
Review

Biological Perspectives on Maillard Reaction Products

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Preface

In 1912, the French scientist Louis-Camille Maillard (1878-1936) described the reaction between amino acids and reducing sugars. [Maillard LC. **Action of amino acids on sugars. Formation of melanoidins in a methodical way.** *Compt Rend.* 1912; 154: 66-68]. He predicted that the implications of this reaction would fall into three categories: (1) formation of humic substances in soil; (2) browning of cooked foods; and (3) aging of the living body. In the 100 years since, the Maillard reaction has attracted soil chemists, food chemists and biochemists in the predicted research areas, and extensive findings have accumulated.

While the Maillard reaction was originally specified as a reaction between amino acids and reducing sugars, it has since become synonymous with the reaction between amino and carbonyl groups; amino-carbonyl reactions. In general, amino compounds are amines, amino acids, peptides, proteins, nucleic acid bases and phospholipids. On the other hand, carbonyl sources include reducing sugars, aldehydes, ketones, polyphenols, ascorbic acid and steroids. The nonenzymatic glycosylation of proteins via the Maillard reaction is known as glycation.

The Maillard reaction gives rise to a number of complex reactions via dehydration, oxidation, cyclization and scission during heating, and this results in discoloration (browning) of the reaction mixture, which not only occurs in processed and stored foods, but also plays a role in the formation of humic substances in the soil. Since the 1970s, much attention has focused on the Maillard reaction in biological systems from an etiological perspective.

The American scientist John Hodge (1914-1996) proposed a simplified scheme for the Maillard reaction, which remains relevant today and is widely quoted [Hodge JE. **Chemistry of browning reactions**

in model systems. *J Agric Food Chem.* 1953; 1(15): 928-943]. Namiki *et al.* later added the free radical degradation pathway to Hodge's original scheme^{1, 2)}.

The initial stage of the Maillard reaction involves the condensation of a carbonyl group, such as reducing sugar, with a free amino group to form an unstable Schiff base, which spontaneously rearranges into the more stable compound known as the Amadori product. The Amadori product is degraded via various pathways into furfurals, reductones and other fragmentation products. The ultimate stage of the Maillard reaction gives rise to higher polymeric and brown-colored pigments called melanoidins via interaction among reaction intermediates, such as dicarbonyls and amino compounds.

In biological systems, the Maillard reaction is thought to contribute to crosslinking of long-lived proteins, such as lens crystallins and collagen, and to an array of biochemical and biological degeneration pathways in diabetes and aging, leading to complications such as arteriosclerosis, nephropathy, neuropathy, retinopathy and cataracts.

This article reviews recent studies on the biological implications of Maillard reaction products (MRPs) in the following areas:

1. Molecular properties of MRPs
2. Enzymatic activity affected by MRPs
3. Cellular functions affected by MRPs
4. Microbial responses to MRPs
5. Animal experiments related to MRPs
6. Clinical relevance of MRPs

1. Molecular properties of MRPs

Finding of novel oligomers in melanoidin formation

Miura *et al.* discovered a marked blue coloration before the initiation of browning when glycine reacted with excess D-xylose at lower temperatures.

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No blue pigment was seen at the cooking temperature. The molecular weight (MW) of the three blue pigments was estimated to be approximately 800 kDa, 1,200 kDa and 1,600 kDa, and these substances were thought to be consecutive oligomers in the earlier stages of browning³⁾.

The blue pigment formation obeyed the following: $v = k [\text{D-xylose}]^2 \cdot [\text{glycine}]^2$ where v is reaction rate and k is reaction constant. The activation energy was found to be 17.5 kcal/mol, and the transition state theory provided -17.4 cal/deg/mol for the activation entropy⁴⁾.

One of blue pigments from the xylose-glycine system was confirmed to be a dimer of the yellow-colored pyrrolopyrrole-2-carboxaldehyde compounds. This compound, designated as Blue-M1, easily polymerized into brown melanoidins. Blue-M1 also had radical scavenging activity, similarly to melanoidins⁵⁾.

Another blue pigment, designated as Blue-M2, was confirmed to be generated by the reaction between Blue-M1 and di-D-xyloseglycine, and it had a higher MW than Blue-M1. Both of these blue pigments were postulated to be important intermediates in melanoidin formation⁶⁾.

From the xylose-glycine system, two novel red pigments were also isolated; Red-M1 and its diastereomer Red-M2. These red pigments were also assumed to be important intermediates in melanoidin formation. The red pigments showed unique structures, having pyrrolopyrrole, pyrrole and azepine rings, and were thought to form melanoidins by reacting with other pigments, such as Blue-M1 and α -dicarbonyl compounds⁷⁾.

From the 2-deoxy-D-ribose-methylamine system, N-methyl-2-(hydroxymethyl) pyrrole and some derived linear oligomers were detected. In addition, the deformylated dimeric bis-(N-methyl-2-pyrrolyl)-methane and trimeric N-methyl-2,5-bis-(N-methyl-2-pyrrolylmethyl)-pyrrole were characterized. Higher regular oligomers of up to six units of N-methyl-2-pyrrolylmethyl, as well as the corresponding dehydro-oligomers up to 12 units were identified⁸⁾.

Blue pigments were also produced in the glucose-glycine system and xylose-beta-alanine system. These blue pigments consisting of pyrrolopyrrole structures were also readily converted into brown polymers⁹⁾.

In the glucose-fructose-glycine system, glucose-glycine and fructose-glycine reactions proceeded in parallel, sharing a common intermediate, the immediate precursor of melanoidins. Glucose was converted into this intermediate in a two-step process,

whereas fructose was converted in a single step. The effects of glucose and fructose on the rate of browning were found to be synergistic¹⁰⁾.

Physicochemical properties of melanoidins

The melanoidin formed in the glucose-glycine system was an amphoteric polyvalent electrolyte with the isoelectric point at pH 2.5, showing remarkable coagulation in the presence of metallic ions, including Fe^{3+} , Al^{3+} , Cu^{2+} , Zn^{2+} , Co^{2+} and Mn^{2+} . Melanoidin was evaluated as a potent scavenger of metallic ions, and this ionic property was suggested to contribute to its antioxidative role in food¹¹⁾. Thus, melanoidin was assumed to interact with protein as a polyvalent electrolytic polymer and to play a role in affecting the texture of foodstuffs in general. The 10- to 50-kDa melanoidin fraction was found to precipitate ovalbumin at a pH range between the isoelectric points of melanoidin (pH 2.5) and the protein (pH 4.6). Nakatani *et al.* [Annual Meeting of the Agricultural Chemical Society of Japan in Sapporo, July 23, 1975] reported on the stiffening of soybeans after the addition of Shoyu (soy sauce) during cooking. The melanoidins in Shoyu were considered to induce this stiffening in the texture of soybeans via binding with soy proteins¹²⁾. On heating the glucose-glycine system, furans were detected as major volatile products, accompanied by pyrroles, pyrazines, pyridines, and some oxazoles¹³⁾.

A tentative method for measuring brown pigment in food was proposed and applied to 25 commercially available browned foods. The principle was based on the assumption that any brown pigment with general absorption in the visible spectrum was quantitatively equivalent to melanoidin prepared from the glucose-glycine system¹⁴⁾. Melanoidins were quantified by measuring the concentration of incorporated sugar using ^{14}C -labeled sugar. An increase in the number of sugar molecules for each reactive amino group on heating the glucose-casein and fructose-casein mixtures was observed by the radiochemical method¹⁵⁾.

