



IMARS Highlights

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What's cooking? Notes from the Editor's pot

The contributing editors to *IMARS Highlights* selects the topic he or she would like to discuss, based on their own idea of what is a timely subject for comment. It is striking that in the current issue, an editor from both the food and biomedical sciences each independently chose a similar topic. Two articles directly address the often overlooked potential for glycated protein, containing primarily the Amadori-product, to have significant effects *in vivo*. These include induction of gene transcription and expression of pro-inflammatory proteins. For the food processing industry these effects may be an important food safety issue, and for biomedical scientists an understudied source of pathology in diabetes. At the same time the Amadori-product may serve as a source of advanced-glycation end-products (AGEs), and there is a growing body of evidence showing that some AGEs or AGE precursors also have deleterious effects *in vivo*.

The other contributions from our Editorial Board members also accomplish a major goal of the journal – highlighting 'hot' papers and emerging concepts in our field. We hope that you find their insights helpful.

IMARS Highlights solicits comments or queries from the membership on any of the current or previous articles. The journal also welcomes contributions in the form of queries, letters, short reports, book reviews and topical reviews from IMARS members. Correspondence can be sent to the editor at the address below.

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“Dry” glycation: An issue for food safety?

By Timo Buetler

Glycation in the “dry” state (or solid state) refers to the Maillard reaction between food proteins and various sugars in the dry state with well defined water activity (50-80% relative humidity). This type of Maillard reaction is used to bestow specific functional properties upon food proteins, as recently reviewed by Oliver *et al.* (1).

1. *Gelling properties*: This feature may be more related to the formation of cross-links and, hence, advanced stages of the Maillard reaction and is in general accompanied by browning and reduced nutritional value. The breaking strength of the gels depends on the choice of protein, the sugar used, temperature and time of incubation and is concentration dependent. Since it involves the formation of cross-links and advanced Maillard reaction products, this application may be less relevant for food processing.
2. *Emulsifying properties*: Glycation can modify proteins with increased emulsifying properties where the hydrophilic sugar side chains form contact with the aqueous phase while the hydrophobic regions of the protein are in contact with the lipid phase. These properties depend on the protein and sugar source, reaction time and sugar branching. Interestingly, it appears that during the reaction an “optimal” glycation is reached above which the emulsifying properties decrease, probably by “overcharging” the protein with hydrophilic residues.
3. *Solubility*: The Maillard reaction can also increase the solubility of proteins. For this property, glycation does not need to be extensive and is probably due to the addition of hydrophilic sugar side chains to the protein.
4. *Stability*: Glycation has also been reported to increase protein stability that may, in part, be due to the blocking of lysine and arginine residues, target sites of certain proteolytic enzymes.
5. *Taste*: Glycation has been shown to improve taste presumably due to the addition of sugar moieties to the protein.

During the “dry” Maillard reaction, the reaction essentially stops at the level of the Amadori product (AP) with little formation of advanced glycation endproducts (AGEs). Because no AGEs are formed during this reaction, these products are generally considered to be safe.

However, a word of caution appears appropriate at this point as some recent, largely ignored evidence indicates that the AP may directly activate cellular pro-inflammatory events independently of RAGE (2-8). Most of these studies were done using a glycated serum albumin purchased from Sigma (A8426 or A8301) that is low or essentially free of AGEs and containing mainly Amadori-type modifications. The most recent study by Higai *et al.* (2) shows that glycated human serum albumin (HSA) was able to stimulate E-selectin expression in cultured human umbilical vein endothelial cells (HUVECs). In the same experiments, HSA modified by glyoxal, glycolaldehyde or methylglyoxal were not able to stimulate these responses. They show that glycated HSA stimulation of E-selectin expression was dependent on the generation of free radicals and activation of the NF- κ B pathway. The fact that the “classical” AGEs (glyoxal-, methylglyoxal- and glycolaldehyde-HSA) were not able to stimulate HUVECs suggests that RAGE was not present / activated in these cells and that Amadori-glycated HSA stimulated a different signaling mechanism.

In an earlier report, Hattori *et al.* (4) also showed that Amadori-glycated HSA stimulated NF- κ B activation in rat vascular smooth muscle cells. This activation did not depend on RAGE since the soluble form of RAGE (sRAGE) had no effect on Amadori-glycated-HSA induced NF- κ B activation. Downstream, treatment of vascular smooth muscle cells with Amadori-glycated HSA resulted in the increased expression of two inflammatory marker proteins, MCP-1 and IL-6. The increased expression of both of these effects was blocked by the antioxidant *N*-acetylcysteine

and an inhibitor of the Erk pathway (4). In addition, these authors showed that Amadori-glycated-HSA stimulated two other transcription factors, AP1 and Elk-1. Another report demonstrated that Amadori-glycated HSA, but not AGE-HSA, stimulated the expression of another pro-inflammatory marker protein (PAI-1) in human peritoneal mesothelial cells (5). Also this effect appeared to be mediated by activation of the NF- κ B and AP-1 pathways. Finally, Ichiki *et al.* reported similar results in vascular smooth muscle cells (7).

