Amino Acids (2003) 25: 275–281
DOI 10.1007/s00726-003-0017-9

Amino Acids
Printed in Austria

Maillard reaction products in tissue proteins: New products and new perspectives

Review Article

S. R. Thorpe[1] and **J. W. Baynes**[1,2]

[1] Department of Chemistry and Biochemistry, University of South Carolina, Columbia, South Carolina, U.S.A.
[2] School of Medicine, University of South Carolina, Columbia, South Carolina, U.S.A.

Received December 7, 2002
Accepted May 8, 2003
Published online July 29, 2003;

Summary. The chemical modification of protein by nonenzymatic browning or Maillard reactions increases with age and in disease. Maillard products are formed by reactions of both carbohydrate- and lipid-derived intermediates with proteins, leading to formation of advanced glycation and lipoxidation end-products (AGE/ALEs). These modifications and other oxidative modifications of amino acids increase together in proteins and are indicators of tissue aging and pathology. In this review, we describe the major pathways and characteristic products of chemical modification of proteins by carbohydrates and lipids during the Maillard reactions and identify major intersections between these pathways. We also describe a new class of intracellular sulfhydryl modifications, Cys-AGE/ALEs, that may play an important role in regulatory biology and represent a primitive link between nonenzymatic and enzymatic chemistry in biological systems.

Keywords: Advanced glycation end-product (AGE) – Advanced lipoxidation end-product (ALE) – N^{ε}-(carboxymethyl)lysine – Glyoxal – Maillard reaction – Methylglyoxal

Abbreviations: AAOP, amino acid oxidation product; AGE, advanced glycation end-product; ALE, advanced lipoxidation end-product; CEL, N^{ε}-(carboxyethyl)lysine; CMC, S-(carboxymethyl)cysteine; CML, N^{ε}-(carboxymethyl)lysine; 1DG & 3DG, 1- & 3-deoxyglucosone; GA, glycolaldehyde; GLO, glucosone; GO, glyoxal; GOLD, glyoxal-lysine lysine dimer, imidazolium salt; MGO, methylglyoxal; MOLD, methylglyoxal-lysine lysine dimer, imidazolium salt; HNE, 4-hydroxynonenal; MDA, malondialdehyde; OxS, oxidative stress; RCS, reactive carbonyl species; RNS, reactive nitrogen species; ROS, reactive oxygen species

Introduction

The discovery of glycated hemoglobin in the late 1970's launched a novel, still rapidly growing area of research on spontaneous, nonenzymatic reactions in biological systems, what Golubev (1996) described as "the other side of metabolism." That spontaneous, unprogrammed chemical reactions occur in highly organized biological systems is not surprising, considering the complexity and temperature of the biochemical milieu. The original focus of biomedical research on nonenzymatic chemistry was on the Maillard reaction, the browning of proteins by carbohydrates, and the relevance of this reaction to pathology associated with aging and diabetes. Today, glycation is recognized as only one of many nonenzymatic modifications of proteins that not only contribute to the aging of body proteins, but may also have an important regulatory role in both physiological responses and pathological processes.

A major spin-off of studies on glycation during the 1980's was the recognition that oxidative reactions, and by inference, oxidative stress (OxS) and reactive oxygen species (ROS), catalyzed the chemical modification of proteins by Maillard reactions *in vivo* (Baynes, 1991). Reactive carbonyl species (RCS) formed on oxidation of carbohydrates, lipids and amino acids were identified as intermediates in the formation of irreversible, advanced glycoxidation and lipoxidation end-products (AGE/ALEs) on protein. ROS, RCS and reactive nitrogen species (RNS) are now recognized important transducers in biological systems. In this article, we will focus on the Maillard reaction, the chemical modification of proteins by carbohydrates, including metabolic intermediates. We also propose two arguments for extended discussion: 1) that Maillard or browning reactions *in vivo* includes pathways of chemical modification of protein by both carbohydrates and lipids and that there are major intersections between these pathways

and other pathways of chemical modification of proteins; and 2) that intracellular sulfhydryl AGE/ALEs (Cys-AGE/ALEs), a novel class of AGE/ALEs, are likely to play an important role in regulatory biology and may serve as a primitive link between nonenzymatic and enzymatic chemistry in biological systems.