Antioxidant activity of melanoidins

Soy sauce was found to possess a peroxyl radical scavenging activity, thus suggesting the presence of melanoidins¹⁶⁾. The livers of rats fed a brownish "soybean paste" (Miso) or glucose-peptide reaction mixture showed lowered TBA (thiobarbituric acid) and chemiluminescence values when compared with controls. Miso and glucose-peptide products were thus estimated to have antioxidative effects in vivo.

Amadori products, melanoidins, modified proteins and hydrolysates, and brown pigments isolated from Miso and other foodstuffs all showed potent radical scavenging activity¹⁷⁾.

Lager beer was assayed for its melanoidin content and correlations with antioxidant activity. Antioxidant activity, measured as N,N-dimethyl-p-phenylenediamine-dihydrochloride concentration, was strongly correlated with total polyphenol and melanoidin contents¹⁸⁾. Melanoidins, which constitute up to 25% of dry matter in coffee beverages, were also assessed for antioxidant activity against linoleic acid peroxidation¹⁹⁾. No significant differences were found between melanoidins and purified melanoidins in coffee roasted to different degrees. More than 50% of the antioxidant activity of melanoidins is due to low-MW compounds, and their chelating activity was estimated to be related to their antioxidant effects²⁰⁾. The high-MW melanoidins, extracted from coffee, barley coffee, and dark beer, inhibited lipid peroxidation during the simulated gastric digestion of turkey meat, decreasing lipid hydroperoxides and secondary lipoxidation products, and Fe²⁺-chelating capacity and radical-scavenging activity were proposed as antioxidant mechanisms²¹⁾.

Photosensitizing activity of melanoidins

When seven melanoidins from a model system consisting of different amino and carbonyl compounds were irradiated in a photoreactor or exposed to sunlight, varied ultraviolet-visible spectra and photobleaching were observed, and reactive oxygen species were quantified. The data suggest UV-dependent production of singlet oxygen and low levels of superoxide radicals²²⁾.

Glycated proteins

Freshly purified human serum albumin reacted with sodium [3H]borohydride, followed by aminoethylation and tryptic digestion. The amino acid composition of the major peptide was: Thr, Glu2, Ala, Val2, Leu2, Lys, lysino-1-deoxysorbitol. This composition corresponded precisely to residues 525-534 of human serum albumin. Glucitol-lysine, the NH₂-terminal residue of this peptide, was completely resistant to cleavage by trypsin. Thus, lysine-525 was suggested to be the predominant site of nonenzymatic glycosylation of human serum albumin *in vivo*²³⁾.

Fluorescence of the reaction of bovine serum albumin with 3-deoxyglucosone was higher than that with an equivalent amount of glucose. Similarly, the rate of polymerization of lysozyme in the presence of 3-deoxyglucosone was also greater than with glucose,

and collagen incubated with 3-deoxyglucosone was less digestible than collagen incubated with glucose. These results suggest that 3-deoxyglucosone accelerated the advanced stages of the Maillard reaction²⁴⁾.

The formation of advanced glycation end product (AGE) crosslinks by the Maillard reaction was reduced by several drugs (aminoguanidine, OPB-9195, tenilsetam and aspirin), substances of natural origin (carnosine and homocarnosine) and antioxidants. These compounds are considered to have an inhibitory effect on the Maillard reaction, but did not appear to break AGE-derived protein crosslinks formed in patients with a long history of disease²⁵⁾. AGEs are thought to play an important pathophysiological role in diabetes and uremia²⁶⁾. Thus, AGE inhibition by established inhibitors, such as aminoguanidine, and by novel inhibitors specifically acting on post-Amadori pathways of AGE formation was thoroughly investigated. Pyridoxamine and, to a lesser extent, thiamine pyrophosphate proved to be novel and effective post-Amadori inhibitors. AGE inhibition is now showing therapeutic potential for preventing initial sugar attachment or scavenging highly reactive dicarbonyl intermediates²⁷⁾.

2. Enzymatic activity affected by MRPs Trypsin

Melanoidin from the glucose-glycine system was found to exert a potent inhibiting effect on trypsin, and this was comparable to soybean trypsin inhibitor and leupeptin. On the other hand, chymotrypsin was not affected²⁸⁾. The inhibitory effects were found to follow a non-competitive mode and to be exerted through an electrostatic interaction between melanoidin and trypsin. The inhibition mechanism of melanoidins might be due to an allosteric effect, which is different from proteinaceous inhibitors, such as ovomisin. The melanoidin molecule was thought to trap a great number of trypsin molecules, binding them and reducing their activity²⁹⁾. Brown-colored foods, including soy pastes, soy sauces, fish sauce, sauces, teas, coffees, and others, were assayed in association with trypsin inhibition. Most of these were effective in inhibiting trypsin activity, while being ineffective on chymotrypsin, except for black tea and cola. The color intensity of foods did not appear to be correlated with the extent of inhibition, except for soy sauce³⁰⁾. Melanoidins from the glucose-asparagine system inhibited trypsin activity, and this inhibition can be described as a linear/mixed type³¹⁾.

The protein structure of enzymes was found to be altered by the Maillard reaction. Thus, enzyme activity was thought to be affected by covalent modification of various amino acids and by inter- and intramolecular crosslinking³²⁾.

Amylase from human saliva

Melanoidin from the glucose-glycine system was found to have an adverse effect on the activity of alpha-amylase from human saliva. The effect was attributed to non-competitive inhibition. The following factors were suggested to be involved in the inhibitory effects of melanoidin: interaction between the enzyme molecule and melanoidin; the substrate and melanoidin; and Ca ions and melanoidin. Melanoidin was also found to resist the thermal instability of alpha-amylase³³⁾.

Brown-colored foods, including soy paste, soy sauce, sauce and tea, were investigated with regard to their inhibitory effects on alpha-amylase from human saliva. However, no correlations between inhibition and color intensity of food components were confirmed³⁴⁾.

Angiotensin I converting enzyme

The browned solutions resulting from a mixture of glucose-amino acid inhibited the activity of ACE (angiotensin I converting enzyme). In the case of glucose-Trp systems, the ~3-kDa fraction showed the strongest inhibitory effects. Browned processed foods, including soy paste, soy sauce, sauce and tea, were also found to inhibit ACE to varying degrees³⁵⁾.

Food melanoidins (from coffee, beer and sweet-wine) were evaluated based on in vitro ACE-inhibiting activity. Inhibition by coffee melanoidins was significantly higher under more severe heating conditions. This inhibition was partly due to the low-MW compounds bound to the melanoidin structure. These compounds were naturally phenolic compounds present in the green beans or intermediary MRPs³⁶⁾.

Amadoriase

Amadori compounds have been implicated in aging and several chronic diseases. Fructosylamine oxidases (FAOXs) are a relatively new class of enzymes that cleave Amadori compounds and had been found in fungi, yeast and bacteria. FAOXs are expected to have applications in diabetes diagnosis, detergents and food processing³⁷⁾.

