



# IMARS Highlights

## Research Commentaries for Members of The International Maillard Reaction Society

A Non-profit Research and Education Organization in Biomedicine and Food Science: <http://imars.case.edu>

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

This issue of IMARS Highlights contains the award winning articles from the Junior Scientist Writing Competition held at the end of 2006. The Society extends warm congratulations to the authors, graduate students Ashay Bhatwadekar and Suji George and post-doctoral fellow Ina Nemet. Since written communication skills are such an integral part of success in research we are happy to have a forum for junior investigators to express themselves. We encourage junior scientists to start preparing for the next round of competition which will be held later this year.

In this issue of Highlights, several of our contributing editors (Sitt, Ames, Pischetsrieder), have commented independently on the effects of dietary Maillard reaction products on human health – both positive and negative effects. Although potential pathology associated with dietary AGEs was originally focused on diabetes and renal disease, independent of diabetes, the current articles focus on AGE/ALE-related disease in the gastrointestinal tract. The underlying theme appears to be that the extent to which AGE/ALEs contribute to pathology has a strong relationship to their activity in eliciting inflammatory reactions. Understanding mechanisms of action by which AGE/ALEs do damage should provide the opportunity to develop methods to limit the damage, as discussed in the articles by Baynes and Stitt. In addition to understanding the effects of Maillard products in vivo, it is important to know their chemical identity and their mechanism of formation. The number of Maillard products that are formed in vitro and in vivo is ever expanding and several articles in this issue also discuss new structures and/or ways to detect them (Baynes, Yaylayan).

There is one change you will have noticed on the list of contributing editors for IMARS Highlights. Unfortunately Dr. Angelika Bierhaus has had to resign from the board, but the good news is that she has promised to send some contributions in the near future. More good news is that Dr. Josephine Forbes, Baker Heart Research Institute, Melbourne, Australia has agreed to join the board. We welcome her to IMARS Highlights and look forward to her contributions.

In order to expand the range of topics covered by the journal we would like to start a LETTERS section in IMARS Highlights. If there are comments that members have about any of the current or past articles, please address a letter to the editor at the address below.

Susan Thorpe  
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## **Puzzle between PPAR $\gamma$ and advanced glycation**

*by Ashay Bhatwadekar*

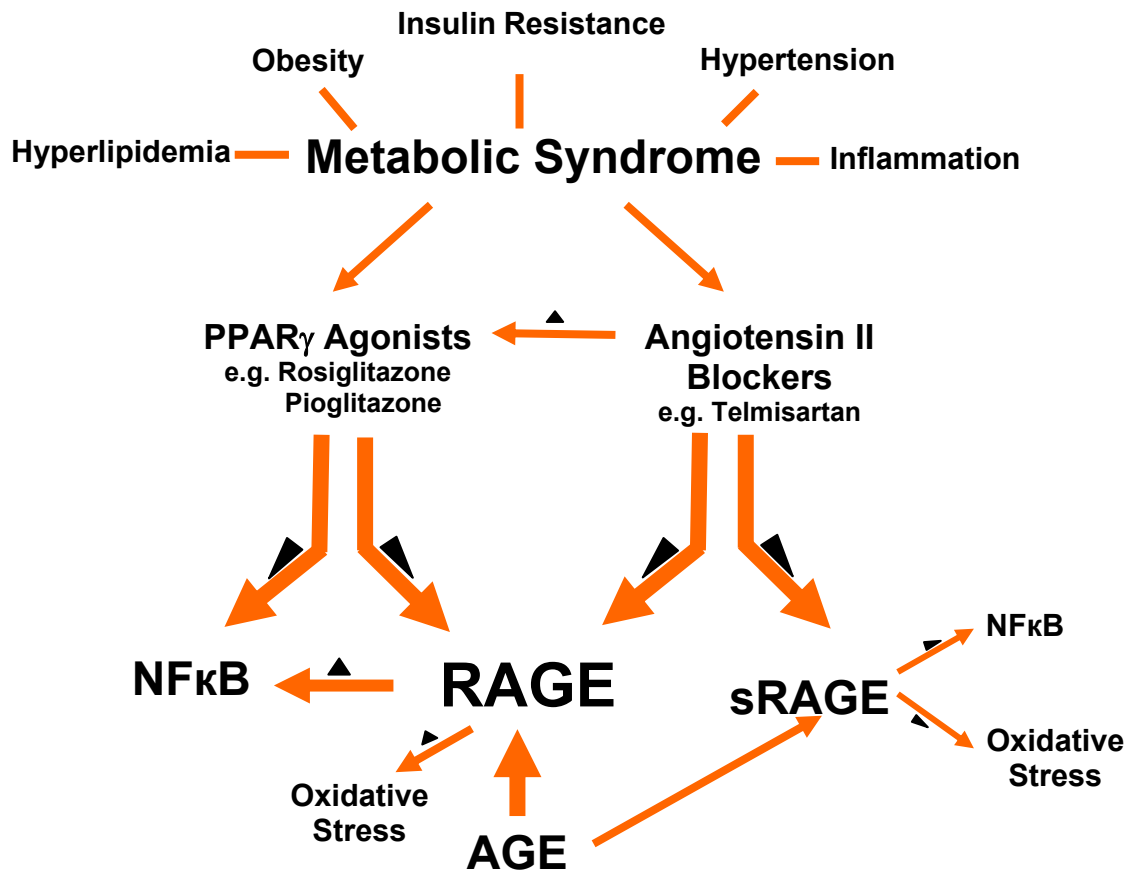
Peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) serves as a receptor for insulin sensitising drugs like rosiglitazone, and is expressed in many cells including vascular smooth muscle, endothelium and macrophages. PPAR $\gamma$  agonists have well recognised clinical properties for counteracting the type 2 diabetic state (1, 2) but these agents are also known to reduce glycoxidized low density lipoproteins and inhibit long term glycation by interfering with metal ion mediated oxidation (3).

