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Review

Toxicity of the AGEs generated from the Maillard reaction: On the relationship of food-AGEs and biological-AGEs

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Advanced glycation end products (AGEs) are generated in the late stages of Maillard reaction in foods and biological systems. These products are mostly formed by the reactions of reducing sugar or degradation products of carbohydrates, lipids, and ascorbic acid. AGEs exist in high concentration in foods, but in relatively low concentrations in most of the biological systems. Recently, some AGEs have been reported to be toxic, and were proposed to be causative factors for various kinds of diseases, especially diabetes and kidney disorder, through the association with receptor of AGE (RAGE). It has also been reported that food-derived AGEs (food-AGEs) may not be a causative factor for prooxidation. However, the relationship of food-AGEs and biological-derived AGEs (biological-AGEs) is not clear. In this review, the following issues are discussed: the formation of AGEs in foods and biological systems; identification of the main AGEs in foods and biological systems; absorption of food-AGEs; the effects of AGEs *in vivo*; relationship between food-AGEs and biological-AGEs; possible defense mechanism against AGEs *in vivo* and finally, the problems to be solved concerning the toxicity of AGEs.

Keywords: Advanced glycation end product / Aminocarbonyl reaction / Diabetes / Maillard reaction / Pentosidine

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Maillard reaction [1] is a chemical reaction involving primary and secondary aliphatic amino groups of amino acids, peptides, amines, proteins, some phospholipids, and carbonyl groups of reducing sugars as well as degradation products of carbohydrates, lipids, and ascorbic acid. This reaction occurs readily in foods and biological systems. The reaction products include low molecular products such as aldehydes, ketones, dicarbonyls, acryl amides, heterocyclic amines, advanced glycation end products (AGEs), *etc.* and high molecular products such as melanoidins, modified proteins, *etc*. Since the Maillard reaction products (MRPs) exist abundantly in many foods, the effects of these products on consumers of such foods are of concern.

The Maillard reaction mechanism is generally divided into three stages. In the early stage, condensation occurs between an amino group and a carbonyl group, leading to the formation of a Schiff base followed by an Amadori rearrangement ketosamine. The advanced stage starts with the degradation of Amadori products to reactive carbonyls such as keto-aldehydes, dicarbonyls, reductones, *etc*. In the late stage, the reactive carbonyls react with amino groups of amino compounds to form melanoidins or AGEs. In recent years, toxicity of many of the low molecular weight products from Maillard reaction, such as keto-aldehydes, glyoxal, methylglyoxal, 3-deoxyglucosone (3-DG), heterocyclic amines, and acryl amides has been reported [2, 3].

This review concentrates on the toxicity and properties of AGEs and the relationship between food-derived AGEs (food-AGEs) and biological-derived AGEs (biological-AGEs).

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Abbreviations: 3-DG, 3-deoxyglucosone, **AGEs,** advanced glycation end products; **CML,** *N*-(carboxymethyl)lysine; **CEL,** *N*-(carboxyethyl)lysine; **ELISA,** enzyme-linked immunosorbent assay; **FN3K,** fructosamine-3-kinase; **MG,** methylglyoxal; **GOLD,** glyoxal-lysine dimmer, **GOLDIC,** (2-ammonio-6-([2-[(4-ammonio-5-oxido-5-oxopentyl)amino]-4,5-dihydro-1H-imidazol-5-ylidene]amino)hexanoate), **MOLD,** methyglyoxal-lysine dimmer; **MODIC,** (2-ammonio-6-([2-[(4-ammonio-5-oxido-5-oxopentyl)amino]-4-methyl-4,5-dihydro-1H-imidazol-5-ylidene]amino)hexanoate); **MRX,** 8-hydroxy-5-methyldihydrothiazolo(3,2-alpha)pyridinium-3-carboxylate; **MRPs,** maillard reaction products; **TBA,** thiobarbaturic acid

1 Formation of AGEs in foods and biological systems

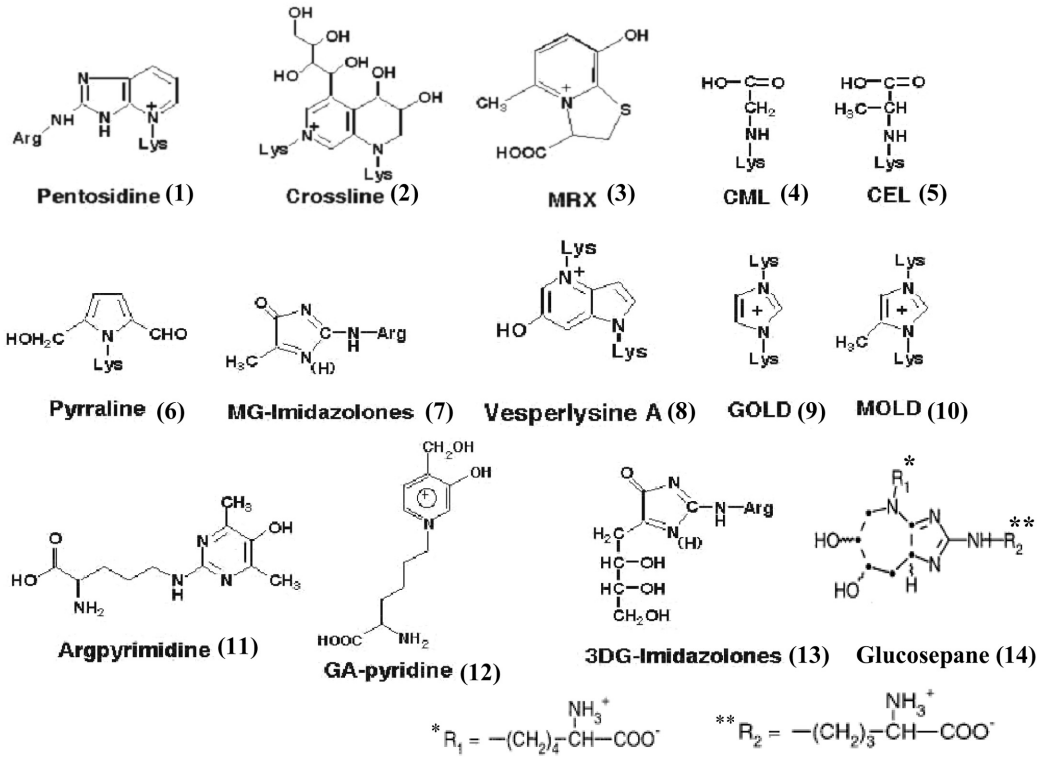
Heating, by either cooking, roasting, or other means is very important in food processing with temperatures ranging from 100 to 250°C. This thermal treatment enhances the formation of Maillard products including Amadori compounds, dicarbonyls, melanoidins, and AGEs.