Maillard reactions involving carbohydrates – the classical Maillard reaction

The first stage in the classical Maillard reaction is the formation of Schiff base and Amadori adducts to protein

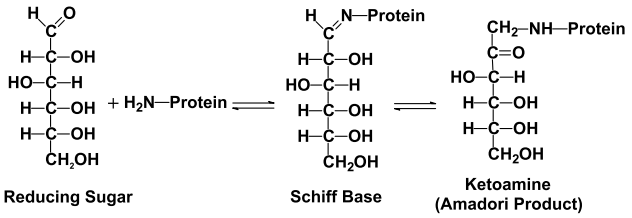

Fig. 1. The classical pathway of the Maillard reaction. The open chain form of glucose reacts with lysine to form a Schiff base (imine) adduct that undergoes an Amadori rearrangement to form a ketoamine (fructosamine) adduct to protein. Both the Schiff base and ketoamine adducts are stabilized in cyclic furanose and pyranose configurations

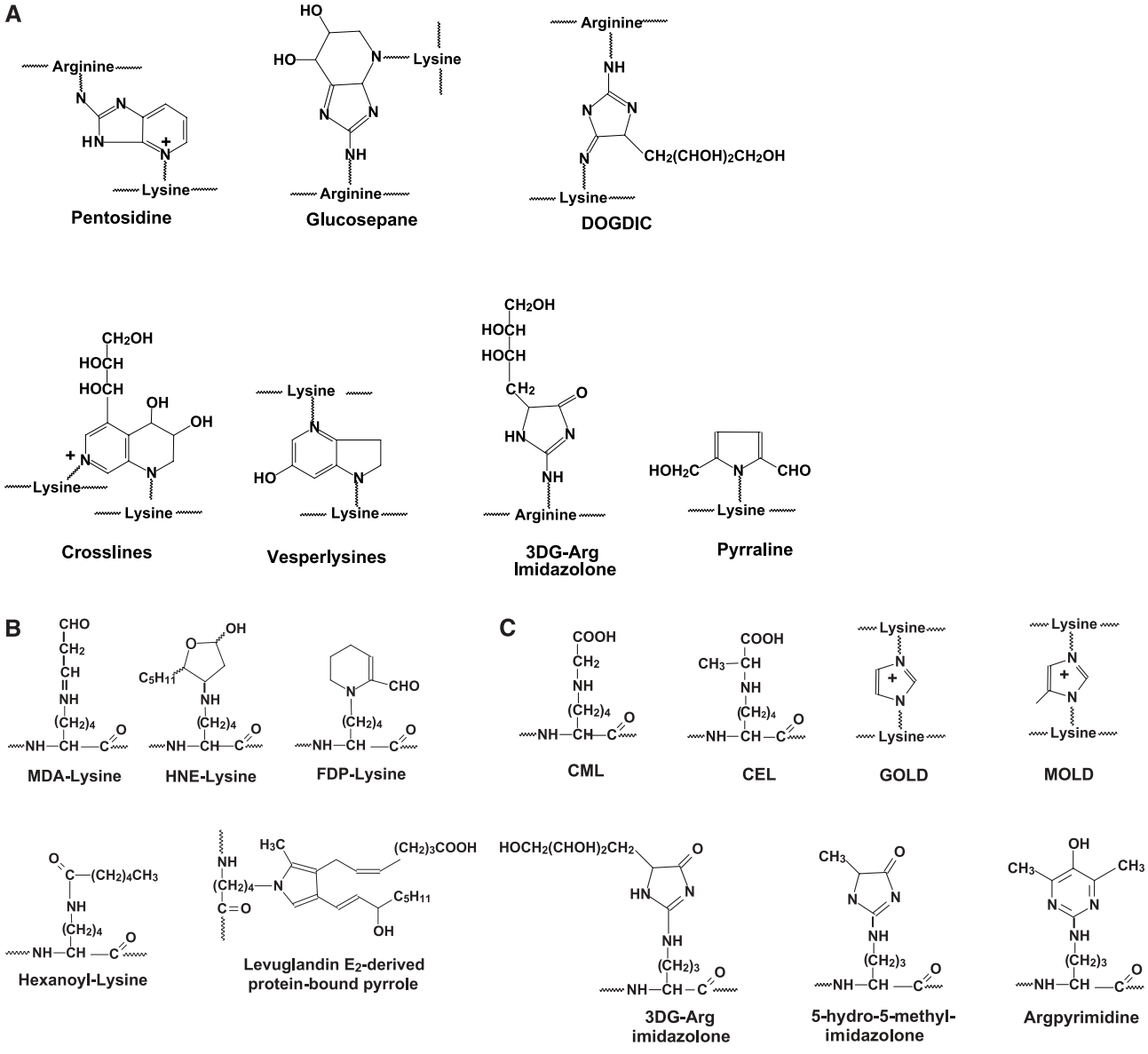

Fig. 2. Structures of (**A**) AGEs, (**B**) ALEs, and (**C**) AGE/ALEs that have been detected in tissue proteins. All of these compounds are derivatives of lysine and arginine residues in protein. It is likely that similar compounds are formed on reaction with other nucleophilic groups in proteins, including the side chains of histidine, serine and threonine, tryptophan and tyrosine. Most recently described structures are cited in the following references: Nakamura et al., 1997; Oya et al., 1999; Uchida et al., 1998; Biemel et al., 2001; Glomb and Lang, 2001

(Baynes and Thorpe, 1999) (Fig. 1). The Amadori adduct formed from glucose *in vivo* undergoes non-oxidative rearrangement and hydrolysis reactions, forming 1- and 3-deoxyglucosones (1DG, 3DG) preserving the carbon skeleton of the sugar. The Schiff base and Amadori adduct also undergo facile oxidation, especially in the presence of transition metal ions, and fragment to yield shorter chain sugars and reactive intermediates, such as glyoxal (GO) and methylglyoxal (MGO). Both glucosone (GLO) and GO are also produced on peroxynitrite-mediated oxidation of glucose (Nagai et al., 2002), and a protein-bound dicarbonyl intermediate in protein crosslinking has also been described (Chen and Cerami, 1993). These reactive dicarbonyl compounds, described as intermediates formed during the second stage of the Maillard reaction, react with lysine and arginine residues in protein to produce a wide range of protein-bound