This IMARS editorial is based on a recent study by Wang et al. (4) who have provided new information on the action of the PPAR $\gamma$  agonist rosiglitazone by demonstrating that this agent induces significant down regulation of expression of the receptor for advanced-glycation end-products (RAGE) whilst inhibiting smooth muscle cell proliferation in diabetic animals. It is interesting that co-administration of sRAGE, an endogenous soluble form of RAGE which serves as a decoy for AGEs (5) did not produce any additive effect (4). Thus it was evident that reduction in neointimal hyperplasia was not due to the blockade of the AGE-RAGE mediated pro-inflammatory pathway and PPAR $\gamma$  agonists produced independent downregulation of RAGE. Although the mechanism of this action is not completely explored in the aforementioned study, it may be partially explained by previous observations that rosiglitazone-treated endothelial cells show impaired NF- $\kappa$ B transcriptional activity (6). Taken together, this may indicate that PPAR $\gamma$  agonists significantly suppress NF- $\kappa$ B transcriptional activity and upstream activation of pro-inflammatory pathways, a mode of action that is shared with AGE-RAGE binding.

How PPAR $\gamma$  agonists modulate RAGE expression could be related to angiotensin II (Ang II) which up regulates both RAGE expression and increases levels of sRAGE. These actions are blocked by the Ang II type 1 receptor antagonist telmisartan which is also a selective PPAR $\gamma$  modulator (7). It may be significant that telmisartan also decreases both AGE-induced generation of reactive oxygen species and RAGE expression; actions that are blocked by an inhibitor of PPAR $\gamma$  and therefore indicating cross talk between RAGE signaling, PPAR $\gamma$  and Ang II (8). However, Ang II blockers also reduce expression of RAGE and modulate PPAR $\gamma$  activity irrespective of alterations in sRAGE (7). This indicates that the actions of Ang II blockers are also independent of sRAGE which might be partially mediated through downregulation of reactive oxygen species. Some of these potential interrelationships among various pathways are summarized in the Scheme below.

From the featured paper and related studies, it can be surmised that both PPAR $\gamma$  agonists and angiotensin receptor blockers like telmisartan can modulate RAGE bioactivity and serve to reduce pro-inflammatory responses. However, the impact of these agents on sRAGE, either alone or in combination, still needs further elucidation. This is especially important in the treatment of the metabolic syndrome which is characterized by the presence of insulin resistance, hypertension, dyslipidemia, obesity and increased risk of cardiovascular disease (9). Patients with metabolic syndrome are often treated with PPAR $\gamma$  agonists and Ang II inhibitors as an adjunct therapy to obesity control (10). Considering that sRAGE may be a potentially useful therapeutic molecule

for regulating the toxic effects of AGEs, pharmacological strategies should be carefully considered. Moreover, evaluation of the multiple signaling events following PPAR $\gamma$  and RAGE activation will need to be considered in order to solve this puzzle.



Scheme depicting possible interrelationship between PPAR $\gamma$  agonists, Ang II blockers and RAGE with reference to the metabolic syndrome: (▼) indicates down regulation or reduction in the response while (▲) indicates upregulation of expression.

#### References:

1. Mayerson AB, Hundal RS, Dufour S, et al. (2002) The effects of rosiglitazone on insulin sensitivity, lipolysis, and hepatic and skeletal muscle triglyceride content in patients with type 2 diabetes. *Diabetes* 51: 797-802.
2. Gillies PS, Dunn CJ. (2000) Pioglitazone. *Drugs* 60: 333-343; discussion 44-5.
3. Sobal G, Menzel EJ, Sinzinger H. (2005) Troglitazone inhibits long-term glycation and oxidation of low-density lipoprotein. *J Cardiovasc Pharmacol* 46: 672-680.

4. Wang K, Zhou Z, Zhang M, et al. (2006) Peroxisome proliferator-activated receptor gamma down-regulates receptor for advanced glycation end products and inhibits smooth muscle cell proliferation in a diabetic and nondiabetic rat carotid artery injury model. *J Pharmacol Exp Ther* 317: 37-43.
5. Goova MT, Li J, Kislinger T, et al. (2001) Blockade of receptor for advanced glycation end-products restores effective wound healing in diabetic mice. *Am J Pathol* 159: 513-525.
6. Marx N, Duez H, Fruchart JC, Staels B. (2004) Peroxisome proliferator-activated receptors and atherogenesis: regulators of gene expression in vascular cells. *Circ Res* 94: 1168-1178.
7. Nakamura K, Yamagishi S, Nakamura Y, et al. (2005) Telmisartan inhibits expression of a receptor for advanced glycation end products (RAGE) in angiotensin-II-exposed endothelial cells and decreases serum levels of soluble RAGE in patients with essential hypertension. *Microvasc Res* 70: 137-141.
8. Yoshida T, Yamagishi S, Nakamura K, et al. (2006) Telmisartan inhibits AGE-induced C-reactive protein production through downregulation of the receptor for AGE via peroxisome proliferator-activated receptor-gamma activation. *Diabetologia* 49: 3094-3099.
9. Despres JP, Lemieux I. (2006) Abdominal obesity and metabolic syndrome. *Nature* 444: 881-887.
10. van Zwieten PA, Mancina G. (2006) Background and treatment of metabolic syndrome: a therapeutic challenge. *Semin Cardiothorac Vasc Anesth* 10: 206-214.

## Can non enzymatic glycation sites in proteins be predicted?

by Suji George

Pattern recognition computational molecular biology programmes have been successfully used in predicting glycosylation sites (1), signal peptides cleavage sites (2) and phosphorylation sites (3) of proteins. On similar lines, can the sites of glycation be predicted? The hurdle in the prediction process is the nonenzymatic nature of glycation process. In spite of this, many studies have revealed that certain groups and sites in proteins are more susceptible to glycation than others.

Glycation is affected by many factors. Buffers such as phosphate and bicarbonate can influence the rate and specificity of glycation (4). The proximity of lysine to another lysine can promote glycation (4). The occurrence of an acidic amino acids in the vicinity of lysine in either the primary structure or 3D structure catalyzes the Amadori rearrangement making the lysine more reactive for glycation (4). Amino groups of proteins with lower pKa values are prone to glycation due to their high reactivity and nucleophilicity (5). The pKa values of the amino groups in turn are dependent on the proximity to other groups in the 3 D structure of the protein. Groups that are far away from glycation in primary sequence can have a strong influence in determining the reactivity of the glycation site. For example the presence of disulphide intrachain bonds can bring other lysines close to the Glycation site.