Figure 1 shows the representative chemical structures of AGEs known to be generated in food as well as in biological systems (http://srv02.medic.kumamoto-u.ac.jp/dept/biochem2/AGE%20website/structure.html).

Pentosidine [4] is a crosslinker generated by the reaction of pentose with lysine and arginine residues of proteins. It is also suggested that ascorbic acid participates in the reaction. Pentosidine was found by acidic hydrolysis of collagen from diabetic rodents and humans [5]. Crossline [6] was found in the reaction solution of *N*-acetyllysine with glucose. This compound was formed by the reaction of two lysine residues and two molecules of glucose; it was found in the serum and kidneys of diabetic patients by immunological assay.

Glyoxal-lysine dimmer (GOLD, the dilysine-imidazolium salt) [7] is formed by the reaction of glyoxal and two molecules of lysine. Methyglyoxal-lysine dimmer (MOLD, the dilysine-imidazolium salt) [8] is formed by the reaction of methylglyoxal (MG) with two molecules of lysine. These two compounds were found to be increased in the serum of urinal patients. Besides Lederer and Klaiber [9], Biemel *et al*. [10] reported the formation of GOLDIC (2-ammonio-6-([2-[(4-ammonio-5-oxido-5-oxopentyl)amino]-4,5-dihydro-1H-imidazol-5-ylidene]amino)hexanoate) by the reaction of glyoxal with two lysine moieties, while MODIC (2-ammonio-6-([2-[(4-ammonio-5-oxido-5-oxopentyl)amino]-4-methyl-4,5-diydro-1H-imidazol-5-ylidene]amino)-hexanoate) is formed by the reaction of MG with lysine and arginine residues of proteins. GOLDIC and MODIC were found to exist in a high concentration in foodstuffs; especially MODIC which exists in a value of 151 mg/kg protein (equivalent to 0.42 mmol/kg protein) in a buttered biscuit sample [10]. Besides, Biemel *et al*. [11] also reported that glucose and sugar derived dicarbonyl intermediates react with HAS to form lysine-arginine crosslinks such as 1,4-dideoxy-5,6-glucosone-derived glucosepane. This compound was also reported to be a major protein crosslink of the human extracellular matrix. Glucosepane was increased up to 500 pmol/mg of collagen, and was increased in diabetes but not old age [12].

MRX [13] was determined to be 8-hydroxy-5-methyldihydrothiazolo(3,2-alpha)pyridinium-3-carboxylate. It was



Modified from http://srv02.medic.kumamoto-u.ac.jp/dept/biochem2/AGE%20website/structure.html.

Figure 1. Chemical structures of some representative AGEs found in foods and biological systems.

found to be formed by the reaction of cysteine and arginine with glucose. MRX was isolated from the hydrolysate of glycated serum albumin. Significant higher levels of MRX were also from serum and urinary proteins of diabetic OLETF (Otsuka Long-Evans Tokushima Fatty) rats in comparison with those of control LETO (Long-Evans Tokushima Otsuka) rats. MRX was estimated to be a potential biomarker for hyperglycemia.

N-(Carboxymethyl)lysine (CML) [14] is formed by the reaction of glyoxal with an epsilon-amino group of lysine. This compound was also identified in skin collagen, lens crystalline, serum, and senescent erythrocyte. This compound was often found when lipid peroxidation had occurred [15]. *N*-(Carboxyethyl)lysine (CEL) [16] is formed by the reaction of MG with lysine, it was also found in lens crystalline. Pyrraline [17] was reported to be generated by the reaction of 3-DG and lysine. Pyrraline was found to have higher concentration in the serum of diabetic patients than those of healthy subjects.

Argpyrimidine (*N*(delta)-(5-hydroxy-4,6-dimethylpyrimidin-2-yl)-L-ornithine) [18] is an AGE derived from the reaction of MG with arginine residue in protein. Argpyrimidine was found at a concentration of 162.40 ± 9.05 pmol/mg protein in familial amyloiddotic polyneuropathy patients, while it was not detected in control subjects.

Imidazolones, such as MG-imidazolones [19], were found to be generated by the reaction of MG with the arginine residue of protein, while 3-DG-imidazolone is formed by the reaction of 3-DG with arginine residue of protein [20]. The 3-DG-imidazolone was observed in kidney tissues of diabetic patients.

Vesperlysines were isolated from hydrolysate of glycated BSA. Vesperlysines A, B, and C [21, 22] were reported to be the crosslinks of two molecules of lysine with C-2 compounds. Ascorbate, D-pentose, and D-threose were precursors of these compounds. Lenticular levels of vesperlysine A increase curvilinearly with age and reach 20 pmol/mg at 90 years. These levels correlate with the degree of lens crystalline pigmentation and fluorescence and are increased with diabetes.

GA-pyridine [23, 24] was shown to generate through the reaction of glycolaldehyde with lysine. Hayase *et al*. [24] reported the formation of a GA-pyridine named GLAP (1-(5-acetylamino-5-carboxy-methyl)-3-hydroxy-5-hydroxymethyl-pyridinium) from the reaction of glyceraldehyde with *N*-alpha-acetyllysine. This compound showed cytotoxicity *in vitro* against HL-60 cells.

The AGE content of selected popular foods in the USA is shown in Table 1 [25, 26]. These include roasted almonds, butter, broiled chicken, broiled beef, bread, homemade pancakes, *etc.* Their AGE content was determined by the measurement of CML using an ELISA based on an anti-CML mAb. Lipid and protein AGEs are presented in units of AGEs *per* gram of food. The average AGE content for each food group is classified as *per* the American Diabetes Association exchange list. As shown in Table 1, foods high in fat and protein content exhibit the highest AGE levels, about 30- and 12-fold higher AGE content than the carbohydrate group. Temperature and methods of cooking or processing appear to be more important for the formation of AGE than the time of cooking or processing. Thus, cooking or processing at high temperatures such as broiling, grilling, frying, and roasting are the major sources of AGEs. According to Uribarri *et al*. [26] based on analyses of the 3-day food records from 90 healthy subjects, most of the people in the USA are consuming an amount of 16 000 ± 5000 AGE kU/day (mean ± SD).