The nature of the receptor for Amadori-modified proteins has not been clarified although several groups have attempted to characterize the cellular AP-binding protein (3, 6, 8).

While much focus in the recent years has been on the biological/pathological effects of AGEs in the biomedical field, food science has invested much effort to exploit the Maillard reaction to improve food taste and stability as well as other properties of food protein (1). The papers listed above would suggest that there may be a link between early Maillard modified proteins and biological effects that need to be addressed to assure the safety of these food technologies.

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The Maillard reaction: From the glycation marker to the master switch of cellular reactions

By Toshio Miyata

Advanced glycation reactions of proteins were initially unravelled by food and nutrition biochemists. The Maillard reaction, a non-enzymatic process, is initiated by protein exposure to glucose or other carbohydrates. In humans, irreversible advanced glycation of tissue proteins is a part of the ageing process and is markedly amplified in diabetes mellitus as a consequence of hyperglycemia. The resulting advanced glycation end product (AGE) levels in diabetic patients are indeed correlated with those of fructose-lysine, taken as a surrogate marker of prevailing plasma glucose concentration.

Surprisingly, AGEs also accumulate in non-diabetic uremic patients despite their normal serum glucose levels. Among dialysis patients, diabetics and non-diabetics have similar plasma pentosidine and N^ε-(carboxymethyl) lysine levels. AGEs also accumulate locally in other normoglycemic diseases, such as atherosclerosis, Alzheimer's disease, rheumatoid arthritis, and cancer. Clearly, factors other than hyperglycemia also impact on AGE formation. Two competing but not mutually exclusive hypotheses have been proposed and confirmed to account for increased AGE levels in normoglycemic conditions: an increased generation of AGEs resulting from enhanced oxidative stress or a decreased removal (detoxification or clearance) of reactive carbonyl precursors for AGEs. The former hypothesis is supported by several *in vitro* studies and animal experiments demonstrating that AGE accumulation and its pathological consequences can be corrected or prevented by agents with potent hydroxyl radical scavenging and transition metal chelating activities (1). The role of an impaired or accelerated detoxification of carbonyls in AGE metabolisms has been demonstrated in a patient with glyoxalase I deficiency or glyoxalase I transgenic mice/rats, respectively (2, 3).

An interesting link between increased AGEs and lipid abnormalities, insulin resistance, obesity, nutrition, chronic inflammation, immunology, or oncogenesis has been delineated in the last several years (4, 5). A recent study by Brownlee and colleagues identified intra-cellular pathways common to some of these outcomes, i.e., an AGE precursor, methylglyoxal, modifies a transcriptional co-repressor leading to enhanced gene transcription (6), opening new avenues for biomedical research on the Maillard reaction. Known as mere glycation markers a few decades ago, the Maillard reaction and its products are now not only very attractive biomarkers for broad derangements of redox and metabolic status but also may be a master switch in a variety of cellular reactions.

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Do not forget about Amadori-albumin; causation, not just correlation!

By Casper G. Schalkwijk

Numerous studies have clearly established that prolonged exposure to hyperglycaemia is the primary factor associated with the development of vascular disease in diabetic patients. But how does hyperglycaemia induce changes in cells that explain the pathogenesis of vascular complications? A growing body of evidence suggests that many effects are mediated by early glycated proteins and/or advanced glycation end-products (AGEs). Despite the fact that the Amadori-product is a major form of glycated protein, most studies so far have focused on the role of AGEs in diabetic complications and only a limited number of studies have investigated the biological role of Amadori-albumin in the pathogenesis of diabetic complications. Previous studies, particularly by Drs Margo Cohen and Fuad Ziyadeh and co-workers from the University of Pennsylvania have revealed that an increased level of Amadori-albumin is an important contributor to the pathogenesis of diabetic complications such as nephropathy and retinopathy (1). In addition to this work, a recent publication of Zhang et al (2) in *Circulation* indicated an involvement of Amadori-albumin in diabetic heart failure. They report that Amadori-albumin stimulates ROS production in cardiomyocytes through a protein kinase C-dependent activation of NADPH oxidase, which results in activation of the transcription factor nuclear factor-kappa B (NF- κ B).