A recent report has made an attempt to analyze and predict the glycation sites of the protein based on primary sequences (6). Statistical analysis of  $\epsilon$ -lysine amino groups that are glycated in various proteins revealed that acidic amino acids, mainly glutamate and other lysines in the vicinity make the Glycation site more susceptible. The catalytic acidic amino acids have been found to be located mainly C-terminal to the glycation sites, while the basic residues have been found mainly on the N-terminal side. The site specific predictor available at [www.cbs.dtu.dk/services/Netglycate-1.0](http://www.cbs.dtu.dk/services/Netglycate-1.0) is based on the above information.

The paper cited above (6) is the first report on prediction of a nonenzymatic, physiological event that can occur both *in vivo* and *in vitro*. Though the program will be very useful for predicting potential lysines for glycation, there are certain limitations in predicting such non specific events. The authors have based their calculation on primary structure surrounding the crucial lysine. This does not take into account wide variations in reactivities of different sugars. For instance, while glucose and fructose have been shown to overlap in the sites of glycation towards amino groups in proteins (7), fructosylation has also been shown to occur at unique lysines that are not glycated by glucose. Similarly the target of glycation by the potent dicarbonyl compound, methyl glyoxal, is arginine with fewer lysine being modified. Further the role of 3D structure of the protein with respect to the arrangement of other groups in space, the presence disulphide bond in the vicinity and other factors which influence the accessibility of lysine have not been taken into account.

Knowledge about the immediate environment of groups in proteins and the computation of pKa of the groups in addition to the primary sequence can give a

prediction, if not accurate, at least close to the experimental results. Thus the data obtained by pattern recognition should be integrated with results of computational pKa prediction, surface accessibility and structural analysis of the lysine at least for those proteins where the 3 D structure is available. This would make the prediction more efficient.

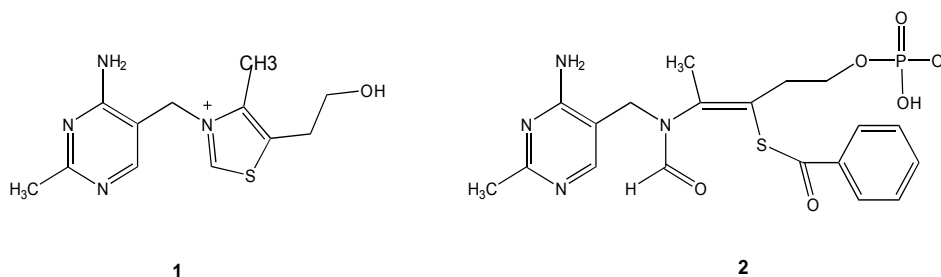
## **References**

1. Julenius K, Molgaard A, Gupta R, Brunak S (2005) Prediction, conservation analysis and structure characterisation of mammalian mucin tyoe O-glycosylation sites. *Glycobiology*15: 153-164.
2. Bendtsen JD, Nielsen H, Von Heijne G, Brunak, S. (2004) Improved prediction of signal peptides: SignalP,3.0. *J.Mol.Biol* 340: 783-795.
3. Blom N, Gammeltoft S, Brunak, S (1999) sequence and structure based prediction of eukaryotic protein phosphorylation sites. *J.Mol.Biol* 294: 1351-1362.
4. Baynes JW, Watkins NG, Fisher CI, Hull CJ, Patrick JS, Ahmed MU, Dunn JA, Thorpe SR (1989) The Amadori product on protein: structure and reactions. *Prog. Clin. Biol. Res* 304: 43-67.
5. Bunn HF, Shapiro R, McManus M, Gariick L, McDonald MJ, Gallop PM, Gabbyay KH. (1979) Structural heterogeneity of human haemoglobin A due to nonenzymatic glycosylation *J.Biol.Chem* 254: 3892-3898.
6. Johansen MB, Kiemer L, Brunak S (2006) Analysis and prediction of mammalian protein glycation. *Glycobiology* 16: 844-853.
7. Zhao HR, Smith JB, Jiang XY, Abraham EC (1996) Sites of glycation of beta B2-crystallin by glucose and fructose *Biochem. Biophys. Res. Commun*, 229:128-133.