Table 1. AGE content of selected food items

Food item	AGE content
Fats	
Almonds, roasted	66.5 kU/g
Oil, olive	120 kU/g
Butter	94 kU/g
Mayonnaise	265 kU/g
Proteins	
Chicken, broiled (15 min)	58 kU/g
Chicken, fried (15 min)	61 kU/g
Beef, boiled (60 min)	22 kU/g
Beef, broiled (15 min)	60 kU/g
Tuna, roasted (40 min)	6 kU/g
Tuna, broiled (10 min)	51 kU/g
Cheese, American	87 kU/g
Cheese, brie	56 kU/g
Tofu, raw	8 kU/g
Tofu, broiled	41 kU/g
Carbohydrates	
Bread, whole wheat	0.5 kU/g
Pancakes, homemade	10 kU/g
Apples	0.13 kU/g
Bananas	0.01 kU/g
Carrots	0.1 kU/g
Green beans	0.2 kU/g

AGE denotes CML-like immunoreactivity, assessed by ELISA (4G9mAb); From [25].

2 Main AGEs in foods and biological systems

AGEs identified to date in foods and biological systems are listed in Fig. 1. They include crosslinked AGEs with fluorescence such as pentosidine, crossline, MRX, vesperlysine A, GOLD, GOLDIC, MOLD, MOLDIC, glucosepane, and noncrosslinked AGEs without fluorescence such as CML,

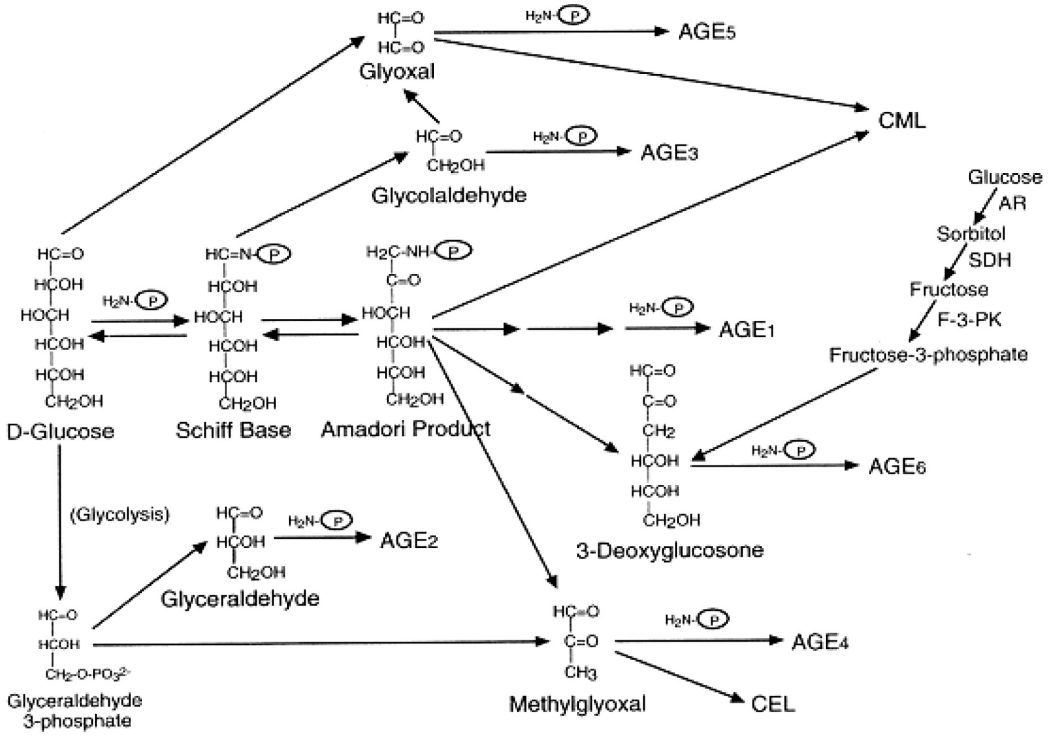


Figure 2. Formation of six distinct AGEs *in vivo*. (P)-NH_2, free amino residue of protein; AR, aldose reductase; SDH, sorbitol dehydrogenase; F-3-PK, fructose-3-phosphokinase [27].

CEL, pyrraline, argpyrimidine, MG-imidazolones, 3-DG-imidazolone, and GA-pyridine.

Beside these AGEs, there are also some other AGEs with structures identified; however, since it is not known whether or not they are present in biological systems they are excluded from the list. These AGEs are generally bound with proteins or high molecular weight compounds. Therefore, even though AGEs are low molecular weight compounds, AGEs mostly bound with macromolecules in foods as well as in biological systems. In recent years, biological-AGEs were thought to be harmful for health and were considered to be causative factors for various kinds of diseases.

Recently, Sato *et al*. [27] proposed and classified these AGEs into six groups as shown in Fig. 2. According to this scheme, AGE-1 group is generated from glucose through Schiff base and Amadori products, AGE-2 from glyceraldehyde, AGE-3 from glycoaldehyde, AGE-4 and CEL from methyl glyoxal, AGE-5 and CML from glyoxal. Takeuchi *et al*. have reported that AGE-2 and AGE-3 are toxic AGEs by contributing to the neuronal cell toxicity [27]. They also proposed that pentosidine, CML, pyrraline, and crossline are nontoxic AGEs [27], however, more evidence is necessary to decide whether their conclusions are valid.

3 The Absorption of food-AGEs

The digestion and absorption of modified proteins were studied intensively by Finot and coworkers [28, 29], Kimiagar *et al*. [30], Oste *et al*. [31], and Chuyen *et al*. [32, 33] in vivo about 20 years ago. At that time, the modified proteins were thought to be just glycated, since the definition of AGEs was not yet proposed. However, with the recent new data, our understanding of the modifications of these proteins has become considerably more sophisticated including the identification of many of the specific AGEs mentioned above.

Recently, Koschinsky *et al*. [34] studied the digestion and absorption of egg cooked with fructose at 90°C for 1–3 h on 38 diabetic patients with or without kidney disease, and five healthy subjects. Serum and urine samples, collected for 48 h, were monitored for AGE immunoreactivity by ELISA and for AGE-specific crosslinking reactivity. The conclusions of this study are that: (i) the renal excretion of orally absorbed AGEs is markedly suppressed in diabetic nephropathy patients, (ii) daily influx of dietary AGEs including glycotoxins appears to constitute an added chronic risk for renal-vascular injury in diabetes mellitus, and (iii) dietary restriction of AGE food intake may greatly

Table 2. Amount of fructoselysine, pyrraline, and pentosidine as supplied with the ingested test meals

	Custard (350 g)	Pretzel sticks (250 g)	Coffee brew (600 mL)
Fructoselysine (mg)	342.0	132.0	ND[a)]
Pyrraline (mg)	0.4	8.4	ND
Pentosidine (mg)	5.0	257.0	12.0
Pentosidine as free amino acid	ND	2.0	9.0

a) ND, not detectable; From [35].