3. Cellular functions affected by MRPs

Caco-2 cells

MRPs from the sugar-Lys system protected cultured Caco-2 cells from exposure to H(2)O(2)-, 2,2'-azobis-

(2-amidinopropane) dihydrochloride (AAPH)-, ferrous- and cupric-induced cytotoxicity. High-MW MRPs exhibited stronger antioxidant activity to scavenge hydroxyl and DPPH radicals, and a greater protective effect against both ferrous- and cupric-induced cytotoxicity in Caco-2 cells than corresponding low-MW MRPs³⁸⁾. Melanoidins from the glucose-glycine mixture exhibited modest but significant genotoxic effects in human lymphocytes, particularly low-MW melanoidins in Caco-2 cells³⁹⁾. A significant protective effect against AAPH radical-induced cytotoxicity, and ferrous- and cupric-induced toxicity in Caco-2 cells was observed for sugar-casein MRPs. These protective effects were not significantly different between the sugar-modified caseins and native casein⁴⁰⁾.

Chinese hamster lung V79 cells

Glucosone formed in the Maillard reaction reduced the survival of Chinese hamster lung V79 cells. Inhibition of this reduction in cell survival by catalase and SOD suggest the involvement of active oxygen species, namely H₂O₂ and O₂⁻, in the biological effects of glucosone. In addition, H₂O₂ was formed in the medium during oxidative degradation of glucosone⁴¹⁾.

COS I cells

The blue pigment formed by the Maillard reaction in the xylose-glycine system scavenged hydroxyl radicals, and prevented the autoxidation of linoleic acid in vitro. The pigment also protected COS-1 cells against AAPH-induced toxicity, and prevented AAPH-induced peroxidation of cell membranes. These results suggest that the blue pigment prevents oxidative cell injury, and protects against oxidative stress in living systems⁴²⁾.

Endothelial cells

Microvascular lesions have been ascribed to the accumulation of AGEs, as demonstrated in diabetic retinopathy or renal glomerulosclerosis. On endothelial cells, ligation of receptor for AGE (RAGE) induces the expression of cell adhesion molecules, tissue factors, cytokines such as interleukin-6, and monocyte chemoattractant protein-1. Diabetes-associated vascular dysfunction in vivo can be prevented by blockade of RAGE⁴³⁾. Endothelial cells incubated in hyperglycemia models in vitro and experimental diabetes in vivo exhibit modified vascular collagen. The increased formation of methylglyoxal and ECM glycation in hyperglycemia impairs endothelial cell survival and angiogenesis, and contributes to vascular dysfunction in diabetes⁴⁴⁾.

HCT-15 cells

Melanoidin extracted from the polysaccharide biological response modifier PSK was cultured together with HCT-15 cells derived from human colon cancer and with AGS cells derived from human gastric carcinoma, leading to significant suppression. Thus, melanoidins present in the immunomodulator PSK were shown to have direct tumor cell growth inhibitory effects⁴⁵).

HepG2 cells

Treatment of HepG2 cells with biscuit melanoidins protected the cells against oxidative challenge⁴⁶. Pretreatment of cultured HepG2 cells with digested coffee melanoidin (DCM) completely prevented cell damage, and partly prevented the decrease in GSH and the increase in MDA and GPx evoked by t-BOOH in HepG2 cells. Treatment of HepG2 cells with DCM protected the cells against oxidative insult⁴⁷).

HL-60 cells

Glyceraldehyde-modified bovine serum albumin and glyceraldehyde-modified casein inhibited the proliferation of HL-60 cells. The reaction products formed from glyceraldehyde and N-alpha-acetyllysine also had cytotoxic effects on HL-60 cells. In addition, the reaction products suppressed intracellular glutathione levels, and induced reactive oxygen species (ROS) production. These results suggest that the glyceraldehyde-related AGEs induced cytotoxicity and oxidative stress⁴⁸).

Human dendritic cells

The effects of AGE derived from the model food allergen ovalbumin (AGE-OVA) on dendritic cells (DCs), their immunostimulatory capacity and the T-cell response were compared with those of regular OVA. AGE-OVA was found to be more immunogenic/allergenic than regular OVA⁴⁹).

Human lymphoblasts

One of the major intermediate products in the Maillard reaction, 5-(hydroxymethyl) furfural (HMF), was found to possess mutagenic and DNA strand-breaking activity. The chemically synthesized sulfuric acid ester, 5-[(sulfooxy)-methyl] furfural (SMF), exhibited direct mutagenicity at both the thymidine kinase and hypoxanthine-guanine phosphoribosyl transferase loci in human lymphoblasts⁵⁰).

Mammalian cells

Genotoxicity (SOS repair) was monitored in *Salmonella typhimurium* (umu assay). HMF induced moderate cytotoxicity in V79 cells and in Caco-2 cells. In primary human colon cells obtained from

biopsy material, DNA damage was not measurable. HMF was weakly mutagenic at the hprt-locus in V79 cells, and the genotoxic potential was not altered by addition of rat liver microsomes. These results suggest that HMF does not pose a serious health risk⁵¹). However, the oxidation products generated by consumption of heated food damaged mammalian cells⁵²).

Myofibroblast

Mice cardiac fibroblasts (CFs) were treated with bread crust extract (BCE), which contained significant amounts of various AGEs. BCE induced a moderate elevation of reactive oxygen species (ROS) production and activation of redox-sensitive pathways but did not alter Akt kinase phosphorylation. Expression of smooth muscle alpha-actin and tropomyosin-1, which represent markers for myofibroblast differentiation, was reduced after BCE treatment, thus suggesting a putative antifibrotic effect for melanoidin-rich food⁵³).

T-cells

During roasting, peanut proteins react with sugars to form MRPs, which could affect allergenicity. The products are recognized by IgE and have an inhibitory effect on IgE binding to peanut allergens, thus suggesting that these MRPs are potentially allergenic⁵⁴). Roasted peanuts exhibit higher levels of AGE and MDA adducts than raw peanuts. IgE was partially inhibited in a competitive ELISA by antibodies against AGE, indicating that IgE has an affinity for peanut AGE adducts⁵⁵). After roasting, increased amounts of AGE adducts were seen in peanuts, while high-oleic peanuts did not differ from normal peanuts in IgE binding and T-cell proliferation. It was concluded that high oleic acid contents have no effect on peanut allergenicity⁵⁶).

The glycation of OVA enhanced T-cell immunogenicity of the allergen. Scavenger receptor class A types I and II (SR-AI/II) were confirmed as mediators of AGE-OVA uptake, whereas the receptor for AGEs and galectin-3 were not responsible. The Maillard reaction might therefore play an important role in the T-cell immunogenicity of food allergens⁵⁷).

Tumor cells

One of the major intermediate products in the Maillard reaction, 5-hydroxymethyl furfural (HMF), was found to possess cytotoxic, genotoxic and tumorigenic activities. HMF exhibited higher skin tumor initiating activity than the parent hydroxymethyl compound⁵⁸). An intensely colored Maillard product, 3-hydroxy-4-

[(E)-(2-furyl) methylidene] methyl-3-cyclopentene-1,2-dione, is formed via the glucose-L-proline system in heated food, and was found to potently inhibit the growth of human tumor cells in vitro. In addition, the compound was found to affect the microtubule skeleton⁵⁹).