AGEs and crosslinks (Fig. 2) during the third and final stage of the classical reaction pathway. The number of AGEs detected in tissue proteins is now increasing at the rate of 2–3 per year, in part because of development of efficient enzymatic hydrolysis methods for recovery of AGEs that are sensitive to protein hydrolysis in strong acid and base solutions.

In addition to the multiplicity of AGE structures, there are multiple pathways for formation of AGEs from reducing sugars (Fig. 3): some proceed from the Amadori compound (Hodge, 1953), while others proceed from the Schiff base (Hayashi and Namiki, 1987) or by direct autoxidation of carbohydrates (*autoxidative glycosylation*) (Wolff and Dean, 1987). Some AGEs, such as the fluorescent vesperlysines and crosslines (Fig. 2A), retain the intact carbon structure of glucose and appear to be derived directly from glucose. In contrast, formation of pentosidine from glucose requires oxidative cleavage and loss of one carbon atom; analysis of the valency of carbon

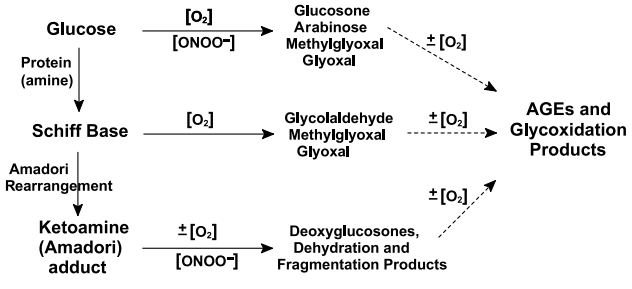

Fig. 3. Alternative pathways of the Maillard reaction from glucose. The formation of AGEs is catalyzed by oxidation and fragmentation of sugars or their adducts to proteins. These reactions are catalyzed by ROS and peroxynitrite

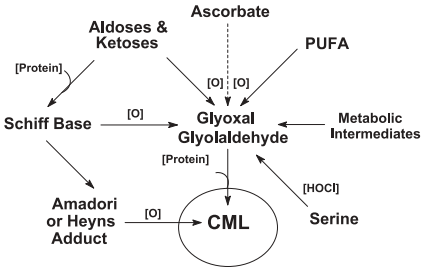

Fig. 4. The AGE/ALE, CML, may be formed from glyoxal or glycolaldehyde, generated by autoxidation of free sugar or sugar adducts to proteins, as in Fig. 3, above. However, there are also a number of alternative pathways for formation of CML, *e.g.* from ascorbic acid, peroxidizing lipids and amino acid degradation products

atoms indicates that formation of pentosidine from pentoses also requires oxidation. In contrast, formation of pyrraline and crosslines from glucose is a non-oxidative process. Other AGEs, such as N^{ε}-(carboxymethyl)lysine (CML) and N^{ε}-(carboxyethyl)lysine (CEL) (Fig. 2C), require oxidative fragmentation of the carbon skeleton of glucose, but may also be formed from other hexoses, pentoses, glycolytic intermediates or ascorbic acid.