## Thiamine and Benfotiamine as potential scavengers of methylglyoxal

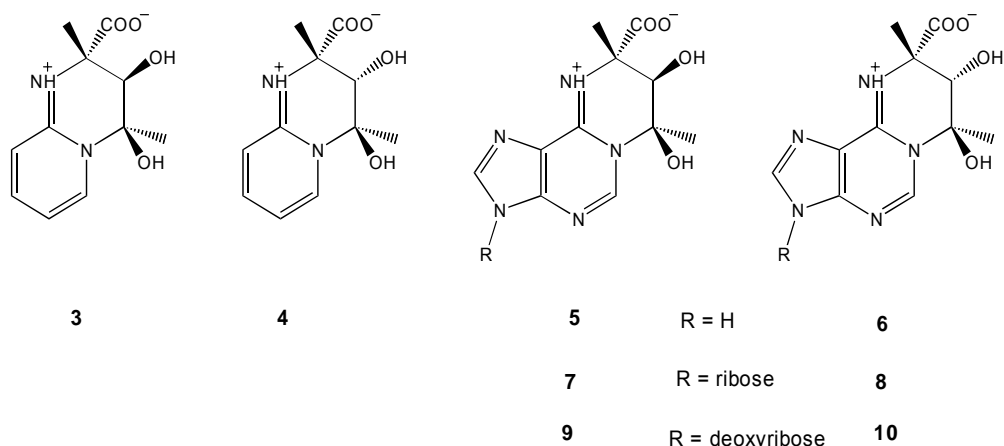
by Ina Nemet

One of the pharmaceutical approaches to prevent or slow down diabetic complications is the inhibition of advanced glycation end product (AGE) formation. In this approach, reactive  $\alpha$ -oxoaldehydes precursors of AGEs, including glyoxal, methylglyoxal (MG) and 3-deoxyglucosone are scavenged with pharmaceutical agents such as aminoguanidine and metformin, among others (1-4). Stimulation of the reductive pentose phosphate pathway with high dose therapy of thiamine (**1**) and the thiamine monophosphate derivate Benfotiamine (**2**) (Figure 1) has recently been suggested as a potential novel strategy for the prevention of diabetic complications (2). Moreover, Thornalley *et al.* have shown that high doses of thiamine (**1**) and Benfotiamine (**2**) prevented the development of incipient nephropathy in experimental diabetes (5). This therapy increased transketolase expression in renal glomeruli, increased conversion of triosephosphates to ribose-5-phosphate, and strongly inhibited the development of microalbuminuria. This was associated with decreased activation of protein kinase C and decreased protein glycation and oxidative stress – three major pathways of biochemical dysfunction in hyperglycemia (6,7).



**Figure 1.** Chemical structures of thiamine (**1**) and Benfotiamine (**2**).

As shown in Figure 2 below, however, Routaboul *et al.* (8) have shown that MG stereoselectively reacts with 2-amino-pyridine as well as with adenine derivatives and forms a new family of imino heterocyclic adducts **3-10** under mild conditions (phosphate buffer, 37°C, pH 7). Because of structural similarities between thiamine (**1**) or Benfotiamine (**2**) and 2-aminopyridine and adenine, it is possible that the imino acid derivatives could also be formed by reaction of MG with these vitamins suggesting the possibility of using **1** and **2** as scavengers for MG *in vivo*. The observed lower concentrations of MG and the MG derived AGE MG-H1, in diabetic animals treated with high doses of thiamine (**1**) and Benfotiamine (**2**) in comparison with non-treated animals are thought to be the result of decreased glycolytic flux by activation of the pentose phosphate pathway (5). However, a decrease in MG and MG-H1 content could also be the result of the reaction of MG with **1** and **2** especially in the extracellular milieu. If this is possible, thiamine (**1**) and Benfotiamine (**2**) could be used for lowering MG concentration caused not only by hyperglycemia (increased flux through glycolytic pathway), but also MG formed by other pathways such as oxidation of ketone bodies formed during low carbohydrate diet and/or diabetic ketoacidosis (9,10).



**Figure 2.** Chemical structures of the methylglyoxal (MG) adducts formed from 2-aminopyridine (3 and 4), adenine (5 and 6), adenosine (7 and 8) and 2'-deoxyadenosine (9 and 10).

## References

1. Brownlee M, Vlasara H, Kooney A, Ulrich P, Cerami A. (1986) Aminoguanidine prevents diabetes-induced arterial wall cross-linking. *Science* (232: 1629-1632).
2. Wu X, Monnier VM. (2003) Enzymatic deglycation of proteins. *Arch. Biochem. Biophys.* 419: 1-15.
3. Thornalley PJ (2003) Use of aminoguanidine (Pimagedine) to prevent the formation of advanced glycation endproducts. *Arch. Biochem. Biophys.* 419, 31-40.
4. Rahbar S, Figarola JL. (2003) Novel inhibitors of advanced glycation end-products. *Arch. Biochem. Biophys.* 419: 63-79.
5. Babaei-Jadidi R, Karachalias N, Ahmed N, Battah S, Thornalley PJ (2003) Prevention of incipient diabetic nephropathy by high-dose thiamine and benfotiamine. *Diabetes* 52: 2110-2120.
6. Brownlee M (2001) Biochemistry and molecular cell biology of diabetic complications. *Nature* 414: 813-820.
7. Brownlee M (2005) The pathobiology of diabetic complications: a unifying mechanism. *Diabetes* 54: 1615-1625.
8. C. Routaboul, L. Dumas, I. Gautier-Luneau, J. Vergne, M.-C. Maurel, J.-L. Décout, (2002) New stereoselective reaction of methylglyoxal with 2-aminopyridine and adenine derivatives: formation of imino acid-nucleic base derivatives in water under mild conditions. *Chem. Commun.* 10: 1114-1115.
9. Beisswenger BG, Delucia EM, Lapoint N, Sanford RJ, Beisswenger PJ (2005) Ketosis leads to increased methylglyoxal production on the Atkins diet. *Ann. N. Y. Acad. Sci.* 1043: 201-210.
10. Turk z, Nemet I, Varga-Defterdarovic L, Car N (2006) Elevated levels of methylglyoxal during diabetic ketoacidosis and its recovery phase. *Diabetes Metab.* 32: 176-180.

## Pyridoxamine Therapy for Type 2 Diabetes in a Mouse

by John Baynes

Advanced glycation and lipoxidation end-products (AGE/ALEs) are thought to be involved in the pathogenesis of diabetic complications. For this reason, a number of drug candidates have been developed to limit or prevent the formation of these protein-bound adducts. For preclinical studies, most AGE/ALE inhibitors are tested in type 1 diabetic animal models; the favorite is the streptozotocin-induced diabetic rat. There are numerous limitations to this model – the generally severe hyperglycemia and hyperlipidemia, the toxicity of streptozotocin, the short term of many studies, and the fact that AGE/ALE inhibitors are most frequently administered in animals from the onset of diabetes, rather than in an intervention model. However, most diabetic patients have type 2 diabetes and have probably had this disease for several years prior to diagnosis. Thus, it is important to test AGE/ALE inhibitors in type 2 diabetic models and to assess the efficacy of the drug as an intervention strategy.