The study of Zhang again demonstrated a putative role of Amadori-albumin in vascular complications. In this regard it is important to emphasize that the concentration of Amadori-products exceeds that of AGEs, not only in serum proteins, but also in *in vitro* protein preparations that contain AGEs. For example, AGE-albumin as prepared under standard conditions by incubating albumin at 37°C in phosphate buffer with 0.5 M D-glucose for 8 weeks, contained ~300nmol/ mg protein fructosamine, i.e. a marker of Amadori glycation, and only ~5 pmol/ mg protein of the AGE pentosidine. Therefore, most *in vitro* and *in vivo* experiments with AGE-preparations prepared under conditions similar to those described above, the proteins contain much more early Amadori-products than AGEs. Therefore, it cannot be excluded that numerous effects that have been ascribed to AGEs are in fact due to effects induced by Amadori-glycated proteins. On the other hand, it cannot be excluded that effects *in vitro* that have been ascribed to Amadori-products, are due to effects induced by AGEs since Amadori-products can undergo further oxidative reactions during the incubation of cells to N^ε-(carboxymethyl)-lysine or to AGE-precursors methylglyoxal, glyoxal and 3-deoxyglucosone.

Importantly, Zhang et al demonstrated that an anti-RAGE (receptor for AGE) antibody was unable to modify the Amadori-albumin mediated responses in cardiomyocytes, emphasizing that the effect of Amadori-albumin on cardiomyocytes seems to be due to Amadori-albumin and not to AGEs. Of note, the binding proteins for Amadori-glycated albumin reported so far do not show any homology with various AGE receptors and Amadori-glycated albumin did not compete with AGE-albumin for binding to RAGE. In agreement with this, a recent study demonstrated that glycated-albumin induced E-selectin expression on HUVECs through a non-RAGE signalling pathway (3). The glycated-albumin up-regulation of E-selectin gene transcription was mediated via the activation of NADPH oxidase and activation of PKB-IKK, JNK and activation of NF- κ B and AP-1.

On the basis of this and previous studies, blocking the formation or effects of Amadori-albumin holds promise as a valuable therapeutic adjunct for the prevention and treatment of complications in human diabetes (4). In this respect, recently described novel approaches to detect

Amadori-modified proteins (5) may provide helpful tools to study the role of these post transcriptional modifications in vascular complications in more detail.

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Molecular gastronomy and the Maillard reaction

By Rosario Zamora

Molecular gastronomy is the scientific discipline that explores the mechanisms of culinary transformations from raw ingredients to eating the final dish (1). The Maillard reaction has a major role in explaining many of these changes because it is responsible for many of the flavor and color changes produced in food during culinary processing, therefore influencing consumer's acceptance. In addition, it also plays a major role in one of nowadays most popular subjects in Maillard-related food safety: acrylamide formation (2).

Many studies have appeared in recent years trying to reduce acrylamide concentration in food through innovative processing according to FAO/WHO recommendations (3). These approaches try either to reduce the amount of reactants or to make unfavourable reaction conditions. Thus, blanching of potatoes before frying, lowering pH, and increasing moisture have been shown to reduce acrylamide levels (4-6).

A new attempt in this sense has appeared recently. Baardseth et al. (7) have proposed a lactic acid fermentation with *Lactobacillus plantarum* strain NC8 as an alternative procedure that may be accompanied or not by potato blanching. Lactic acid fermentation of non-blanching potato rods for 45 min reduced acrylamide level by 48% and by 71% after 120 min of fermentation. When blanching potato rods were employed, reductions in acrylamide were 79% and 94% after fermentation for 45 and 120 min, respectively. This fermentation reduced the browning of the French fries and it was a consequence of the reduced levels of reducing sugars in the treated potatoes rather than reduction of available asparagine. In addition, although pH was reduced and sugar level declined during fermentation, no distinguishable taste differences were observed between the fermented French fries and the unfermented control. These results suggest that it is possible to reduce the Maillard reaction in foods without compromising the taste of the dish. Is this a new step in molecular gastronomy?

