from the oxidation of glucose and cleavage of the Amadori product. Presently, the only AGE known to form from GLO is CML.

Protein — NH_2

HC=O
H—C—OH
HO—C—H
H—C—OH
H—C—OH
CH_2OH

Glucose

Protein
NH^+
CH
H—C—OH
HO—C—H
H—C—OH
H—C—OH
CH_2OH

Schiff Base

Amadori
Rearrangement

Protein
NH
CH
H—C—OH
HO—C—H
H—C—OH
H—C—OH
CH_2OH

Amadori Product

[O_2]

HC=O
C=O
HO—C—H
H—C—OH
H—C—OH
CH_2OH

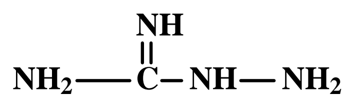
Glucosone

H_2N— Protein

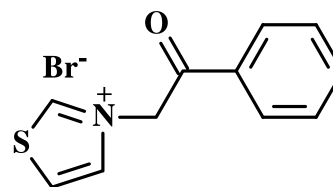
COOH
CH_2
NH
$(CH_2)_4$
O
NH—CH—C

N^{ε}-(carboxymethyl)-Lysine
CML

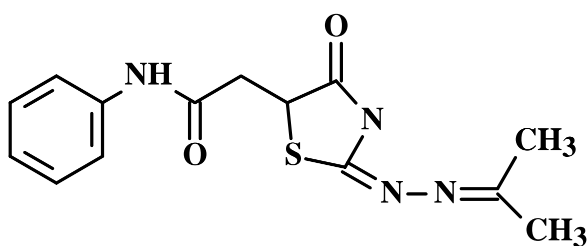
Figure 9: Structures of AGE inhibitors and AGE breakers. Aminoguanidine, phenylacylthiazolium bromide (PTB), OPB-9195, and pyridoxamine inhibit AGE formation.



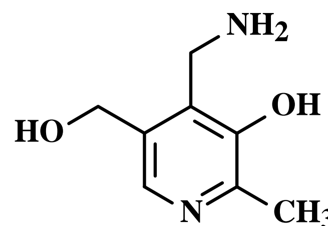
Aminoguanidine



Phenylthiazolium bromide (PTB)



OPB-9195



Pyridoxamine

Editor: Hiroko Hama
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