Vero cells

Fumonisin B(1) (FB(1)) content in corn products is reduced during the heating process in foods containing reducing sugars, mainly due to the formation of N-(carboxymethyl) fumonisin B(1). In monkey kidney cells (Vero cells), cytotoxicity and lipid peroxidation were suppressed by the transformation of fumonisin B(1) to N-(carboxymethyl) fumonisin B(1)⁶⁰).

Others

Melanoidins from the glucose-glycine system, in both the low-MW and high-MW fractions, showed no genotoxicity on Ames test, Vitotox test and micronucleus test. At high concentrations, however, the low-MW fraction showed toxicity, while the volatile fraction produced in this reaction showed genotoxicity in the Vitotox test⁶¹). RAGE is a multiligand member of the immunoglobulin superfamily of cell surface molecules whose repertoire of ligands includes AGEs, amyloid fibrils, amphotericins and S100/calgranulins. RAGE-ligand interaction is a propagation factor in chronic disorders, based on the enhanced accumulation of the ligands in diseased tissues⁶²). Mitochondrial alteration is involved and plays a central role in various oxidative stress-related diseases. The potential application of antioxidants to reduce glycation and to increase antioxidant defence by targeting mitochondria is expected⁶³). The protective activity of coffee against cells is underpinned by the antiradical properties, reducing power, and metal chelating activity⁶⁴).

Tissue

MRPs were produced 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 are indicators of tissue aging and pathology⁶⁵). Collagen molecules in articular cartilage with an exceptionally long lifetime are susceptible to the accumulation of AGEs. In comparison with other collagen-rich tissues, articular cartilage contains relatively high amounts of the AGE pentosidine. Protein turnover is a major determinant in AGE accumulation in different collagen types⁶⁶). Melanoidins obtained from the glucose-arginine system (MW > 3.5 kDa) provokes

concentration-dependent contraction of gastric smooth muscles, whereas muscle relaxation is seen at high concentrations⁶⁷). Most of the total MRPs applied evoked contraction of gastric smooth muscles; among these, the products obtained from the glucose-arginine system produced the most powerful contractions⁶⁸).

Organs

Melanoidins from coffee reacted with salivary nitrite and SCN(−) in the gastric lumen, reducing nitrous acid to nitric oxide⁶⁹). Amino acids (glycine, lysine, alanine) were incubated with glucose and galactose, and the action of the incubation products reduced oxygen radical generation⁷⁰).

4. Microbial responses to MRPs

Intestinal microbes

Every day for 4 months, rats were orally administered a given amount of unfractionated MRPs from the glucose-glycine system. Dietary MRPs caused an increase in the growth of both aerobic and anaerobic *Lactobacilli* in the microflora of the rat. With respect to the other organisms examined, no significant differences in growth were noted⁷¹).

The effects of fecal bacteria were also determined in batch cultures containing melanoidins in order to examine the effects on microbial diversity. Melanoidins affected the growth of gut bacteria during mixed culture growth, causing a non-specific increase in the anaerobic bacterial population. In addition, melanoidins were found to affect the growth of human large-bowel bacteria⁷²).

Escherichia coli were found to grow on fructoselysine at a rate of about one-third of that observed on glucose. Kinase and deglycase activities were both induced when *E. coli* were grown on fructoselysine, and reached activities sufficient to account for the rate of fructoselysine utilization⁷³).

Six ruminally cannulated Angus-cross steers were used to determine effects of supplementing MRPs on acid-resistant *E. coli* and coliform populations. The ruminal populations of *E. coli* and total coliforms decreased at pH 4, lowering fecal pH. Dietary MRPs had limited effectiveness at decreasing acid-resistant coliforms in the rumen and feces of cattle⁷⁴). Melanoidins from different types of bread were investigated for their potential prebiotic activity by static batch culture. Anaerobic bacteria, particularly *Bifidobacteria* strains, were able to use bread melanoidins as a carbon source⁷⁵). Raw, pre-roasted and roasted Cocoa samples were separated into four

different MW fractions (> 30 , 30-10, 10-5 and < 5 kDa) and were tested for their antibacterial effects, which were observed by incubating the fractions with several strains of *Bifidobacteria*, *Enterobacter* and *Escherichia*. All fractions reduced bacterial growth⁷⁶).

Food poisoning

No antimicrobial effects for any glycated form of β -lactoglobulin (BLG) against *E. coli*, *Bacillus subtilis*, *Listeria innocua*, or *Streptococcus mutans* was observed⁷⁷). Glycomacropeptide (GMP), and its conjugates with xylooligosaccharide (XOS) and carboxymethyl dextran (CMD), prepared by the Maillard reaction showed the ability to bind with *Salmonella enteritidis* and enterohemorrhagic *E. coli* O157:H7 (EHEC O157), leading to prevention of intestinal infection. Carbohydrate moieties such as sialic acid in GMP are involved in binding to *S. enteritidis* and EHEC O157. GMP inhibited the adhesion of EHEC O157 to Caco-2 cells in a dose-dependent manner, although it was not a potent inhibitor of the adhesion of *Salmonella*. GMP is expected to be a promising agent for preventing intestinal infection⁷⁸).

Some beverages and foods protect tooth surfaces against *S. mutans* colonization. Barley coffee (BC), a beverage made from roasted barley, interferes with *S. mutans* adsorption onto hydroxyapatite (HA), and has been examined for antiadhesive components⁷⁹). The high-MW (> 10 kDa) fraction of melanoidins displayed strong anti-adhesive properties towards *S. mutans*. It was found that BC and its fractions, at concentrations that were devoid of antimicrobial activity, inhibited *S. mutans* biofilm formation. In general, the melanoidin fraction was more active than the low-MW fraction⁸⁰). The high-MW fraction of water-soluble melanoidins (> 10 kDa) exerted the highest antimicrobial activity against a Gram-negative reference pathogenic bacterium (*E. coli*). Water-soluble melanoidins also killed pathogenic bacteria strains (*E. coli*) by causing irreversible changes in both the inner and outer membranes. Likewise, it allows for interference with biosynthetic processes, such as the inhibition of nutrient transport and macromolecular precursors⁸¹). Coffee melanoidins suppressed the growth of different pathogenic bacteria due to their metal-chelating properties. At low concentrations, melanoidins exerted bacteriostatic activity mediated by iron chelation of Fe from the culture medium, while melanoidins at high concentrations removed Mg from the outer membrane, thereby disrupting the cell membrane⁸²).

Helicobacter pylori

Extracellular urease on the surface of *H. pylori* facilitates gastric mucin-targeted adhesion. The inhibitory activity of MRPs on the microbe was measured using plate-immobilized urease. Various protein-derived melanoidins strongly inhibited urease-gastric mucin adhesion. In particular, dietary melanoidin from the casein-lactose system suppressed colonization of *H. pylori* in mice. Foods containing protein-derived melanoidins are thus expected to be an alternative to preventing *H. pylori* colonization⁸³). Anti-*H. pylori* effects of aminoreductone (AR), an MRP, were also assayed. AR was found to inhibit the growth of all 24 strains tested (19 clinical isolates and 5 isogenic mutants). The bactericidal activity of AR was significantly higher than that of its derived melanoidin, an inhibitor of *H. pylori* urease-gastric mucin adherence. Foods containing AR, such as milk and dairy products, have also been evaluated as sources for preventing *H. pylori* colonization⁸⁴).