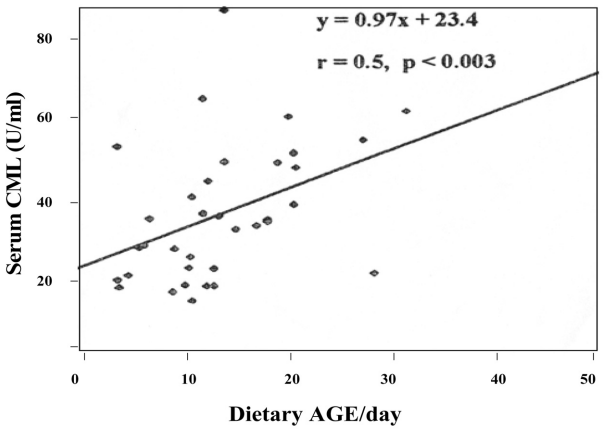


Figure 3. Serum AGE correlates with dietary AGE consumption in a cross-section of patients with chronic renal failure [36].

reduce the burden of AGEs in diabetic patients and possibly improve prognosis. They also concluded that AGEs such as CML, pyrroles, imidazoles, and pyridines are not reactive (nontoxic) and excrete to urine, whereas, AGEs generated from 1 and 3-DG and MG are toxic since they can react with serum or tissue components to form new AGEs that in turn lead to eventual pathological consequences.

Forster *et al*. [35] studied the influence of nutrition on urinary excretion of MRPs such as fructoselysine, pyrraline, and pentosidine on 18 healthy volunteers with a test meal that included pretzel sticks, brewed coffee, or custard containing a defined amount of MRPs. The amount of fructoselysine, pyrraline, and pentosidine present in these test meals is detailed in Table 2.Urine samples taken over a 24-h period were analyzed for MRPs using chromatographic means. Consumption of pretzel sticks and coffee resulted in increased amounts of pyrraline and pentosidine in urine samples. Of the pyrraline present in the pretzel sticks about 50% was recovered in the urine. The recovery of pentosidine was 60% of the ingested free derivative from coffee brew and only 2% of the peptide-bound adducts, indicating a better bioavailability for free pentosidine compared to the protein-bound form. As a result of the MRP-free diet, urinary excretion of free fructoselysine, which was calculated from furosine analysis, was lowered by about 90%. Besides, for fructoselysine ingested with the test meals, no effect on the corresponding urinary excretion was observed. In a study on the relationship between ingested AGEs and serum AGEs, Vlassara [36] reported a strong association between dietary AGEs and serum AGE levels in patients with chronic renal failure (Fig. 3). Finally, Somoza *et al*. [37] found that AGEs such as CML, CEL, and pentosidine from bread crust activate p38-MAP kinase. From a comparison of healthy control and 5/6 subtotally nephrectomized rats (Table 3), it is clear that dietary AGEs are readily absorbed into the plasma, liver, and kidneys and then rapidly excreted to urine. Thus, overall we can safely conclude that those dietary AGEs such as CML, CEL, pentosidine, *etc*. are absorbed readily *in vivo*.

4 Effects of AGEs *in vivo*

To date, the toxicity of AGEs in biological systems was mostly reported by Vlassara [36]. Based on animal studies, the injurious impact of diet-derived AGEs to vascular and kidney tissues is estimated to rival or even exceed that caused by hyperglycemia or hyperlipidemia. They also considered that restriction of AGEs was associated with suppression of several immune defects, insulin resistance, and diabetic complication.

They propose that AGEs and the analogous lipoxidation end products (ALEs) exert their toxic effects by contributing to oxidant and carbohydrate stress by enhancing redox-sensitive transcription factor activity, and by impairing innate immune defense that lead, over time, to inappropriate inflammatory responses. Vlassara *et al*. [38] also reported that inflammatory mediators were induced by dietary glycotoxins (toxic AGEs), a major risk factor for diabetic angiopathy. In agreement with the conclusions of Vlassara *et al.*, Zheng *et al*. [39] have reported that a diet low in AGEs prevent diabetic nephropathy in mice. They used a standard mouse food (AIN-93G) rich in AGEs in parallel with a similar diet that contained six-fold lower AGEs content but equal calories, macronutrients, and micronutrients for nonobese diabetic mice with type 1 diabetes (T1D) and db/db mice with type 2 diabetes (T2D) for either 4 or 11 months. They concluded that the intake of high level of food-AGEs is a major contributor to diabetic nephropathy in T1D and T2D mice. Similarly, Lin *et al*. [40] reported that lowering of dietary AGEs reduces neointimal formation after arterial injury in genetically hypercholesterolemic mice. In another study, Uribarri *et al*. [41] reported that dietary glycotoxins (CML- and MG-derived AGE) correlate with circulating AGE levels in renal failure patients. They proposed that the toxicity of dietary glycotoxins was

Table 3. Content of AGEs in plasma, liver, and kidney of healthy control and 5/6 subtotally nephrectomized rats fed on either 25% w/w wheat starch or bread crust for 6 wk

	Control rats			Subtotally nephrectomized rats		
	+WS	+BC	ΔWS *vs.* BC (%)	+WS	+BC	ΔWS *vs.* BC (%)
Plasma						
CML	31.7 ± 4.57	41.9 ± 8.31	32.9	32.66 ± 4.51	41.8 ± 3.56	28.9
CEL	4.66 ± 0.66	5.80 ± 0.29	24.5	4.16 ± 0.38	4.18 ± 0.36[a)]	0.4
PENT	0.17 ± 0.02	0.31 ± 0.03[b)]	82.4	0.27 ± 0.02[c)]	0.48 ± 0.04[a,b)]	77.8
Liver						
CML	23.8 ± 1.36	21.5 ± 0.89	−9.66	18.5 ± 1.17	25.0 ± 1.29[d,e)]	35.1
CEL	27.3 ± 1.79	24.7 ± 7.68	− 9.5	17.2 ± 0.99[a)]	23.7 ± 1.41[b)]	37.8
PENT	0.67 ± 0.11	0.56 ± 0.08	−16.4	0.48 ± 0.05	0.71 ± 0.13	47.9
Kidneys						
CML	55.7 ± 2.42	39.3 ± 1.88[f)]	−29.4	51.9 ± 1.58	34.8 ± 1.45[f)]	−32.9
CEL	11.1 ± 0.61	11.4 ± 0.48	2.7	12.0 ± 0.41	10.1 ± 0.35[b)]	−15.8
PENT	2.06 ± 0.22	1.66 ± 0.13	−19.4	2.83 ± 0.16[e)]	1.64 ± 0.11[b)]	−42.1

WS, wheat starch; BC, bread crust; CML, N^{ε}-(carboxymethyl)lysine (μmol/mol lysine); CEL, *N*-(carboxyethyl)lysine (μmol/mol lysine); PENT, pentosidine (μmol/mol lysine).
Results are given as mean ± SEM; n = 10 *per* group; From [37].
a) $p < 0.01$ *vs.* control group on the same diet.
b) $p < 0.01$ *vs.* the same group on the WS diet.
c) $p < 0.05$ *vs.* control group on the same diet
d) $p < 0.05$ *vs.* the same group on the WS diet.
e) $p < 0.05$ *vs.* control group on the same diet.
f) $p < 0.001$ *vs.* the same group on the WS diet.

due to interactions between AGEs and the RAGE. Takeuchi *et al*. [42] confirmed the existence of glyceraldehyde-derived AGE in Alzheimer's disease. They also studied on the acetaldehyde-derived AGE and proposed that this AGE is an important toxic moiety for neuronal cells in alcoholism [43].