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Dietary antioxidants as “anti-glycating” agents

By Timo Buetler

Several recent publications have evaluated the ability of dietary antioxidants to prevent protein glycation *in vitro* and *in vivo* (1-6). The only *in vivo* study investigated green tea extract (GTE) in streptozotocin-induced diabetic rats (1). Oral GTE administration (300 mg/kg bodyweight/d) for 4 weeks significantly reduced the diabetes-induced increase in systolic blood pressure, blood glucose, lipid peroxidation and glycation of aortic collagen. In addition, GTE prevented the diabetes-induced decrease in vitamin C and reduced glutathione levels in serum. The other articles mostly deal with cell lines or *in vitro* glycation reactions. Cervantes-Laurean (2) show that rutin, a plant derived flavonoid glycoside composed of quercetin and the disaccharide rutinose, and its metabolites (including quercetin) were able to inhibit glucose oxidation as well as the glycation of isolated collagen measured as fluorescence, CML and pentosidine. Zhang *et al.* (3) show that vitamin E was a potent inhibitor of glucose- and AGE-BSA-induced inhibition of proliferation of cultured bovine aortic endothelial cells. From the same group, Ahmad and Ahmed (4) show that aged garlic and its ingredient S-allylcysteine, known for its antioxidant properties, were able to inhibit the *in vitro* glycation of lysozyme. Hsieh *et al.* (5) show that several herbs used in Asian traditional medicine were able to prevent glucose- or glyoxal-induced glycation of low-density lipoprotein (LDL), as well as the formation of conjugated dienes and TBARS, two measures of lipid oxidation. Finally, Reddy *et al.* (6) summarize data on the anti-glycating and antioxidant activities of carnosine an endogenous antioxidant present in muscle and nervous tissues.

These articles add to a long list of synthetic and natural antioxidants that were shown to possess anti-glycating activity as summarized by Monnier (7). With respect to the above article by Babu *et al.* (1), it is interesting to note that a recent retrospective epidemiological study with 17,413 Japanese adults showed an inverse correlation between green tea and coffee consumption and the development of type II diabetes (8). Although in the article the link was made between caffeine consumption and reduction in diabetes it is likely that caffeine is correlated with the polyphenol content of green tea and coffee and, hence, antioxidant activity. Does this prove that prevention of AGEs will prevent type II diabetes? The answer is very likely, no. The papers on dietary antioxidants show that oxidative stress favors protein glycation *in vitro* and *in vivo* and antioxidants can interfere with the oxidative formation / maturation of AGEs. On the other hand it is becoming clear that diabetes, and in fact most if not all inflammatory diseases, are associated with increased oxidative stress (9). In bringing things together, we start to realize how important oxidative stress probably is in the development of diabetes and other diseases and what power dietary antioxidants have in fighting against these diseases. In this scenario, AGEs may just be another marker of oxidative stress.

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Inhibition of advanced glycation end products (AGEs): an implicit goal in clinical medicine?

By Toshio Miyata

The discovery that proteins are progressively modified with advanced glycation end products (AGEs) has opened new avenues for research in medical sciences. AGEs play critical roles in various pathological processes including diabetic and uremic complications, atherosclerosis, and oncogenesis. Inhibition of their formation has thus become a therapeutic goal.

Various *in vitro* inhibitors including aminoguanidine, OPB-9195, and pyridoxamine have been developed. Their activities rely to different extents on several mechanisms, such as transition metal chelation, hydroxyl radicals scavenging, and entrapment of reactive carbonyl precursors for AGEs through their amino, hydrazine and/or guanidino groups. The effects of these inhibitors are indeed beneficial in experimental animal models. In humans, clinical benefits have sometimes been hampered by side effects, but some promising compounds (e.g., pyridoxamine) are currently under investigation in clinical trials.

It should be noted that a potent AGE inhibitory activity has also been recently discovered in drugs already widely used in humans. These include anti-hypertensive agents (angiotensin receptor blockers, ARB; angiotensin converting enzyme inhibitors, ACEI; hydralazine), glucose lowering agents (biguanides, glitazones), statins, edaravone (a drug used to treat cerebral infarction), some antibiotics, and a carbonic anhydrase inhibitor. These drugs have been shown to inhibit AGE formation either directly by chelating transition metals, scavenging hydroxyl radicals or entrapping reactive carbonyls, or indirectly by modulating AGE-mediated signaling pathways (1-3). Surprisingly, the *in vitro* AGE inhibitory potential of some of them (e.g., ARB) markedly exceeds those of compounds originally designed as AGE inhibitors (2).

Currently, the treatment of diabetic nephropathy includes anti-hypertensive agents (ARB/ACEI), glucose lowering agents, statins, anti-oxidants, and diet. Of particular interest is the clinical evidence that the benefits of ARB/ACEI and statins to protect the kidney are independent of their intended pharmacological actions (blood pressure or lipid lowering). Recent papers by Izuhara et al (2) and Yamagishi et al (3) demonstrating that these drugs and treatments also lower advanced glycation of proteins, have provided therapeutic perspectives. An important question thus arises: is AGE inhibition an implicit goal in clinical medicine?