The term, glycoxidation product (Baynes, 1991), was originally introduced to characterize products formed by sequential glycation and oxidation reactions. However, some glucose-derived glycoxidation products may be derived from other precursors by non-oxidative routes, *e.g.* the formation of CEL from triose phosphates or MGO, which are products of anaerobic metabolism (Ahmed et al., 1997). CML may also be formed from a variety of non-carbohydrate sources, including lipid (Fu et al., 1996) and amino acid (Anderson et al., 1999) oxidation products (Fig. 4). Thus, the term glycoxidation, although it is useful to describe the synergistic processes of glycation and oxidation, cannot be readily applied to specific products, such as CEL or CML, unless their chemical origin is known. When CML is formed from lipids, it is described as an advanced lipoxidation end-product, and, in those cases where its origin is uncertain, for example in tissue proteins, CML and the related compound CEL, GOLD and MOLD (Fig. 2C), are best described as AGE/ALEs.

Maillard reactions involving lipids – the broader context of the Maillard reaction

Until the late 1970s, the scope of research on the Maillard reaction was limited to nonenzymatic browning reactions of carbohydrates with protein during food processing and storage. When diabetes researchers began to study the

reaction *in vivo*, the focus was initially on the reaction of protein with glucose, and then expanded to include the study of reactions of the protein with ascorbate (or dehydroascorbate) and intermediates in carbohydrate metabolism. However, when foods are cooked or stored, it is the lipids, especially in milk products and meats, that are often the major contributors to the changes in color, taste, aroma and texture of the food product. Similarly, there is increasing evidence that lipids are as important as carbohydrates in the chemical modification of tissue proteins and development of pathology (Baynes and Thorpe, 2000).

Both diabetes and atherosclerosis, two diseases that are intimately linked to one another, may be described as *diseases of concentration*, pathologies resulting from chronically elevated levels of plasma glucose and/or lipids. Hyperglycemia, whether caused by lack of insulin, insulin resistance or diet, or in animal models by amplification of gluconeogenic enzymes or knock-out of GLUT-4 is a risk factor for diabetic complications. Similarly, hyperlipidemia is a risk factor for macrovascular disease, regardless of the genetic or dietary origin of the increase in plasma lipids. There are several animal models of atherosclerosis, *e.g.* knock-out of apolipoprotein E or the LDL-receptor, which are characterized by hyperlipidemia and an increase in susceptibility to atherosclerosis that is exacerbated by lipid-rich diets. The ability to maintain homeostasis and to control substrate concentration in plasma declines with age, consistent with a role for *excess substrate*, carbohydrate or lipids, in both aging and age-related chronic diseases. The common link between these diseases of concentration, diabetes and atherosclerosis, is that the chemical modification of proteins is, in both cases, primarily the result of carbonyl-amine chemistry or, in a broader sense, the reaction of nucleophilic groups on proteins (*e.g.* the side chains of lysine, arginine, histidine and cysteine) with electrophilic carbohydrates and lipids and their derivatives (*e.g.* hydroxyaldehydes, dicarbonyls, hydroxyalkenals and epoxides). The fact that CML and CEL, among the major chemical modifications of tissue proteins, may be derived from GO and MGO, respectively, and that these compounds may be derived from both carbohydrate and lipid precursors, emphasizes the intersection between carbohydrate and lipid chemistry. In both Maillard reactions and metabolism there are common intermediates derived from carbohydrates and lipids. There is another interesting parallel between the nonenzymatic chemistry and the enzymatic metabolism of sugars and lipids – the formation of AGEs, like the metabolism of sugars, may be either non-oxidative or oxidative, *i.e.* anaerobic or aerobic, while the formation of ALEs, like the metabolism of lipids, requires oxidative chemistry to form intermediates.

Metabolism is an important contributor to nonenzymatic glycoxidation and lipoxidation chemistry. Intermediates in carbohydrate metabolism, including products of glycolysis (triose phosphates) and the polyol pathway (fructose or fructose 3-phosphate), are potent modifiers of protein or precursors of reactive intermediates (Hamada et al., 1996). Similarly, in addition to the nonenzymatic formation of isoprostanes, cyclooxygenase and lipoxygenases are important sources of endo- and hydro-peroxide precursors of ALEs in tissues. Thus, Maillard reactions may be seen as unintended, or at least uncatalyzed, chemical reactions of proteins with substrates and intermediates in metabolism. While the above discussion has focused on Maillard reactions of proteins, nucleic acids are an equally attractive, though better protected, target. Nucleic acids are also aggressively repaired by multiple pathways, so that damage does not accumulate with age or in disease (Baynes, 2002).