Mutagenicity

Three pyrrole MRPs (2-acetylpyrrole, pyrrole-2-carboxaldehyde and pyrrole-2-carboxylic acid) were found to react with nitrite. The reaction mixtures were tested for mutagenicity using *Salmonella typhimurium* strains TA97, TA98, TA100, TA102 and TA104. The methylene chloride extract of the 2-acetylpyrrole/nitrite reaction mixture showed strong mutagenicity in all the strains, both with and without S-9 mix⁸⁵).

Some mutagenic activity was demonstrated using a dichloromethane extract of a glucose-ammonia reaction mixture. Potent mutagens were also isolated from pyrolyzed amino acids or proteins⁸⁶).

A DNA strand-breaking MRP was identified as 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP). DNA strand breaking by DDMP was inhibited by superoxide dismutase, catalase, scavengers for hydroxyl radicals, spin trapping agents and metal chelators, and the breaking was enhanced by Fe(III) ions. DDMP was found to be mutagenic in *Salmonella typhimurium* TA100 without metabolic activation⁸⁷).

MRPs from casein-sugars (glucose, fructose, galactose, tagatose, lactose and lactulose) were compared with regard to mutagenicity of *Salmonella typhimurium* strain TA100. Mutagenicity strongly varied with sugar type. Mutagenicity differed among the sugar-casein systems. Disaccharides showed no mutagenic activity (lactose) or lower mutagenic activity (lactulose)

than their corresponding monosaccharides. Ketose (fructose and tagatose) showed markedly higher mutagenicity when compared with their aldose isomers (glucose and galactose)⁸⁸).

5. Animal experiments related to MRPs

Metabolic transit of MRPs

Dietary melanoidins from the glucose-glycine system increased the specific activities of small intestinal disaccharidases, and simultaneously caused exfoliation of epithelial cells from rat villi. These morphological changes were considered to be caused by mechanical abrasion rather than inflammation. When a sucrose solution containing melanoidins was injected by means of stomach probes, fluctuations in blood sugar levels were reduced. The melanoidins present in the incubation medium were found to have an adverse effect on the uptake of sucrose through the everted sac of the small intestine. There were no differences between basal diet and melanoidins with respect to uptake of sucrose through the everted sac⁸⁹).

In order to elucidate the metabolic pathway of 3DG, [14C]3DG was synthesized from [14C]-glucose, and was then orally and intravenously administered to rats. Two hours after oral administration of [14C]3DG, the percentages of radioactivity in the stomach, small intestine and urine were 3.9, 60 and 6.4%, respectively, while those in the liver, kidney, spleen, blood and CO₂ were less than 0.5%. The absorption rate of 3DG was obviously lower in comparison with that of glucose. Three hours after intravenous administration of [14C]3DG, the percentage radioactivity in urine was 72% and those in the liver, kidney, spleen, blood and CO₂ were less than 1%. It therefore appears that the absorbed 3DG was not biologically utilized by the rats, but was rapidly excreted in the urine. Metabolites of [14C]3DG were also detected in urine by TLC-autoradiography⁹⁰).

Synthesized pentosidine, an AGE, intravenously injected into rats with normal renal function was rapidly eliminated from the circulation through glomerular filtration, but was undetectable in the urine. Pentosidine transiently accumulated in the proximal renal tubules disappeared from the kidney after 24 hours. Free pentosidine (another AGE) was suggested to be filtered by renal glomeruli and reabsorbed into the proximal tubules, where it was degraded or modified, and eventually excreted in the urine⁹¹).

The radical scavenging activity of skimmed milk for 2,2-diphenyl-1-picrylhydrazyl radicals was enhanced by heating, and further increased by fermentation with the *Lactobacillus casei* strain Shirota. In normal hamsters fed these milk products, potential radical scavenging activity per unit dry weight of feces and cecal content was in the order of fermented product-fed hamsters > heated product-fed hamsters > control hamsters, reflecting the radical scavenging activity of the corresponding diets. Some of the radical scavenging activity was suggested to reach the colon⁹²).

For rats and humans, the percentages of orally taken free versus protein-bound fructose-lysine excreted in the urine were found to range from 60 to 80% and 3 to 10%, respectively. Ingested radioactivity absorbed and excreted in the urine was found at levels ranging from 16 to 30% and from 1 to 5% for low-MW melanoidins and high-MW melanoidins, respectively. The metabolic transit was thought to vary based on the mechanisms involved: (i) intestinal degradation by digestive and microbial enzymes; (ii) absorption of these compounds or their degradation products; and (iii) tissue retention⁹³).

The Cost Action 919 program started to investigate the role of melanoidins in food and health in 1999, while the chemical structures of dietary melanoidins were poorly understood. Structure-specific health-promoting effects have been described in terms of their antioxidant and chemopreventive activity in cell culture investigations as well as in animal feeding studies and human trials. Dietary melanoidins have been investigated for their ability to promote glycation reactions in vivo, which are involved in several diseases, such as diabetes mellitus, cardiovascular complications, and Alzheimer's disease. However, further studies are required to distinguish between chemically identified harmful and beneficial melanoidins⁹⁴).

AGEs and aging

With aging, the aorta becomes stiffer and shows reduced elasticity. Ring-shaped samples were prepared from the thoracic aorta of three groups of rats, representing young, adult and old animals. The diameter and stiffness of aortic samples from old rats increased when compared with adult rats, while the aortic wall was reduced by 20% and the elastin of the aortic wall was reduced by 19%. Collagen- and elastin-associated fluorescence was conformed to be a marker of AGEs. Both parameters were increased by 42% and 17%, respectively, in old rats when

compared with adult rats. A positive correlation between collagen-associated fluorescence and maximum stiffness was observed⁹⁵).

Senile cataracts are known to be associated with progressive oxidation, fragmentation, cross-linking, insolubilization, and yellow pigmentation of lens crystallins. The Maillard reaction occurs between the lens proteins and the highly reactive oxidation products of vitamin C. A mouse that selectively overexpresses the human vitamin C transporter SVCT2 in the lens exhibited lenticular levels of vitamin C, and showed 5- to 15-fold higher levels of oxidation products, resulting in a highly compressed aging process and accelerated formation of several AGEs identical to those in the aging human lens⁹⁶).

The addition of a chemically defined AGE (methyl-glyoxal-BSA) to low-AGE mouse chow increased serum levels of AGEs and oxidant stress (OS), demonstrating that dietary AGEs were oxidants that could induce systemic OS. Pair-fed mice were provided with either a CR diet or a high-AGE CR diet in which AGEs were elevated by brief heat treatment (CR-high). Old CR-high mice developed high levels of AGEs coupled with insulin resistance, marked myocardial and renal fibrosis, and shortened lifespan. In contrast, old CR mice had low levels of OS and AGEs⁹⁷).

AGE formation in the living body

A typical fluorophore, designated Maillard reaction product X (MRX), was isolated from the hydrolysate of glycated proteins formed by incubation of bovine serum albumin with glucose. The structure of MRX was determined to be 8-hydroxy-5-methyl dihydrothiazolo [3,2- α]-pyridinium-3-carboxylate, and formation of MRX was confirmed in genetically diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats. Significantly higher levels of MRX were observed from the serum and urinary protein of OLETF rats, suggesting that MRX is a potential biomarker for hyperglycemia⁹⁸).