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What are the real effects of AGEs on endothelial cells? *Discrepancies between different in vitro studies*

By Casper G. Schalkwijk

A biological relevant characteristic of extracellular AGEs is the binding of these AGEs to endothelial cells and macrophages and, subsequent activation of these cells. The consequences thereof are believed to play a pivotal role in the pathogenesis of vascular complications in diabetes. However, in contrast to numerous reports demonstrating expression of many genes including those for adhesion molecules and cytokines by AGE-protein prepared *in vitro*, it is apparent from several recent reports that the interaction between well-characterized AGE-proteins and endothelial cells is absent under various experimental conditions. Thus, the relationship between AGE-protein and endothelial cells is not as straight forward as many think.

Hui and co-workers reported a study which suggests caution in the interpretation of *in vitro* experiments using AGE-proteins (1). In an attempt to identify the AGEs responsible for the increase in endothelial permeability, they demonstrated that traces of transition metals in the serum-free incubation buffer could explain the increased permeability observed with the AGE-protein preparations. They concluded that the presence of transition metals acts as a confounder in effects mediated by AGE-proteins and suggested, for the first time, caution in the interpretation of experiments using these proteins in cell studies. In another study, AGE preparations produced by incubation with glucose, fructose or ribose, which were essentially endotoxin free, were incapable of the expression of inflammatory genes, regardless of RAGE binding affinity on endothelial cells (2). In two other recent studies with endothelial cells, well characterized AGE-proteins such as CML-albumin and albumin incubated with glyoxal and methylglyoxal, also did not induce activation of endothelial cells, measured by the expression of adhesion molecules, while, under the same conditions, TNF- α did (3;4).

What are possible explanations for the discrepancies found between different *in vitro* experiments? The omission of non-glycated protein as control preparations, contamination of endotoxin in the AGE-preparations, the presence of transition metals in AGE-preparations and the existence of splice variants of the receptor for AGE (RAGE) are likely possibilities. These factors must be taken into consideration before conclusions can be drawn from *in vitro* experiments. Furthermore, there is no standard *in vitro* AGE-protein preparation and a limitation of many studies is that the composition of the final AGE-protein is largely unknown or not specified. It might be that some model AGEs do not reflect the chemical composition of AGEs formed *in vivo*. Finally, differences in the standard culture conditions of endothelial cells (i.e. with or without serum) and the use of endothelial cells from different vascular origin (i.e. microvascular vs. macrovascular) and species (human vs. bovine) are of importance and may provide additional explanations for the differences in biological effects.

Insofar as the role of endothelial cells is concerned, identifying and clarifying these processes as they occur in the different settings is essential for our understanding how and which specific AGEs are involved in vascular disease in diabetes, and other chronic diseases.

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Can pro-oxidants inhibit and antioxidants promote carbonyl chemistry?

By Rosario Zamora

Lipid oxidation originates a cascade of reactions that compete with Maillard reaction in the generation of reactive carbonyls. In the case of lipids, the formation of these compounds, which are able to modify macromolecules in both foods and living beings, is generally assumed to be promoted in the presence of pro-oxidants and to be delayed with the use of antioxidants. However, a recent paper by Mark et al. (1) shows some surprising data. They studied the formation of furan and methylfuran in three systems submitted to heat treatment: ascorbic acid, Maillard precursors, and polyunsaturated lipids. The results showed that ascorbic acid exhibited the highest potential to generate furan followed by unsaturated lipids. However, when they studied the effect of pro-oxidants and antioxidants in the assayed systems, some unexpected results were obtained.

Furan formation from oxidized lipids is supposed to involve oxidative steps to produce 4-hydroxy-2-butenal which, after cyclization and dehydration, is the precursor of furan (2). Therefore, as expected, the presence of ferric ions increased the furan production in samples containing *n*-6 lipid derivatives. However, when *n*-3 lipid samples were heated in the presence of ferric ions, production of furan and methylfuran decreased (up to 60%), which was – in part – in accordance with previous results of Becalski and Seaman (3). Furthermore, linoleic acid (*n*-6) produced a 155% higher furan content in the presence of the antioxidant BHT, and this value was a 40% higher than that produced in the linoleic acid sample containing ferric ions.

These surprising results are explained by suggesting the existence of different mechanisms for furan formation that require distinct reaction conditions. However, although the existence of precursors other than 4-hydroxy-2-butenal may be hypothesized, lipid oxidation seems to be a necessary step in the formation of the reactive carbonyls that later will produce the food toxicant. The fact that pro-oxidants may decrease or antioxidants may either increase the carbonyl content in foods or facilitate the cyclization of reactive carbonyls to produce furan is not only surprising but an important point to be taken into account. The authors have promised new experiments to explain these results. We are looking forward to hearing from them.

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