There are two recent reports that demonstrate the efficacy of the AGE/ALE inhibitor pyridoxamine (PM) in prevention of nephropathy in type 2 animal models. Striker and colleagues (1) showed that PM prevented the development of nephropathy in the *db/db* mouse, and, more importantly, that it inhibited the progression of nephropathy in mice with established renal disease. In both the *de novo* and intervention model, PM inhibited the increase in albuminuria and changes in renal morphology, including glomerular sclerosis and hypertrophy and mesangial expansion. PM also inhibited both renal cortical  $\alpha 1$  type IV collagen mRNA expression and glomerular collagen accumulation in the *db/db* mice. In a more recent article, Tanimoto *et al.* (2) show that PM inhibits the development of nephropathy in the KK- $A^y$ /Ta mouse, also a type 2 model. In these studies, PM also had profound effects on renal function, including decreased albumin excretion, urinary and plasma creatinine, and substantially reduced immunostaining for both CML and nitrotyrosine in the kidney. In a dose-dependent fashion, PM also reduced plasma triglycerides and 3-deoxyglucosone. Decreases in plasma insulin and glucose, and HbA1c concentration indicate that PM also improved insulin sensitivity, possibly through an anti-inflammatory action. This was consistent with potent inhibition of expression of TGF- $\beta 1$  and laminin- $\beta 1$  in the kidney. Overall, these two studies in type 2 animal models strengthen the evidence that PM is an effective agent for inhibiting the progression of complications in type 2 diabetes. The next step is to confirm the work of Striker and colleagues in the *db/db* mouse by showing that PM inhibits or reverses the progression of complications in the KK- $A^y$ /Ta mouse. The growing evidence from studies in animal models and from Phase II clinical trials strengthen the argument for testing of PM in clinical trials for treatment of type 2 diabetes in humans.

### References

- 1 Zheng F, Zeng Y-J, Plati, A-R, Elliot SJ, Berho M, Potier M, Striker LJ, Striker GE (2006) Combined AGE inhibition and ACEi decreased the progression of established diabetic nephropathy in B6 *db/db* mice. *Kidney Int* **70**: 507-514.
2. Tanimoto M, Gohda T, Kaneko S, Hagiwara S, Murakoshi M, Aoki T, Yamada K, Ito T, Matsumoto M, Horikoshi S, Tomino Y (2007) Effect of pyridoxamine (K-163), an inhibitor of advanced glycation end products, on type 2 diabetic nephropathy in KK- $A^y$ /TA mice. *Metabolism* **56**: 160-167.

## Soothing Melanoidins

*by Monika Pischetsrieder*

When food science first got interested in Maillard products, the focus was mainly on their favourable properties, such as flavour, browning and antioxidative properties, which prolong shelf life. Later on, reports were added on the physiological effects of Maillard products, which were mostly bad news for the consumer: Overly heated food shows mutagenic and carcinogenic properties and also leads to a loss of essential amino acids. Recently, the hypothesis was added that dietary Maillard products considerably add to endogenously formed AGEs and increase the overall systemic AGE load. As a consequence, AGEs from nutrition could trigger pro-inflammatory reactions in a similar way as described for in vivo formed AGEs (1). Thus, food rich in Maillard products got the reputation that it appeals to our senses, but it is bad for our health.

In the meantime, however, food science looked again more closely on the bright side of Maillard products: Beneficial physiological properties of thermally treated food were described, primarily related to the antioxidative activity of Maillard products and melanoidins. A recent work of Rufián-Henares and Morales adds another flavor to the valuable health effect of Maillard products: they report that coffee brews, as well as melanoidins, isolated from coffee brew, beer or sweet wine inhibit Angiotensin-I converting enzyme (ACE) (2). ACE is a peptidase, which cleaves angiotensin I into angiotensin II and inactivates bradykinin. Both events result in a rise of blood pressure. Therefore, food components with ACE inhibiting activity are of interest as a means to normalize blood pressure in the population by nutrition. Functional food with ACE inhibiting peptides is already commercialized, for example in Finland and Japan. The present paper is yet the first report about the ACE inhibiting activity of melanoidines.

Food melanoidines are very complexly composed, containing not only Maillard products, but also proteins, polyphenols and polysaccharides (3). At present, it can therefore only be hypothesized about the chemical nature of the active component and the inhibitory mechanism. Thus, further studies will give an insight into the origin of the ACE inhibiting activity of melanoidins, determining their possible application as functional food ingredients. Furthermore, it will be interesting to see, if (decaffeinated) coffee, beer or sweet wine has a potential to reduce blood pressure in intervention studies.

### **References**

1. Vlassara H, Cai W, Crandall J, Goldberg T, Oberstein R, Dardaine V, Peppas M, Rayfield E (2002) Inflammatory mediators are induced by dietary glycotoxins, a major risk factor for diabetic angiopathy. *Proc Natl Acad Sci USA* 99: 15596 – 15601.
2. Rufián-Henares J, Morales F (2007) Angiotensin-I converting enzyme inhibitory activity of coffee melanoidins. *J Agric Food Chem* 55: 1480 – 1485.
3. Koen Bekedam E, Marieke P, De Laat C, Schols H, van Boekel M, Smit G. Arabinogalactan proteins are incorporated in negatively charged coffee brew melanoidines (2007) *J Agric Food Chem* 55: 761 – 768.

## **AGE-modification of the extracellular matrix – insights into pathogenesis and implications for new therapeutic strategies**

*by Alan Stitt*

Vascular basement membrane (BM) thickening of capillaries is a well-recognized lesion of diabetic microvasculopathy in both patients and experimental models. This progressive, diabetes-related pathological change is a consequence of increased protein expression combined with reduced proteolytic degradation by vascular cells. Although BM thickening has been used as a robust indicator of disease progression, there have been doubts expressed whether the lesion has major pathogenic significance or if it is simply another epiphenomenon, a consequence of the diabetic milieu. However, over the last 10 years our appreciation of vascular BMs as complex, dynamic matrices that regulate cell function has grown considerably. As a consequence, disease and age-related changes in this highly specialized extracellular matrix would be expected to have a profound influence on vascular cells which are reliant on substrate interactions for proper function and survival.

There is abundant evidence that advanced glycation endproducts (AGEs) accumulate on vascular BMs during both diabetes and aging (1). This occurs in parallel with the AGE formation on the more readily obtained, and oft examined, structural collagens in skin and tendons. It has been speculated that vascular BM-modifications can contribute to microvasculopathy by changing the properties of the matrix and leading to vascular wall stiffening, attenuated endothelial – pericyte communication, and/or appropriate vascular cell interaction with constituent BM proteins (1).