Levels of glycation (fructose-lysine, FL) and advanced glycoxidation and lipoxidation end-products (AGE/ALEs) were measured in total skeletal (gastrocnemius) muscle and myofibril protein. Levels of FL in total muscle and myofibril protein were 3-5% those in skin collagen. The AGE/ALEs N(epsilon)-(carboxymethyl)lysine (CML) and N(epsilon)-(carboxyethyl)lysine were also significantly lower in total muscle and myofibril protein, approximately 25% of the levels in skin collagen. FL and AGE/ALEs were also elevated in

muscle protein in diabetes⁹⁹).

In diabetes, isolated renal mitochondria produced significantly higher quantities of superoxide and showed evidence of oxidative damage. Administration of aminoguanidine improved mitochondrial respiration and decreased oxidative damage to mitochondrial proteins. Post-translational modifications of mitochondrial proteins by Maillard reaction might represent pathogenic events leading to mitochondria-induced oxidative stress in the kidney in chronic diabetes¹⁰⁰).

Dietary AGEs played a role in the progression of insulin resistance, when C57/BL/KsJ db/db mice were randomly placed for 20 weeks on a diet with either a low AGE content (LAD) or a 3.4-fold higher AGE content (high AGE diet [HAD]), including CML and methylglyoxal (MG). LAD-fed mice exhibited significantly improved responses to both glucose and insulin tolerance tests. When compared with the severe hypertrophy and morphological disorganization of islets in HAD-fed mice, LAD-fed mice had a better-preserved islet structure¹⁰¹).

In the nondiabetic, Zucker obese rats, AGE/ALEs formation was increased two- to three-fold in skin collagen of obese rats when compared with lean rats. Pyridoxamine (PM) inhibited increases in AGE/ALEs in collagen. Lipids are an important source of chemical modification of tissue proteins, even in the absence of hyperglycemia. PM inhibited AGE/ALE formation and hyperlipidemia, and protected against renal and vascular pathology in a nondiabetic model¹⁰²).

Carcinogenicity

Acrylamide, an acknowledged potential genetic and reproductive toxin with mutagenic and carcinogenic properties in experimental mammals, has been detected in various heat-processed foods, and is apparently formed by the Maillard reaction. There have been numerous reports with regard to the correlation between the Maillard reaction and acrylamide, the formation mechanisms, the main pathways of formation and impact factors on formation, including cultivar¹⁰³).

HMF is known to initiate aberrant crypt foci in rat colon, and skin papillomas and hepatocellular adenomas in mice. Its derivative, 5-sulfoxymethylfurfural (SMF), was found to have stronger mutagenicity. HMF injected intravenously was found to be converted to SMF and to reach the circulation. HMF-related carcinogenicity is considered to be mediated by its reactive metabolite SMF¹⁰⁴).

Blood lipids

Melanoidin from the glucose-glycine system affects cholesterol metabolism in rats. Dietary melanoidins over 10 kDa suppress elevation of cholesterol level in both plasma and liver, and lead to decreases in cholesterol levels in feces and cecum contents. On the other hand, fecal recovery of radioactivity from orally ingested 26 [27]-14C cholesterol is promoted by melanoidin, although the radioactive species were not identified. This suggests that cholesterol levels decrease due to interference of intestinal cholesterol absorption¹⁰⁵).

Volatile MRPs, including monomethylpyrazine, 2,3-, 2,5-, and 2,6-dimethylpyrazine, trimethylpyrazine, and tetramethylpyrazine, are metabolized to a corresponding pyrazinoic acid such as non-substituted pyrazinoic acid, 3-, 5-, or 6-methylpyrazinoic acid, 3,5-, 3,6-, and 5,6-dimethylpyrazinoic acid, and trimethylpyrazinoic acid, respectively. Furthermore, pyrrole-2-carboxaldehyde is metabolized to pyrrole-2-carboxylic acid. Non-substituted and 5-methylpyrazinoic acid and 2-furoic and 5-hydroxymethyl-2-furoic acid show significant non-esterified fatty acid-lowering effects, while 5-methyl and 6-methylpyrazinoic acid and 2-furoic acid show significant triacylglycerol-lowering effects in rats¹⁰⁶).

6. Clinical relevance of MRPs

Biomarker for hyperglycemia

Human hemolysate contains several minor components designated HbA1a, HbA1b, HbA1c, which are post-translational modifications of the major hemoglobin component A0. Individuals with diabetes mellitus are known to have elevated levels of HbA1c, hemoglobin modified with a glucose moiety at the NH₂ terminus of each beta chain. HbA1c is formed by the direct reaction of hemoglobin with glucose. Thus, McDonald *et al.* suggested that hemoglobin could serve as a model system for nonenzymatic protein glycosylation¹⁰⁷).

The subfraction HbA1c identified as a minor "abnormal fast-moving hemoglobin band" in diabetic patients during routine screening for hemoglobin variants later turned out to be an important biomolecular marker with clinical and pathological applications. Measurement of HbA1c in diabetic patients is an established procedure for evaluating long-term control of diabetes, allowing assessment of quality of care in diabetic patients. More importantly, HbA1c is the first example of *in vivo* nonenzymatic glycation of proteins. However, much

remains poorly understood with regard to clinical application of AGE inhibitors and breakers¹⁰⁸).

Two-dimensional gel electrophoresis, Western blot, and mass spectrometry have been utilized to identify Amadori-modified plasma proteins in patients with type 2 diabetes and to assess the impact of short-term insulin treatment on glycation of these proteins. Patients with type 2 diabetes exhibit several proteins with higher glycation than in control groups, and insulin treatment reduces Amadori modification of albumin, fibrin, the Ig heavy chain constant region, transferrin, and the Ig light chain¹⁰⁹).

AGEs and aging

Higher levels of the AGE pentosidine have been associated with a stiffer collagen network in cartilage. Pentosidine itself represents < 1 cross-link per 20 collagen molecules. However, the levels are higher in cartilage collagen than in skin collagen. The digestibility of articular collagen by bacterial collagenase decreases linearly with age, in proportion to the extent of glycation. A variety of MRPs accumulate in cartilage collagen with age, leading to greater stiffening of cartilage¹¹⁰). Altered proteins are associated with numerous age-related pathologies, and sources of these altered proteins include glycation and cross-linking by glucose and more reactive metabolites. Glycated proteins may damage mitochondria to increase production of ROS, while highly oxidized/cross-linked polypeptides may resist proteolysis, inhibit proteasome function and induce a permanent stress response¹¹¹).

Diabetic complications

Glycated and lipoxidated proteins and peptides in the body are associated with activation of RAGE. Activation of RAGE, a member of the immunoglobulin superfamily of cell surface molecules, is associated with a series of diseases ranging from allergy and Alzheimer's disease to rheumatoid arthritis and urogenital disorders. Heat treatment, irradiation, and ionization of foods increase the content of damaging molecules. Consumption of antioxidants, particularly polyphenols, apparently counteracts such pathogenesis¹¹²). Analysis of glycated proteins by mass spectrometry has greatly contributed to proteomics, leading to the identification of new markers of disease¹¹³). The pathophysiological effects of α -oxoaldehydes in diabetes and related complications has attracted attention with regard to endogenously produced carbonyls such as methylglyoxal, glyoxal and 3-deoxyglucosone¹¹⁴).