There are a number of compounds that are uniquely AGEs, *e.g.* pentosidine, crosslines, vesperlysines and 3DG-imidazolones (Fig. 2A). Similarly, there are characteristic compounds that appear to be uniquely ALEs: malondialdehyde (MDA) and acrolein adducts to lysine; hydroxynonenal (HNE) adducts to lysine, histidine and cysteine; hexanoic acid amide derivatives of lysine; and levuglandin adducts to protein (Fig. 2B). Like AGEs, ALEs, such as MDA-Lys and HNE-Lys, have been detected at higher concentration in diseased tissues, particularly in the vascular wall in atherosclerosis and in protein deposits in neurodegenerative and amyloid plaque. In contrast to studies arguing for enzymatic reversal of glycation (Szwergold et al., 2001), there is no evidence for enzymatic reversal of ALE formation. However, there is also no evidence for age-dependent accumulation of ALEs in tissue proteins. The failure to detect a relationship between ALEs and age may be a technical problem. Several of the ALEs, *e.g.* MDA-Lys and HNE-Lys, have residual carbonyl groups which may participate in further reactions, and others, which are labile to acid and base hydrolysis, have not been studied in detail. It is likely, however, that ALEs will be found at high concentrations in lipofuscin, an intracellular, proteolipid age-pigment; both CML and pentosidine have been detected in lipofuscin granules by immunohistochemistry (Kimura et al., 1998).

Other chemical modifications of proteins

In addition to AGEs and ALEs, there are a number of other indicators of chemical damage to proteins in biological systems. These compounds, discussed elsewhere in this volume, are products of oxidation of amino acids in protein (AAOPs). They include oxidized amino acids, such as cystine, glutathionyl-cysteine, adipic acid semialdehyde and 2-oxohistidine, methionine sulfoxide and *o*- and *m*-tyrosine; modified amino acids, such as chloro- and nitro-tyrosine; and crosslinks such as dityrosine. Like AGE/ALEs, AAOPs are formed by a combination of enzymatic and nonenzymatic mechanisms. The oxidants H_2O_2 and $O_2^{\bullet -}$ may be formed by various enzymes, or $O_2^{\bullet -}$ may be formed in a side reaction of mitochondrial electron transport, then dismutate spontaneously or enzymatically to form H_2O_2. NO, a product of nitric oxide synthase may react with $O_2^{\bullet -}$ to form $ONOO^-$, which reacts spontaneously with protein to form nitrotyrosine residues; $ONOO^-$ may also catalyze glycoxidation and lipoxidation reactions (Nagai et al., 2002). Similarly, the formation of HOCl is enzymatic and the chlorination of proteins by HOCl is nonenzymatic. Enzymes, such as cyclooxygenase, lipoxygenases and myeloperoxidase also contribute to formation of lipid peroxides whose oxidation initiates chain reactions forming both ALEs and AAOPs. These reactions are catalyzed by traces of redox-active metal ions (copper or iron) in tissues, but also proceed spontaneously. Arachidonic acid, for example, autoxidizes spontaneously under air in the dark in the absence of metal ions. This nonenzymatic chemistry, leading to formation of AGEs, ALEs and AAOPs, is checked by ubiquitous and redundant detoxification systems for inactivation of reactive carbonyl intermediates and inhibition or quenching of ROS and radical intermediates. When things go wrong, as they do during inflammatory processes when ROS and RNS are generated in high concentrations, it is not surprising that many things go wrong together. Thus, protein deposits in neurodegenerative, amyloid and atherosclerotic plaque are simultaneously rich in AGEs, ALEs, and oxidized and nitrated proteins (Sayre et al., 2001). With the exception of glutathionylation reactions, formation of cystine, and oxidation of methionine to methionine sulfoxide, these modifications appear to be irreversible.