A recent study by Dobler et al. (2) demonstrated that methylglyoxal (MGO) derived from endothelial cells exposed to high glucose leads to significant hydroimidazolone adduct formation on arginine residues in type IV collagen (a component protein of vascular BMs). Moreover, the authors provide evidence that this arginine modification occurs on the RGD and GFOGER motifs on collagen IV (2). This is significant because these sequences are key recognition motifs for integrins which are important membrane proteins involved in cell interaction with substrate components and initiation of signal transduction events. MGO-derived hydroimidazolone formation on RGD and GFOGER can cause endothelial dysfunction, as evidenced by impaired attachment and reduced tubulogenesis (a component of angiogenesis). Ultimately endothelial cells growing on this modified collagen IV substrate die by anoikis (Greek for “homelessness”) which is a form of apoptosis induced by anchorage-dependent cells detaching from their matrix.

Dobler et al. (2) have added further evidence that BM modifications by advanced glycation during diabetes are important for pathogenesis of disease. Significantly, MGO derived from the overlying endothelial cells themselves could play an important role in this process, which could lead to AGE adducts forming on the substrate at much earlier stages of disease than was first appreciated. In vivo, AGE-modification of vascular BMs has important implications for upcoming “therapeutic angiogenesis” strategies that seek to re-perfuse organs damaged by capillary loss. Many of these are based on gene delivery of angiogenic

growth factors (3) or introduction of marrow-derived endothelial progenitor (stem) cells (EPCs) (4) in an effort to “re-endothelialise” defunct vessels. The presence of AGE-modifications on redundant BM tubes could significantly impair the ability of “new endothelium” to re-form viable capillaries. Therefore the impact of these matrix-immobilised AGEs on new capillary formation, combined with the ability of EPCs to operate in a vasoreparative capacity warrants more study as we seek to both prevent and reverse the consequences of long-term diabetes.

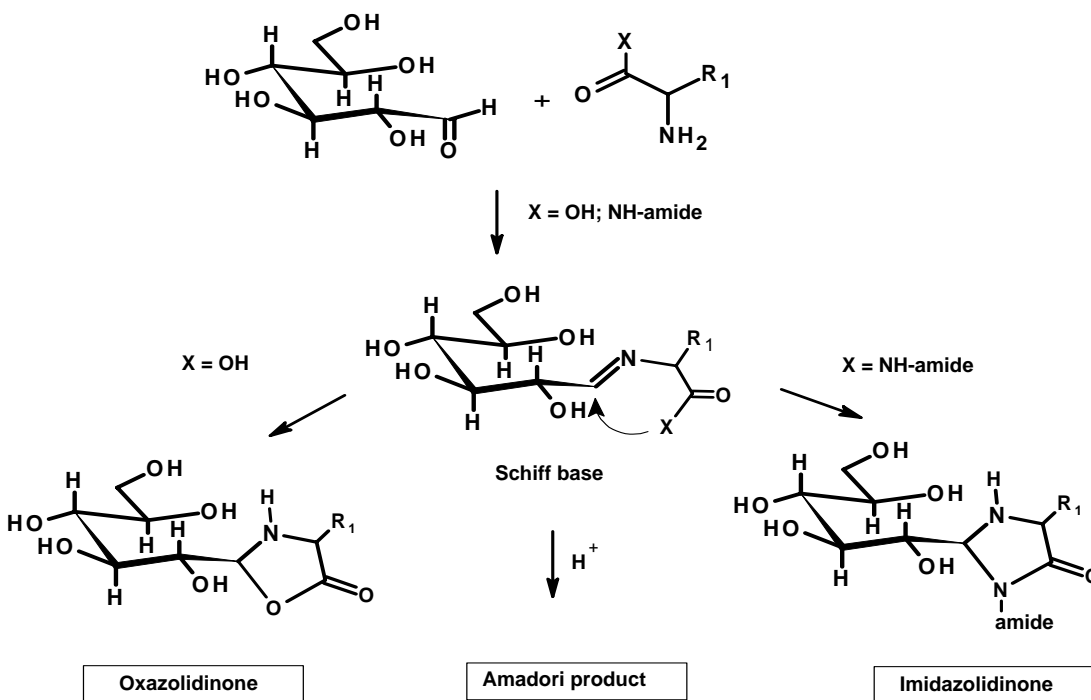
### **References**

1. Tsilibary, E. C. (2003) Microvascular basement membranes in diabetes mellitus. *J Pathol* 200, 537-546.
2. Dobler, D., Ahmed, N., Song, L., Eboigbodin, K. E., and Thornalley, P. J. (2006) Increased dicarbonyl metabolism in endothelial cells in hyperglycemia induces anoikis and impairs angiogenesis by RGD and GFOGER motif modification. *Diabetes* 55, 1961-1969.
3. Syed, I. S., Sanborn, T. A., and Rosengart, T. K. (2004) Therapeutic angiogenesis: a biologic bypass. *Cardiology* 101, 131-143.
4. Loomans, C. J., de Koning, E. J., Staal, F. J., Rookmaaker, M. B., Verseyden, C., de Boer, H. C., Verhaar, M. C., Braam, B., Rabelink, T. J., and van Zonneveld, A. J. (2004) Endothelial progenitor cell dysfunction: a novel concept in the pathogenesis of vascular complications of type 1 diabetes. *Diabetes* 53, 195-199.

## GLYCATION OF PEPTIDES: IMIDAZOLIDINONE FORMATION IN COMPETITION WITH AMADORI REARRANGEMENT

by Varoujan A. Yaylayan

The formation of imidazolidinones (not to be confused with cross-link structures imidazolinones formed from dicarbonyls and arginine) from sugar peptide interaction (see the scheme below) has been known for some time. Recently the same research group (1) that identified and characterized imidazolidinones for the first time, has also studied their formation in relation to the Amadori rearrangement. All their model systems consisting of different sugars and bioactive peptides generated both Amadori products and imidazolidinones when incubated at 37 or 50°C in phosphate buffer at pH 7.4 or in methanol.