Receptors for AGEs were believed to play a critical role in AGEs related biology and the pathology associated with diabetic complications and aging disorders [44–47]. Some known popular receptors are: the RAGE [48], oligosaccharyl transferase-48 (AGE-R1) [49], galectin-3 (AGE-R3) [50], CD36 [51], and macrophage scavenger receptor types I and II [52].

Yamamoto *et al*. [44] created transgenic mice that overexpress human RAGE in vascular cells, and crossbred them with another transgenic line which develops insulin-dependent diabetes early after birth. They found that the resultant double transgenic exhibited accelerated kidney changes compared with single transgenic littermales. From these results, they concluded that RAGE engagement by AGE plays an active role in the development of diabetic nephropathy, and the AGE-RAGE system is an effective target for the intervention of this disease.

In our own studies, we investigated the effect of glycated proteins on diabetic rats [53] using glycated casein (GC) and glycated soy protein (GS). A mixture of 20% glycated proteins (GC/GS = 1:1) diet was fed to streptozotocin diabetic rats for 11 wk. Our results indicate that (i) fructoselysine was readily detectable in the hepatic portal veins, arteries, and femoral veins of rats fed with glycated proteins after 2 h of feeding; (ii) blood sugar of glycated protein-fed rats was lower than that of diabetic rats fed with intact protein, while hemoglobin A1C (HbA1C) in blood and glucose in urine of both groups were similar; (iii) lipid peroxidation status in serum, liver, and kidney of both groups was similar (Table 4); (iv) superoxide dismutase (SOD) and glutathione-*S*-transferase (GST) enzymatic activity in erythrocyte and liver of both groups were also similar; (v) there were no significant differences in the degree of cataract formation and concentration of glucose, fructose, sorbitol, and lipid peroxide in the lenses of both groups (Table 5). Our results with this model are somewhat discordant with the conclusions of Vlassara *et al*. since it appears that, in our studies, food-AGEs did not accelerate the glycation or oxidation in biological systems, and the reactive oxygen species that increase in diabetic rats is not caused by glycated proteins, but instead by other pathways.

Another aspect of the effect of dietary AGEs is the fact that proteins modified by Maillard reaction are lower in nutritional value, since amino acid residues in the proteins, such as lysine, arginine, tryptophan, and methionine, are

Table 4. Chemiluminescence (CL) intensity and lipid peroxide (MDA) of liver and kidney of diabetic rats

	Liver		Kidney	
	MDA (nmol/mg protein)	CL intensity (count/min)	MDA (nmol/mg protein)	CL intensity (count/min)
Control group	22.8 ± 8.5	3161.3 ± 399.1	1.1 ± 0.3	8277.9 ± 965.8
Glycated protein group	27.5 ± 7.8	3266.6 ± 1439.0	1.6 ± 0.9	8203.2 ± 954.0

Values are mean ± SD ($n = 8$). CL intensity of liver and kidney homogenate were measured at 40°C by CL counter (CLD-110, Tohoku Electronic Industrial, Japan); From [53].

Table 5. Glucose, fructose, sorbitol, and lipid peroxide in the lens of diabetic rats

	Glucose (mg/g lens)	Fructose (mg/g lens)	Sorbitol (mg/g lens)	Lipid peroxide (nmol MDA/g lens)
Control group	22.3 ± 14.4	16.9 ± 10.0	16.9 ± 10.0	7.56 ± 2.78
Glycated protein group	25.1 ± 15.2	17.7 ± 8.9	17.7 ± 8.9	6.79 ± 2.43

Values are mean ± SD ($n = 8$). Glucose, fructose and sorbitol were measured by the enzymatic method. Lipid peroxide was measured by spectrofluorophotometer with excitation at 515 nm and emission at 553 nm; From [53].

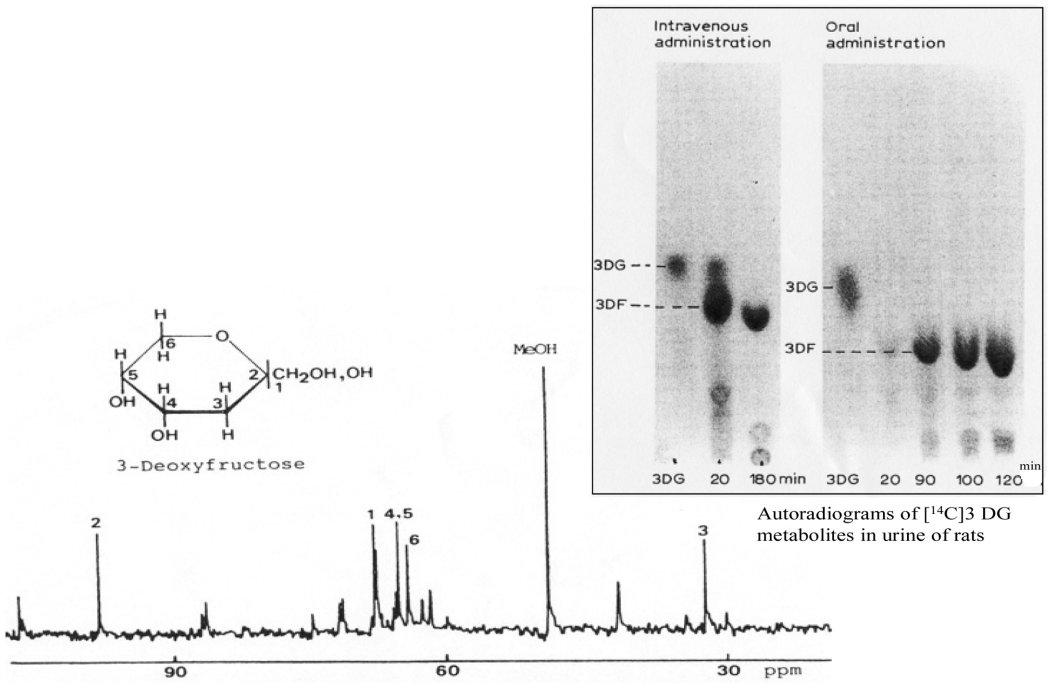


Figure 4. ^{13}C NMR spectrum (complete decoupling) of the purified main 3-DG metabolite [61].