Pathological significance of Maillard reactions

One of the major obstacles to understanding the role of AGEs, ALEs and AAOPs in pathology is the fact that these compounds are present at only trace levels, even in long-lived proteins. Which, if any, of these chemical modifications of protein are important; do they have an individual or a collective impact? Lysine and arginine, the amino acids modified to the greatest extent during Maillard reactions, are commonly located on the surface of proteins and have a limited role in the active site of most enzymes. There may be ''hot spots'' on proteins, *e.g.* lysines adjacent to acidic or basic amino acids that would accelerate site-specific modification of lysine residues in proteins. However, there is limited evidence that such specificity is of any biological consequence. In addition, although AGE-proteins and oxidized lipoproteins prepared *in vitro* are known to induce biological, regulatory responses, these responses are observed only with highly modified proteins, rich in protein carbonyls and lipids peroxides, which are unlikely to be found *in vivo*. Even when AGEs and ALEs are present at high concentration in a tissue, they are not always pathogenic. Older, healthy individuals have levels of AGEs in extracellular proteins comparable to those found in younger diabetic patients with complications, yet older people do not have typical diabetic complications. Overall, the argument for a causative, quantitative role of AGEs in biology and pathology is still an uphill battle.

A role for the Maillard reaction in regulatory biology

For several years now, Maillard researchers have been changing their focus from AGEs formed on extracellular proteins from the relatively inert precursor glucose, to AGEs formed on intracellular proteins from more reactive intermediates (Hamada et al., 1996). These RCS, including glycolytic intermediates such as triose phosphates, MGO, and possibly GO and glycolaldehyde (GA), increase in cells during hyperglycemia. In contrast to the extracellular space, the intracellular space is a reducing environment, rich in cysteine residues that are maintained in the reduced state by an array of reduced intermediates, including thioredoxins, periredoxins, metallothioneins and glutathione (GSH); the latter is present at millimolar concentrations in the cell. The sulfhydryl group of cysteine is more nucleophilic than the side chain amino groups of lysine and arginine. Thus, in the intracellular compartment, sulfhydryl residues are logical targets for modification by RCS. Many of the enzymes involved directly or indirectly in metabolism or detoxification of RCS are thiol enzymes, *e.g.* aldose reductase, glucose-6-phosphate dehydrogenase, glyceraldehyde-3-phosphate

dehydrogenase and acetaldehyde dehydrogenase. Glyoxalase I uses the thiol compound glutathione as a sulfhydryl coenzyme. Like the ionic and electrical gradients across cell membranes, the maintenance of a ''redox gradient'' across the plasma membrane and a reduced intracellular environment is essential for cell survival. Oxygen is sequestered by binding proteins, such as myoglobin, hemoglobin and cytochromes, which control and channelize intracellular oxidative chemistry. When ROS are formed in response to external stresses, intracellular cysteine residues are vulnerable targets.

Szent-Györgyi proposed that MGO and other ketoaldehydes were important regulators of cell growth and division (Szent-Györgyi et al., 1967). He implicated the – SH groups of enzymes in reactions with these dicarbonyl compounds, but, with the tools of his time, was unable to explain at the molecular level the mechanisms involved in carbonyl regulation of metabolism. In support of Szent-Györgyi's hypothesis, we have recently detected what we think is the first member of a new class of intracellular AGEs formed by reaction of small RCS (in this case, GO and GA) with cysteine residues in proteins. S-(carboxymethyl)cysteine (CMC) is formed by nucleophilic addition of the thiol group of cysteine to GO or GA. As shown in Fig. 5, reaction of GO with a sulfhydryl group sets the stage for a Cannizzaro reaction that leads to formation of CMC. Reaction of GA with thiol groups also yields CMC, although the mechanism has not been elucidated. CMC is present in intracellular proteins at absolute concentrations comparable to CML – despite the much lower concentration of cysteine, compared to lysine, residues in protein. CMC is also increased in tissue proteins in diabetes and increase in cells in culture in response to oxidative stress and growth in high-glucose medium (Wang, 2002). Interestingly, monocarbonyl species, such as glucose, form thiohemiacetal adducts to cysteine, which cannot undergo ene-diol, Amadori-type rearrangements and subsequent cleavage reactions, so that CMC appears to be a unique product of reaction of GO (or GA) with protein. Thus, CMC and other Cys-AGE/ALEs may serve as unique integrators of a cell's exposure to dicarbonyl species and provide a historical record of cumulative intracellular oxidative and carbonyl stress.