Vascular complications

Thirty-eight diabetic patients with or without kidney disease (KD) and five healthy subjects consumed a single meal comprising egg whites, cooked with (AGE-diet) or without fructose (100 g). Serum and urine samples were monitored for AGE immunoreactivity and for AGE-specific crosslinking reactivity. The AGE-diet produced distinctly elevated levels of serum AGE in direct proportion to amount ingested, and this was correlated with the severity of KD. These results suggest that: (i) renal excretion of orally absorbed AGEs is markedly suppressed in patients with diabetic nephropathy; (ii) daily influx of dietary AGEs includes glycotoxins that may constitute added chronic risk for renal-vascular injury in diabetic patients; and (iii) dietary restriction of AGE food intake may reduce AGEs in diabetic patients and possibly improve prognosis¹¹⁵.

The Maillard reaction acts as an amplifier of oxidative damage in aging and diabetes. Antioxidants can exert beneficial effects in diabetes, and some antidiabetic drugs may have antioxidant properties in addition of their main role on glycemia control¹¹⁶.

AGEs play multifunctional roles in the development of atherosclerosis in NIDDM. AGEs form in low density lipoproteins (LDL), HDL and VLDL particles and increase their oxidative modification. They aid deposition of cholesterol and its esters in macrophages as well as in creation of foam cells. Reaction of LDL-AGE particles with proteoglycans in the blood vessel intima results in its thickening. AGEs promote prothrombotic changes, and they block formation of nitric oxide, which results in impaired vessel relaxation¹¹⁷.

Experimental studies have indicated that the binding of AGEs to RAGE activates cells, particularly monocytes and endothelial cells. Activated endothelial cells produce cytokines, and express adhesion molecules and tissue factors. AGEs may also play a role in increased oxidative stress. The microvascular retinal, glomerular and nerve lesions induced by diabetes are prevented by inhibitors of AGE formation, such as aminoguanidine¹¹⁸.

The interaction between AGEs with their receptors (RAGE) has been implicated in the development of vascular complications. This interaction elicits marked vascular cell changes analogous to those observed in diabetes mellitus¹¹⁹.

In the glycation of human aortic elastin in healthy subjects, two approaches have been attempted: (1) assessment by the fluorescence method in vivo AGEs

in elastins, purified from human aortas, obtained from different age groups; (2) in vitro glycation of elastins from different age groups and investigation of their capacity to form early and late AGEs. Human insoluble elastins were prepared from macro- and microscopic unaltered regions of thoracic aortas, obtained from 68 accident victims, distributed in 15 age groups. The direct assessment of Maillard reaction-related fluorescence in the age groups showed increased fluorescence with age¹²⁰.

Within the vessel wall, collagen-linked AGEs may “trap” plasma proteins, quench nitric oxide (NO) activity and interact with specific receptors to modulate large numbers of cellular properties. AGEs initiate oxidative reactions that promote the formation of oxidized LDL. Interaction between AGEs and endothelial cells, as well as with other cells accumulating within atherosclerotic plaques, such as mononuclear phagocytes and smooth muscle cells, provides a mechanism to augment vascular dysfunction¹²¹.

The formation and accumulation of AGEs progress during normal aging, and at an extremely accelerated rate under diabetes, and are thus involved in the pathogenesis of various diseases, such as diabetic vascular complications and neurodegenerative diseases. Inhibition of AGE formation may thus be a promising target for therapeutic intervention in AGE-related disorders¹²².

There are few epidemiological studies that have shown that glycated hemoglobin, fructosamine, an index of total non-enzymatic glycated proteins in the blood, and glycated apolipoprotein B and other non enzymatic glycated proteins in the blood in non diabetic subjects are associated with cardiovascular diseases. Non enzymatic glycation of protein in the blood is also associated with cardiovascular disease in non-diabetic subjects, and could be used to define dietary risk factors of cardiovascular disease¹²³.

Protein damage by the formation of early glycation adducts is limited to lysine side chain and N-terminal amino groups whereas later stage adducts, AGEs, modify these, as well as arginine and cysteine residues. Metabolic dysfunction in vascular cells leads to increased formation of methylglyoxal, which disproportionately adds to the glycation damage in hyperglycemia. The formation of AGEs is suppressed by intensive glycemic control, and may in future be suppressed by thiamine and pyridoxamine supplementation, and several other pharmacological agents¹²⁴.

Both glycoxidation products and oxoaldehydes

have been implicated in the complications of diabetes, while Amadori products have not been associated with complications in most animal experiments. It is thought that glucose-derived AGEs, such as glucosepane, inflict sustained damage to the extracellular matrix in diabetes and contribute to tissue stiffening and accelerated sclerosis in arteries, kidneys and other organs. Preliminary experiments with nucleophiles show that aminoguanidine and other compounds block glucosepane in vitro¹²⁵⁾.

AGEs act directly to induce cross-linking of long-lived proteins such as collagen to promote vascular stiffness, and thus alter vascular structure and function; on the other hand, AGEs can interact with certain receptors, such as RAGE, to induce intracellular signaling that leads to enhanced oxidative stress and elaboration of key proinflammatory and prosclerotic cytokines¹²⁶⁾.

Direct interaction between MRPs and NO has been suggested as a pathophysiological mechanism involved in enhanced diabetic arteriosclerosis. However, native NO concentrations were not affected by the MRPs tested¹²⁷⁾.

As oxidative stress and inflammation are closely associated with insulin resistance, the AGEs-RAGE system may play a role in the pathogenesis of insulin resistance and the subsequent development of diabetes¹²⁸⁾. AGEs are directly implicated in the development of complications in diabetes such as nephropathy, retinopathy, neuropathy, and other related diseases such as atherosclerosis, heart disease, stroke, and peripheral vascular disease¹²⁹⁾.

In comparison with a steamed diet, a high-heat-treated diet induced significantly lower insulin sensitivity and plasma concentrations of long-chain n-3 (omega-3) fatty acids and vitamins C and E. A diet based on high-heat-treated foods increases markers associated with a risk of type 2 diabetes and cardiovascular diseases in healthy people. Replacing high-heat-treatment with mild cooking may help to positively decrease risk of diabetes mellitus and cardiovascular diseases¹³⁰⁾.

Complications in Alzheimer's disease

The Maillard reaction is also thought to be associated with Alzheimer's disease, as AGEs, such as pyrraline and pentosidine, were demonstrated to be localized in lesions of the disease. Tenilsetam, an antidementia drug, inhibits glucose- and fructose-induced polymerization of lysozyme in vitro. In streptozotocin-induced diabetic rats, AGE fluorescence and pyrraline in the renal cortex and aorta of diabetic rats were

suppressed by administration of tenilsetam¹³¹⁾. Various AGE inhibitors have been developed in recent years, and their mechanism is based on the attenuation of glycoxidation and/or oxidative stress by the sequestration of metal ions, reactive 1,2-dicarbonyl compounds, and reactive oxygen and reactive nitrogen species¹³²⁾.