decreased and crosslinks are formed. Besides, the digestibility of modified proteins are also considered to be decreased. Consequently, we studied [54] the digestibility of glucose modified lysozyme *in vitro* and *in vivo*, and found that when lysine residue of lysozyme was modified by glucose at a level of 60%, the digestibility of this modified protein was decreased *in vitro* with pepsin-pancreatin. However, *in vivo*, the digestive peptide patterns of native or modified lysozymes in the rats small intestine were also similar to that of the control dietary AGEs, and can also have beneficial effects. Thus, when browned casein or browned soy-bean was fed to rats for 6 wk, liver thiobarbaturic acid (TBA) of rats fed with browned casein or browned soy-protein were significantly of lower value than that of the control (Table 6) [55]. From these results, it appears that browned protein showed antioxidative effect *in vivo*. In confirmatory studies, it was found that the digestive products of browned proteins, such as peptides, showed

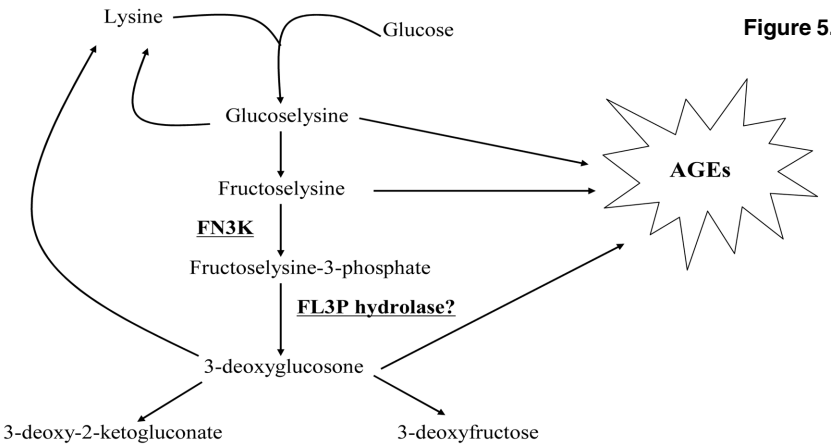


Figure 5. Deglycation theory of Szwergold *et al.* [59].

FN3K (Fructosamine-3-kinase); **FL3P** (Fructolysine-3-phosphate)

strong scavenging activity against reactive oxygen species [56, 57].

In another example of the beneficial effects of dietary AGEs, Hiramoto *et al*. [58] also studied melanoidin, a casein-lactose derived advanced MRP. They found that this lactose modified casein strongly inhibited urease-gastric mucin adhesion in the concentration range of 10 μg/mL. This modified protein suppressed colonization of *Helicobacter pylori* in mice when given for 10 wk *via* the diet. Furthermore, 8 wk daily intake of 3 g lactose-modified casein significantly decreased the OD of HpSa (*H. pylori* stool antigen) in two volunteer males and 12 females. From these results, it appears likely that that lactose-modified casein can be used as a dietary factor for the prevention of *H. pylori*.

5 The defense mechanism against AGEs *in vivo*

As mentioned above, Maillard reaction appears to play an important role in the development of diabetic complications. The reaction starts from the formation of glucoselysine (Schiff base) followed by fructoselysine (FL, Amadori product). Then, Amadori product will degradate to form reactive dicarbonyl compounds such as 3-DG, glyoxal, MG, *etc*. that can again react with amino compounds leading to the generation of AGEs.

Consequently, defense mechanism against the Maillard reaction such as the deglycation of protein-bound Maillard products and the detoxification of dicarbonyl compounds to nonreactive compounds would be beneficial.

Recently, Szwergold *et al*. [59] proposed that intracellular nonenzymatic glycation is controlled by an enzymatic deglycation process, catalyzed by fructosamine-3-kinase

Table 6. TBA values[a)] of rats fed with browned casein, browned soy-protein or control

Group	TBA value (nmol MDA/1 g wet liver)
Browned casein	71.5 ± 4.4
Control casein	95.8 ± 4.2[b)]
Browned soy-protein	74.0 ± 3.8
Control soy-protein	95.9 ± 4.0[b)]

MDA, malondialdehyde; From [55].
a) mean ± SE ($n = 5$).
b) statistically significant ($p < 0.01$) against control.

(FN3K). FN3K phosphorylates FL to fructolysine-3-phosphate (FL3P) and then decompose and generate an unmodified lysine residue. With the purification, sequencing, and cloning of FN3K [60] the concept of enzymatic deglycation has received considerable experimental support. Szwergold *et al*. proposes a new theory on the development of diabetic complication, *i.e.*, the nonenzymatic glycation and the enzymatic deglycation hypothesis. They postulated that enzymatic deglycation is an essential defense system in mammalian cells. In diabetes, this system is stressed and often overwhelmed by the phenomenon of extreme hyperglycemia during which nonenzymatic glycation preferentially proceeds. Thus, for the consequence of diabetic complication there are at least two factors: severity of the hyperglycemic stress and ability of the deglycating system to cope with that stress. The hypothesis of Szwergold *et al*. is shown in Fig. 5.

During the advanced stage of the Maillard reaction, Amadori products are decomposed into 3-DG which is a highly reactive 2-oxoaldehyde, and can attack lysyl, arginyl, and tryptophanyl residues of proteins, leading to the formation of AGEs. We studied on the metabolism of [^{14}C]3-DG by administration of [^{14}C]3-DG to rats orally or intravenously, and found that 3-DG was rapidly reduced to 3-deoxyfruc-

tose [61] (Fig. 4). Later, a new enzyme, NADH-dependent 2-oxoaldehyde reductase, was isolated from porcine kidney by Hayase *et al*. [62]. This enzyme catalyzes the reduction of reactive 3-DG to nonreactive 3-deoxyfructose, and reactive MG to nonreactive acetol. Thus, we postulate that this enzyme prevents the advanced stage of Maillard reaction as a selfdefense enzyme in biological systems.