The authors have proposed that before the protonation of the Schiff base that yields Amadori product, the imine undergoes a nucleophilic attack by the amide nitrogen to form two stereoisomers of imidazolidinone at the anomeric carbon of the sugar. The relative distribution and amounts of Amadori versus imidazolidinone were dependant on the structure of reactants, solvent and temperature. In methanol, the formation of imidazolidinone was inhibited in comparison with the aqueous models. Furthermore, the addition of a tertiary amine such as N-ethylmorpholine significantly enhanced the formation of imidazolidinones. However, in most cases the Amadori adduct was the main product except when using carboxy-protected peptides. In this case only traces of Amadori product was formed with good yields of imidazolidinones.

These findings indicate the increased stability of imidazolidinones relative to oxazolidinones that form from amino acids instead of peptides, in a similar fashion to imidazolidinones (see the above Scheme). Although numerous carbonyl-amino acid based oxazolidinones have been synthesized no carbohydrate derived characterized in the literature. It is likely that imidazolidinones are also formed in food and may contribute to their overall aroma and color.

## **References**

1. Roščić, M. and Horvat Š. (2006) Transformations of bioactive peptides in the presence of sugars –Characterization and stability studies of the adducts generated via the Maillard reaction. *Bioorganic and Medicinal Chemistry* 14: 4933-4943.

## Probing the Size of the Iceberg

by John Baynes

The argument that advanced glycation and lipoxidation end products (AGE/ALEs) contribute to pathology in aging and disease has always been weakened by the fact that these compounds are present in only trace amounts in tissue proteins. Maillard researchers have argued, however, that the known advanced glycation and advanced lipoxidation end products (AGE/ALEs) are only “the tip of the iceberg”, that many more AGE/ALEs are present in tissues, as in foods, but their structure is still unknown. This somewhat defensive position is supported by the continuing discovery of new AGE/ALEs, but it is also hassled by the suggestion that the known AGE/ALEs are only the “tip of an ice-cube”, and that the total AGE/ALE burden in the body is still too low to be pathologically significant. Notwithstanding the fact that even low levels of AGE/ALEs can initiate inflammatory responses through interaction with RAGE and other receptors, it would be helpful if we could gain some insight into the size of the Maillard ice-pack.

Beryl Ortwerth and colleagues (1) have recently used a combination of low-tech and hi-tech methods to do the equivalent of an ultrasound scan on the Maillard domain in the human lens. Using a large, Bio-Gel P-2 ultrafine column, they resolved a protease digest of human lens proteins into a variety of molecular weight fractions. P-2 has a nominal void volume of 200 amu – it is an old, but reliable matrix for resolving free amino acids from dipeptides and crosslinks. The higher molecular weight P-2 fractions, enriched in crosslinks, were then analyzed by reverse phase HPLC with a quadrupole MS system as a detector. The output is a series of three-dimensional maps with axes of time, molecular weight and signal intensity. Several sets of maps were compared, those from old human lens proteins, from lens cataract proteins, from calf lens proteins, and from calf lens proteins exposed to ascorbate (ACLP) - the ascorbate map is particularly appropriate, considering Ortwerth and Monnier's recent report (2) on the increased rate of accumulation of AGEs in the lens of a mouse model with increased lens ascorbate transporter activity. Comparison of these maps reveals many similarities between the maps of human lens proteins and ACLP. About half of the peaks in the human lens cataract map were present in the ACLP map; some of the differences are attributable to tryptophan derivative in the human lens. Only a few of the peaks have been identified, but the similarities extend beyond the appearance of a peak – MS/MS comparison of a number of the products establishes their identity, based on fragmentation patterns. Interestingly, most of the common peaks represent unknown products, supporting the “iceberg” model. Eventually, comparisons of old and cataractous lenses with calf lenses treated with glucose or methylglyoxal, or even polyunsaturated fatty acids, may yield even more information on the origin of AGE/ALEs in the human lens and other tissues. In the meantime, it looks like we have a lot of ice for future research.

### References

1. Cheng R, Feng Q, Ortwerth BJ (2006) LC/MS display of the total modified amino acids in cataract lens proteins and in lens proteins glycosylated by ascorbic acid in vitro. *Biochim Biophys Acta* 1762: 533-543.
2. Fan X, Reneker LW, Obrenovich ME, Strauch C, Cheng R, Jarvis SM, Ortwerth BJ, Monnier VM (2006) Vitamin C mediates chemical aging of lens crystallins by the Maillard reaction in a humanized mouse model. *Proc Natl Acad Sci* 103: 16912-16917.

## A gut reaction to AGEs

by Monika Pischetsrieder

One of the liveliest topics discussed in the field of the Maillard reaction is currently the question on the health implications of dietary AGEs. On the one hand, it is well established that the concentrations of Maillard products which we take up with our food exceed by far the endogenously formed AGEs. On the other hand, a growing number of reports appear on the bioavailability, metabolism and excretion of dietary AGEs, which raise our hopes that the Maillard products which we consume with our food are not so bad for us after all (1-3). However, a recent paper by Andrassy et al. (4) now indirectly implies that we must consider not only systemic effects, but also direct interactions of food derived Maillard products in the gut.

In this work, gut biopsy tissue from patients with inflammatory bowel disease (Crohn's disease and ulcerative colitis) were studied. The inflamed part of the biopsy tissue showed increased NF- $\kappa$ B activation and RAGE expression as compared to non-inflamed tissue from the same patients. They further prepared protein extracts from the biopsies which they used to stimulate endothelial cells in vitro. The extracts were also applied rectally to mice. The protein extracts from the inflamed tissue, but not from the control tissue led to perpetuated NF- $\kappa$ B activation in vitro and to increased proinflammatory gene expression and inflammation in vivo. The effect was very likely mediated by an interaction between RAGE and CML, which is formed during inflammation, because it was inhibited by CML depletion and the addition of sRAGE. Likewise, inflammation was not detected in RAGE<sup>-/-</sup> mice. The authors concluded that CML which is formed in inflamed gut tissue can evoke an inflammatory response in the intestine. The study gives interesting insights into the pathological mechanism leading to inflammatory bowel diseases. However, it may also have implications in nutritional biochemistry: Taking into account that CML-modified food proteins may not be readily absorbed, they are likely to get in contact with RAGE expressing intestinal endothelial cells. Food derived CML would then be able to promote pro-inflammatory intestinal reactions in a similar way as CML, endogenously formed during inflammatory bowel disease.