As an extension of Szent-Györgyi's hypothesis, we propose that the irreversible modification of sulfhydryl groups on intracellular proteins by RCS places limits on the effectiveness of homeostatic, ROS- and sulfhydryl-dependent regulatory processes and on the cell's ability to respond to external stimuli or stresses. These stressors, including inflammation, osmotic or thermal shock, excessive substrate concentration and hormones, are known to induce reversible regulatory responses involving sulfhydryl groups (oxidation to cystine, glutathionylation, nitrosylation). In contrast, irreversible modification of sulfhydryl groups by RCS could increase the background of inert proteins and limit signal transduction, regulatory adaptations and rescue efforts in response to stress. Thus, nonenzymatic Maillard reactions of dicarbonyl intermediates may serve as a link or mechanism by which ketoaldehydes can regulate biological processes, including the cell's commitment to apoptosis or necrosis. We propose that there are key intracellular regulatory proteins or enzymes with reactive thiols that are highly susceptible to modification by ketoaldehydes. These proteins may have slow rates of turnover and may be present in limited quantity in the cell, but their sensitivity to Maillard reactions provides the mechanism for linking the Maillard reaction to regulatory biology. In a recent article, ''From Life to Death, the Struggle between Chemistry and Biology during Aging'' (Baynes, 2000), we argued that accumulation of chemical damage by Maillard and other nonenzymatic reactions was also a major determinant of the natural lifespan of species.

Now that the first product of ketoaldehyde reaction with proteins has been identified, Szent-Györgyi's hypothesis on the regulatory role of ketoaldehydes in cell biology becomes a testable hypothesis. The testing will require the accurate measurement of Cys-AGE/ALEs in tissue proteins and in cells *in vivo*, with emphasis on changes in AGE formation on intracellular, regulatory proteins in response to stress. The use of modern methods in proteomics to identify major thiol proteins that are targets of the

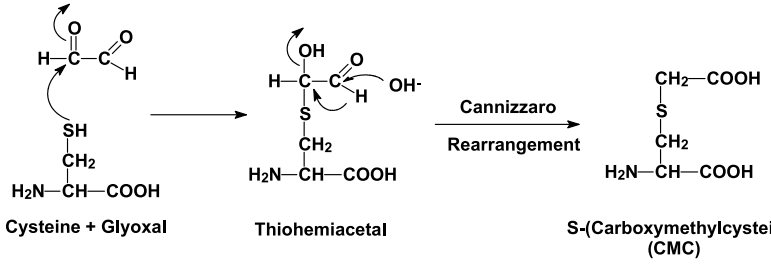

Fig. 5. Structure and proposed mechanism of formation of sulfhydryl-AGE, S-(carboxymethyl)cysteine (*CMC*)

Maillard reaction should lead to a broader understanding of the regulatory role of the Maillard reaction in aging and disease.

Conclusion

The focus of research on the Maillard reaction *in vivo* has changed significantly over the last three decades: from the original focus on glycation, then to structural damage resulting from AGE accumulation; from the original focus on diabetes and aging, to a much broader focus today on the role of AGEs in a wide range of degenerative diseases of aging; from a focus on carbohydrate chemistry, to a broader focus on lipid chemistry and oxidative stress; and from the original focus on damage resulting from Maillard reaction, to an emerging focus on the role of non-enzymatic chemistry in the regulation of metabolism. Evolution has a way of taking advantage of every opportunity, and it is not surprising that biological systems would harness the Maillard reaction for their own advantage. Szent-Györgyi deserves credit for recognizing the relationship between dicarbonyl chemistry and regulatory biology, but much remains to be done to elucidate the role of the Maillard reaction in the intracellular economy.

Acknowledgements

Work in the authors' laboratory was supported by research grants from the National Institutes of Diabetes, Digestive and Kidney Diseases (DK-19971) and the Juvenile Diabetes Research Foundation (JDRF 1-2000-663).

References

Ahmed MU, Brinkmann-Frye E, Degenhardt TP, Thorpe SR, Baynes JW (1997) N^{ε}-(carboxyethyl)lysine, a product of chemical modification of protein by methylglyoxal, increases with age in human lens proteins. Biochem J 324: 565–570

Anderson MM, Requena JR, Crowley JR, Thorpe SR, Heinecke JW (1999) The myeloperoxidase system of human phagocytes generates N^{ε}-(carboxymethyl)lysine on proteins: a mechanism for producing advanced glycation end products at sites of inflammation. J Clin Invest 104: 103–113

Baynes JW (1991) The role of oxidative stress in the development of complications in diabetes. Diabetes 40: 405–412

Baynes JW (2000) From life to death – the struggle between chemistry and biology during aging; the Maillard reaction as an amplifier of genomic damage. Biogerontology 1: 235–246

Baynes JW (2002) The Maillard hypothesis on aging: Time to focus on DNA. Ann NY Acad Sci 959: 360–367