It has been known that glyoxalase I system [63], a system existing in the cytosol of cells, catalyses the conversion of reactive alpha-oxoaldehydes into the corresponding alpha-hydroxyacids. This system was reported to have a critical role in the prevention of glycation reactions mediated by MG, glyoxal, and other alpha-oxoaldehydes *in vivo.*

For the defense against MRPs *in vivo,* peritoneal macrophages and sinusoidal liver cells are also of concern. Takata *et al*. [64] reported the plasma clearance of aldehyde (formaldehyde, glycolaldehyde) modified protein (BSA) by macrophages through a scavenger receptor in rats. The clearance of ^{125}I-AGE-BSA rapidly occurred throughout 12 min. Shinoda *et al*. [65] also reported the uptake of proteins modified [BSA] with 3-DG by the type I macrophage scavenger receptor of murine peritoneal macrophages. These data provide evidence for the biological importance of the scavenger receptor in eliminating senescent macromolecules from the circulation.

6 Challenges

As mentioned above, Maillard reaction occurs readily in the food system as well as in biological systems; this reaction leads to the formation of AGEs. These AGEs exist in foodstuffs in a large quantity, in mg% of foods, while they exist in biological systems in a very low quantity, in pg%.

Food-AGEs were absorbed readily in to the blood stream and a part of them were excreted in urine and were observed. To date, there are substantial number of papers concerning AGEs in food and biological systems. Some of the papers concluded that AGEs such as glyceraldehyde or glycolaldehyde-derived AGEs, glyoxal- and MG-derived AGEs are toxic *in vivo*, while pentosidine, CML, CEL, pyrraline, *etc.* are nontoxic; however, it seemed that these data are no conclusive, especially given the fact that dietary AGEs also appear to have some beneficial effects.

People in Asian countries consume a large quantity of AGEs daily through soy-paste, soy-sauce, cooked, fried, and roasted food coffee, *etc*.

Therefore, we think that for better understanding of the role of dietary AGEs in that population as well as elsewhere, the following questions have to be addressed.

(i) Are the endogenous AGEs in biological systems a result of aging and diseases or are they causative factors for aging and diseases?

(ii) What are the relationships between dietary AGEs and endogenous-AGEs and how are they modified by diets and physiological state of the individual consuming these AGEs?

(iii) Which food-derived AGEs are toxic in biological systems, and what are the mechanisms of this toxicity?

(iv) If food-AGEs are toxic in biological systems, what are the limits for consumption of such toxins and how can we minimize their uptake?

(v) What are the relationships between dietary-AGEs, endogenous-AGEs, and the antiglycation defense mechanisms?

For improvement of the quality of life, and the prevention and/or control of noncommunicable diseases such as diabetes, we need the answers to these questions, or at least we must address them. Effective studies of these issues will require an increased cooperation between food scientists and medical researchers as well as development of new models and paradigms.

7 References

[1] Maillard, L. C., *C. R. Acad. Sci.* 1912, *154*, 66 – 68.

[2] Wong, J. W., Shibamoto, T., in: Ikan, R. (Ed.), *The Maillard Reaction*, Wiley Baffins Lane, Chichester 1996, pp. 129 – 159.

[3] Mottram, D. S., Wedzicha, B. L., Dodson, A. T., *Nature* 2002, *419*, 448 – 449.

[4] Sell, D. R., Monnier, V. M., *J. Biol. Chem.* 1989, *264*, 21597 – 21602.

[5] Grandhee, S. K., Monnier, V. M., *J. Biol. Chem*. 1991, *266*, 11649 – 11653.

[6] Nakamura, K., Hasegawa, T., Fukunaga, Y., Ienaga, K., *J. Chem. Soc. Chem. Commun.* 1992, *14*, 992 – 994.

[7] Wells-Knecht, K. J., Brinkman, E., Baynes, J. W., *J. Org. Chem.* 1995, *60*, 6246 – 6247.

[8] Nagaraj, R. H., Shipanova, I. N., Faust, F. M., *J. Biol. Chem.* 1996, *271*, 19338 – 19345.

[9] Lederer, M. O., Klaider, R. G., *Bioorg. Med. Chem.* 1999, *7*, 2499 – 2507.

[10] Biemel, K. M., Budler, H. P., Reihl, O., Lederer, M. O., *Nahrung* 2001, *45*, 210 – 214.

[11] Biemel, K. M., Reihl, O., Conrad, J., Lederer, O. M., *J. Biol. Chem.* 2001, *276*, 23405 – 23412.

[12] Sell, D. R., Biemel, K. M., Reihl, O., Lederer, O. M. *et al*., *J. Biol. Chem.* 2005, *280*, 12310 – 12315.

[13] Oya, T., Kumon, H., Kobayashi, H., Akiba, M. *et al*., *Biochem. Biophys. Res. Commun.* 1998, *246*, 267 – 271.

[14] Fu, M. A., Requena, J. R., Jenkins, A. J., Lyons, T. J., *J. Biol. Chem.* 1996, *271*, 9982 – 9986.

[15] Glomb, M. A., Monnier, V. M., *J. Biol. Chem.* 1995, *270*, 10017 – 10026.

[16] Ahmed, M. U., Brinkmann-Frye, E., Degenhardt, T. P., Thorpe, S. R., *Biochem. J.* 1997, *324*, 565 – 570.

[17] Hayase, F., Nagaraj, R. H., Miyata, S., Njoroge, F. G., Monnier, V. M., *J. Biol. Chem.* 1989, *264*, 3758 – 3764.

[18] Gomes, R., Sousa Silva, M., Quintas, A., Cordeiro, C. *et al*., *Biochem. J.* 2005, *385*, 339 – 345.

[19] Schmarzenbolz, U., Henle, T., Haebner, R., Klostermeyer, H., *Z. Lebensm. Unters. Forsch. A* 1997, *205*, 121 – 124.

[20] Konishi, Y., Hayase, F., Kato, H., *Biosci. Biotechnol. Biochem.* 1994, *58*, 1953 – 1955.

[21] Nakamura, K., Nakazawa, Y., Ienaga, K., *Biochem. Biophys. Res. Commun.* 1997, *232*, 227 – 230.

[22] Tessier, F., Obrenovich, M., Monnier, V. M., *J. Biol. Chem.* 1999, *274*, 20796 – 20804.

[23] Usui, T., Hayase, F., *Biosci. Biotechnol. Biochem.* 2003, *67*, 930 – 932.

[24] Usui, T., Shizuuchi, S., Watanabe, H., Hayase, F., *Biosci. Biotechnol. Biochem.* 2004, *68*, 333 – 340.

[25] Goldberg, T., Cai, W., Peppa, M., Dardaine, V. *et al*., *J. Am. Diet. Assoc.* 2004, *104*, 1287 – 1291.

[26] Uribarri, J., Cai, W., Sandu, O., Peppa, M. *et al*., *Ann. N. Y. Acad. Sci.* 2005, *1043*, 461 – 466.

[27] Sato, T., Iwaki, M., Shimogaito, N., Xuegang, W. *et al*., *Curr. Mol. Med.* 2006, *6*, 351 – 358.