MG modifies arginine and lysine residues in proteins forming AGEs, which have been identified in vivo and are associated with complications of diabetes and some neurodegenerative diseases. In foodstuffs and beverages, MG is formed during processing, cooking, and prolonged storage. Fasting and metabolic disorders and/or defects in MG detoxification processes cause accumulation of this reactive dicarbonyl in vivo. The intake of low doses of MG over a prolonged period of time can cause degenerative changes in tissues, and can also exert anticancer activity¹³³⁾.

Oxidative stress is a key factor involved in the development and progression of Alzheimer's disease. There are two principal pathways for protein modification: the first involves homolytic hydroxyl radical-like production of protein-based carbonyls; and the second involves electrophilic nitration of vulnerable side chains, particularly the electron-rich aromatic rings of Tyr and Trp¹³⁴⁾.

Nephropathic complications

Perturbation of interactions between cells and extracellular matrix (ECM) in renal glomeruli may contribute to characteristic histopathological lesions in the kidneys of patients with diabetic nephropathy. MG strongly inhibited adhesion of renal glomerular cells, podocytes, and mesangial cells to native collagen IV and laminin-1. Pyridoxamine, a promising drug for treatment of diabetic nephropathy, blocked the inhibition of cell adhesion and integrin binding by MG. The perturbation of integrin-mediated cell-matrix interactions occurs via the modification of critical arginine residues in renal ECM by reactive carbonyl compounds¹³⁵⁾.

Skin collagen glycation and AGEs are considered to predict the risk of progression of microvascular disease. Glycation products were assessed in the skin collagen of 211 Diabetes Control and Complications Trial (DCCT) volunteers starting in 1992, and these patients were followed in the Epidemiology of Diabetes Interventions and Complications study for 10 years. In multivariate analyses, the combination of furosine (glycated collagen) and carboxymethyllysine (CML) predicted the progression of retinopathy and

nephropathy, even after adjustment for mean HbA1c. Glycation and subsequent AGE formation may explain the risk of these complications associated with prior A1C and provide a rational basis for the phenomenon of “metabolic memory” in the pathogenesis of these diabetes complications¹³⁶).

Ocular complications

The AGE glucosepane was found to be the dominant compound in human serum albumin of diabetics, and its levels were correlated markedly with HbA1c¹³⁷).

Allergenicity

Thermal processing provides many beneficial effects, but also brings about major changes in allergenicity. The reaction of protein amino groups with sugars leads to an impressive cocktail of AGE-modified protein derivatives, and may be the most important of these. These compounds are antigenic and many of the important neoantigens found in cooked or stored foods are probably MRPs¹³⁸). The effects of roasting on the allergenic properties of peanut proteins and the contribution of MRPs or AGEs are known. Thermal processing alters specific biophysical and immunological properties of peanut proteins, such as structure, function, solubility, digestibility, immunoglobulin E (IgE) binding and T-cell responses¹³⁹).

Beta-lactoglobulin (beta-LG) in cow's milk (MW, ~18 kDa) is folded into a beta-barrel (or calyx) structure, and is a major allergen in milk. During heating in the presence of reducing sugars, beta-LG is subjected to the Maillard reaction, leading to the formation of melanoidins (brown pigments). The heat-induced denaturation of beta-LG is associated with weaker binding of IgE from CMA patients. Moderate glycation of beta-LG in the early stages of the Maillard reaction has a small effect on recognition by IgE, whereas a high degree of glycation has a clear “masking” effect on the recognition of epitopes¹⁴⁰). Introduction of extensively heated milk and egg proteins into the diets of allergic children may represent an alternative approach to oral tolerance induction¹⁴¹).

Dietary MRPs

Pyrraline[epsilon-(2'-formyl-5'-hydroxymethyl-pyrrolyl)-L-norleucin] belongs to the group of AGEs formed in the Maillard reaction in foods and in vivo. After the first day without dietary restrictions, seven healthy volunteers were asked, starting on the morning of day 2, to ingest a diet virtually free of Maillard compounds (e.g., no cooked or roasted foods, no

baked products, no coffee). Dietary control was stopped on the morning of day 5. The 24-h urine samples for these five days were analyzed for free pyrraline. Urinary excretion of free pyrraline was directly affected by dietary composition. Urinary excretion of pyrraline is strongly dependent on dietary intake¹⁴²). The urinary excretion of MRPs, fructoselysine, pyrraline and pentosidine, was followed for nine days involving 18 healthy volunteers. On day 5, participants were divided into four groups; three receiving a test meal (pretzel sticks, brewed coffee, or custard) containing defined amounts of MRPs, and the fourth group served as a control. The urinary excretion of free pyrraline and fructoselysine were reduced by about 90%, while excretion of pentosidine decreased by about 40%. Thus, differences regarding the excretion rate of individual MRPs point to individual resorption and metabolic pathways¹⁴³).

Restriction of diet-derived AGEs not only blocks the progression of atherosclerosis and renal injury, but also improves insulin resistance in animal models. AGE-poor diets reduce serum levels of inflammatory biomarkers in patients with diabetes or chronic renal failure¹⁴⁴).

Conclusions

The overview of the Maillard reaction appears to be a “labyrinth” under construction, where the pathways are extraordinarily entangled with unknown intermediates, particularly in advanced stages. The entire reaction scheme affects enzymatic or biological controls, leading to extreme complexity and randomization. Despite 100 years of work since Maillard's initial description, vast unknown areas remain.

This review confirms Maillard's third prediction concerning the biological implications, aging and disorders in the living body, in which rapid progress has been made in recent years. Today, beyond Maillard's expectations, the reaction has found itself to be the focus in etiological fields of various degenerative or chronic diseases, including diabetes mellitus, cancer, arteriosclerosis and Alzheimer's disease.

International symposia on the Maillard reaction:

- 1st: Uddevara, Sweden (1979)
- 2nd: Nevada, USA (1982)
- 3rd: Shizuoka, Japan (1985)
- 4th: Lausanne, Switzerland (1989)
- 5th: Minnesota, USA (1993)

6th: London, UK (1997)

7th: Kumamoto, Japan (2001)

8th: South Carolina, USA (2004)

9th: Munich, Germany (2007)

The International Maillard Reaction Society (IMARS) was established in 2004.

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総 説

メイラード反応生成物の生物学的意義とその展望

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L.C. Maillard (仏 1878～1936) は糖とアミノ酸による反応（メイラード反応）を発見した時（1912）、(1) 肥沃土壌における腐植物質の形成 (2) 加熱食品の褐変 (3) 生体の加齢の三つの研究分野の展開を予言した。以来 100 年、彼の予言どおり、おびただしい研究が蓄積されて来た。この総説では、20 世紀末から急速に進展してきたメイラード反応生成物の生体関連研究を以下のカテゴリーに分けて総覧した。

1. 分子的特性
2. 酵素活性に及ぼす影響
3. 生体細胞機能に及ぼす影響
4. 微生物学的応答に及ぼす影響
5. 動物体に及ぼす影響
6. 臨床的意義

非酵素的に進行するメイラード反応は極めて複雑多岐にわたり、その全体像は依然として、あたかも「工事中の迷宮」の観を呈し、膨大な未知の研究領域が残されている。この総説でメイラード反応生成物が、加齢現象、生体における慢性的疾患（糖尿病、ガン、動脈硬化、アルツハイマー氏病など）に関連することを示した。