Baynes JW, Thorpe SR (1999) Glycation and advanced glycation reactions. In: Turtle JR, Kaneko T, Osato S (eds) Diabetes in the New Millennium. Endocrinology and Diabetes Research Foundation, Sydney, pp 337–350

Baynes JW, Thorpe SR (2000) Glycoxidation and lipoxidation in atherogenesis. Free Radic Biol Med 28: 1708–1716

Biemel KM, Reihl O, Conrad J, Lederer MO (2001) Formation pathways for lysine-arginine cross-links derived from hexoses and pentoses by Maillard processes. J Biol Chem 276: 23405–23412

Chen HJ, Cerami A (1993) Mechanism of inhibition of advanced glycosylation by aminoguanidine *in vitro*. J Carbohyd Res 12: 731–742

Fu M-X, Requena JR, Jenkins AJ, Lyons TJ, Baynes JW, Thorpe SR (1996) The advanced glycation end-product, N^{ε}-(carboxymethyl)lysine, is a product of both lipid peroxidation and glycoxidation reactions. J Biol Chem 271: 9982–9986

Glomb M, Lang G (2001) Isolation and characterization of glyoxal-arginine modifications. J Agric Food Chem 49: 1493–1501

Golubev AG (1996) The other side of metabolism. Biokhimiia 61: 2018–2039

Hamada Y, Araki N, Koh N, Nakamura J, Horiuchi S, Hotta N (1996) Rapid formation of advanced glycation end products by intermediate metabolites of glycolytic pathway and polyol pathway. Biochem Biophys Res Commun 228: 539–543

Hayashi T, Namiki M (1986) Role of sugar fragmentation in the Maillard reaction. Dev Food Sci 13: 29–38

Hodge JE (1953) Chemistry of browning reactions in model systems. Agric Food Chem 1: 928–943

Kimura T, Takamatsu J, Miyata T, Miyakawa T, Horiuchi S (1998) Localization of identified advanced glycation end-product structures, N^{ε}-(carboxymethyl)lysine and pentosidine, in age-related inclusions in human brains. Pathol Int 48: 575–579

Nagai R, Unno Y, Hayashi MC, Masuda S, Hayase F, Kinae N, Horiuchi S (2002) Peroxynitrite induces formation of N^{ε}-(carboxymethyl)lysine by the cleavage of Amadori product and generation of glucosone and glyoxal from glucose: novel pathways for protein modification by peroxynitrite. Diabetes 51: 2833–2839

Nakamura K, Nakazawa Y, Ienaga K (1997) Acid-stable fluorescent advanced glycation end products: vesperlysines A, B, and C are formed as crosslinked products in the Maillard reaction between lysine or proteins with glucose. Biochem Biophys Res Comm 6: 227–230

Oya T, Hattori N, Mizuno Y, Miyata S, Maeda S, Osawa T, Uchida K (1999) Methylglyoxal modification of protein. Chemical and immunochemical characterization of methylglyoxal-arginine adducts. J Biol Chem 274: 18492–18502

Sayre LM, Smith MA, Perry G (2001) Chemistry and biochemistry of oxidative stress in neurodegenerative disease. Curr Med Chem 8: 721–738

Szent-Györgyi A, Együd LG, McLaughlin JA (1967) Keto-aldehydes and cell division: glyoxal derivatives may be regulators of cell division and open a new approach to cancer. Science 155: 539–541

Szwergold BS, Howell S, Beisswenger PJ (2001) Human fructosamine-3-kinase: purification, sequencing, substrate specificity, and evidence of activity *in vivo*. Diabetes 50: 2139–2147

Uchida K, Kanematsu M, Sakai K, Matsuda T, Hattori N, Mizuno Y, Suzuki D, Miyata T, Noguchi N, Niki E, Osawa T (1998) Protein-bound acrolein: potential markers for oxidative stress. Proc Natl Acad Sci USA 95: 4882–4887

Wang Y (2002) Modification of cysteine residues in proteins: identification and quantitation of sulfhydryl AGEs. M.S. Thesis, University of South Carolina, Columbia, SC

Wolff SP, Dean RT (1987) Glucose autoxidation and protein modification: the potential role of autoxidative glycosylation in diabetes. Biochem J 245: 243–250

Authors' address: John W. Baynes, Department of Chemistry and Biochemistry, Graduate Science Research Center, University of South Carolina, 631 S. Sumter St., Columbia, SC 29208, U.S.A.,
Fax: 803-777-7272, E-mail: baynes@mail.chem.sc.edu