[28] Finot, P. A., Furniss, D. E., *Prog. Clin. Biol. Res.* 1989, *304*, 343 – 358.

[29] Finot, P. A., Magnenat, E., *Prog. Food Nutr. Sci.* 1981, *5*, 193 – 198.

[30] Kimiagar, M., Lee, T. C., Chichester, C. O., *J. Agric. Food Chem.* 1980, *28*, 150 – 155.

[31] Oste, R. E., Dahlqvist, A., Sjostrom, H., Miller, R., *J. Agric. Food Chem.* 1986, *34*, 356 – 361.

[32] Chuyen, N. V., Utsunomiya, N., Kato, H., *Agric. Biol. Chem.* 1991, *55*, 659 – 664.

[33] Utsunomiya, N., Chuyen, N. V., Kato, H., *J. Nutr. Sci. Vitaminol.* 1990, *36*, 387 – 397.

[34] Koschinsky, T., He, C. J., Mitsuhashi, T., Bucala, R. *et al.*, *Proc. Natl. Acad. Sci.* 1997, *94*, 6474 – 6479.

[35] Forster, A., Kuhne, Y., Henle, T., *Ann. N. Y. Acad. Sci.* 2005, *1043*, 474 – 481.

[36] Vlassara, H., *Ann. N. Y. Acad. Sci.* 2005, *1043*, 452 – 460.

[37] Somoza, V., Lindenmeier, M., Hofmann, T., Frank, O. *et al.*, *Ann. N. Y. Acad. Sci.* 2005, *1043*, 492 – 500.

[38] Vlassara, H., Cai, W., Crandall, J., Goldberg, T. *et al.*, *Proc. Natl. Acad. Sci.* 2002, *99*, 15596 – 15601.

[39] Zheng, F., He, C., Cai, W., Hattori, M. *et al*., *Diabetes Metab. Res. Rev.* 2002, *18*, 224 – 237.

[40] Lin, R. Y., Reis, E. D., Dore, A. T., Lu, M. *et al.*, *Artherosclerosis* 2002, *163*, 303 – 311.

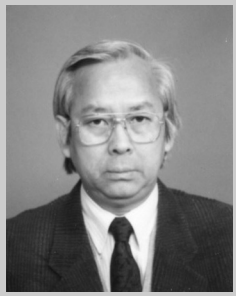
[41] Uribarri, J., Peppa, M., Cai, W., Goldberg, T. *et al.*, *Am. J. Kidney Dis.* 2003, *42*, 532 – 538.

[42] Takeuchi, M., Kikuchi, S., Sasaki, N., Watai, T. *et al.*, *Curr. Alzheimer Res.* 2004, *1*, 39 – 46.

[43] Takeuchi, M., Saito, T., *Alcohol Clin. Exp. Res.* 2005, *29*, 220S – 224S.

[44] Yamamoto, Y., Kato, I., Doi, T., Yonekura, H. *et al.*, *Excerpta Medica, Int. Congr. Ser.*, Elsevier, Kumamoto 2002, *1245*, 45 – 50.

[45] Schmidt, A. M., Yan, S. D., Yan, S. F., Stern, D. M., *Biochem. Biophys. Acta* 2000, *1498*, 99 – 111.



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[46] Vlassara, H., *Diabetes Metab. Res. Rev.* 2001, *17*, 436 – 443.

[47] Ramasamy, R., Vannucci, S. J., Yan, S. S., Herold, K. *et al.*, *Glycobiology* 2005, *15*, 16R – 28R.

[48] Schmidt, A. M., Hori, O., Bret, J., Yan, S. D. *et al.*, *Arterioscler. Thromb.* 1994, *14*, 1521 – 1528.

[49] Li, Y. M., Mitsuhashi, T., Wojciechowiez, D., Shimizu, N. *et al.*, *Proc. Natl. Acad. Sci.* 1996, *93*, 11047 – 11052.

[50] Vlassara, H., Li, Y. M., Imani, F., Wojciechowicz, D. *et al.*, *Mol. Med.* 1995, *1*, 634 – 646.

[51] Ohgami, N., Nagai, R., Ikemoto, M., Arai, H. *et al.*, *J. Diabetes Complicat.* 2002, *16*, 56 – 59.

[52] El Khoury, J., Thomas, C. A., Loike, J. D., Hickman, S. E. *et al.*, *J. Biol. Chem.* 1994, *269*, 10197 – 10200.

[53] Chuyen, N. V., Arai, H., Nakanishi, T., Utsunomiya, N., *Ann. N. Y. Acad. Sci.* 2005, *1043*, 467 – 473.

[54] Umetsu, H., Chuyen, N. V., *J. Nutr. Sci. Vitaminol.* 1998, *44*, 291 – 300.

[55] Chuyen, N. V., Utnomiya, N., Hidaka, A., Kato, H., in: Finot, P. A, Aeschbacher, H. U., Hurrell, R. F., Liardon, R. (Eds.), *The Maillard Reaction in Food Processing, Human Nutrition and Physiology*, Birkhauser Verlag, Basel 1990, pp. 285 – 290.

[56] Chuyen, N. V., *Adv. Exp. Med. Biol.* 1998, *434*, 213 – 235.

[57] Chuyen, N. V., Ijichi, K., Umetsu, H., Moteki, K., *Adv. Exp. Med. Biol.* 1998, *434*, 201 – 212.

[58] Hiramoto, S., Itoh, K., Shizuuchi, S., Kawachi, Y. *et al.*, *Helicobacter* 2004, *9*, 429 – 435.

[59] Szwergold, B. S., Howell, S. K., Beisswenger, P. J., *Excerpta Medica, Int. Congr. Ser.*, Elsevier, Kumamoto 2002, *1245*, 143 – 152.

[60] Szwergold, B. S., Howell, S. K., Beisswenger, P. J., *Diabetes* 2000, *50*, 2139 – 2147.

[61] Kato, H., Chuyen, N. V., Shinoda, T., Hayase, F. *et al.*, *Biochem. Biophys. Acta* 1990, *1035*, 71 – 76.

[62] Hayase, F., Liang, Z. Q., Suzuki, Y., Chuyen, N. V. *et al.*, *Amino Acids* 1991, *1*, 307 – 318.

[63] Thornally, P. J., *Biochem. Soc. Trans.* 2003, *31*, 1343 – 1348.

[64] Takata, K., Horiuchi, S., Araki, N., Shiga, M. *et al.*, *J. Biol. Chem.* 1988, *263*, 14819 – 14825.

[65] Shinoda, T., Hayase, F., Chuyen, N. V., Kato, H., *Biosci. Biotech. Biochem.* 1993, *57*, 1826 – 1831.