### References

1. Bütler T. (2006) Open questions around the bioavailability of Maillard reaction products, IMARS Highlights, Vol 1, Number 5: 1-3.
2. Zamora R.(2006) The relative contributions of in vivo formed and nutrition-derived AGEs, IMARS Highlights, Vol 1, Number 5: 7.
3. Pischetsrieder M. (2006) The human body is still a black box for food derived AGEs, IMARS Highlights, Vol 1, Number 4: 2-3.
4. Andrassy M, Igwe J, Autschbach F, Volz C, Remppis A, Neurath M, Schleicher E, Humpert P, Wendt T, Liliensiek B, Morcos M, Schiekofer S, Thiele K, Chen J, Kientsch-Engel R, Schmidt A-M, Stremmel W, Stern D, Katus H, Nawroth P, Bierhaus A. (2006) Posttranslationally modified proteins as mediators of sustained intestinal inflammation. *Am J Pathol*, 169: 1223 – 1237

## **RAGE activation of pro-inflammatory signalling in the gut – yet another reason to watch what we eat?**

*by Alan Stitt and Jennifer Ames*

It is well known that AGEs interact with various receptors and binding proteins expressed on the surface of many cell-types in various organs. It is thought that this binding can lead to clearance/detoxification of serum/tissue AGEs and/or initiation of signal transduction, depending on the cell-type and receptor system involved. While RAGE is the best characterised AGE-receptor, it should be appreciated that this protein binds many ligands, including amyloid components and pro-inflammatory mediators of the S100/calgranulin family. RAGE acts as a receptor for at least two distinct AGE ligands; CML (1) and hydroimidazolone adducts (2). RAGE interaction with AGEs triggers intracellular signalling cascades and leads to NF- $\kappa$ B transcriptional activation, upstream changes in gene expression and oxidative stress (3) – phenomena that are intimately linked to pro-inflammatory responses and propagation of diabetic vascular complications.

A recent paper by Zen et al. (4) has described a role for RAGE in neutrophil migration across the intestinal epithelium. They show that epithelial cells from the human colon constitutively express low levels of RAGE but upon stimulation with pro-inflammatory cytokines (IFN $\gamma$  and TNF $\alpha$ ) this expression increases, especially at the apical plasma membrane (4). Moreover, RAGE is highly expressed in colonic mucosal epithelium from patients with active ulcerative colitis (UC) and Crohn's disease. The authors report that RAGE modulates migration of neutrophils across epithelial monolayers and could therefore be linked to pro-inflammatory processes in the lower intestine. Interesting reports have been emerging about RAGE acting as a counter-receptor of  $\beta$ 2 integrin (Mac-1; CD11b/CD18) which may modulate inflammatory processes via enhanced leukostasis (5). Zen et al. demonstrate that this mechanism may also be important for binding and transcellular migration of neutrophils across colonic epithelial monolayers (4).

What implications does this have for dietary AGEs and their role in human health? An answer to this question may reside in the fact that levels of AGEs (and ALEs) in food increase markedly during conventional cooking and that CML, a major ligand for RAGE, has been identified at very high levels in evaporated milk (up to 0.5 g/kg) and bakery products (6). Remarkably, it has been estimated that consumption of a conventional Western diet constitutes a daily intake of 25-75 mg AGEs with CML likely to be a major contributor. Of course, when ingested, these adducts may be released from protein by digestive enzymes or gut bacteria and some of them may subsequently be absorbed into the bloodstream, thereby contributing to the body's AGE burden (7). Amadori rearrangement products (ARPs) are largely metabolised by the colonic microflora (8) and this may also be the fate of some AGEs. However, dietary AGEs would also be expected to interact with RAGE expressed on the apical membranes of the colonic epithelium (4).

Thus, with increasing evidence for RAGE playing a significant role in inflammatory bowel disease, perhaps it should be considered that certain foods with high-AGE content could exacerbate this condition? Further, it may be appropriate for

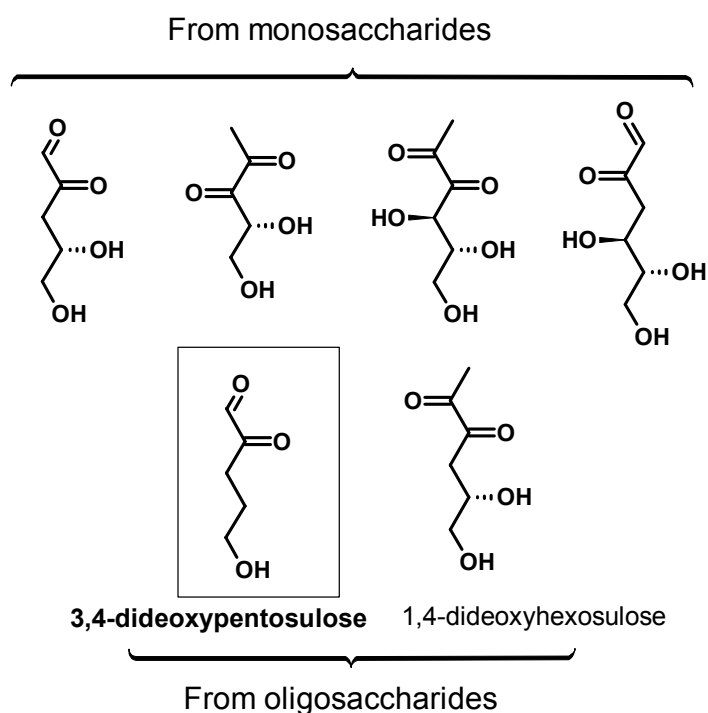
well-designed in vitro, pre-clinical and clinical studies to be conducted on food-derived AGEs in order to establish a clear picture about what adducts actually reach the colon. Although considerable research is needed in this arena, we suggest that it may be beneficial for patients susceptible to inflammatory bowel disease to limit their intake of dietary AGEs and thereby reduce pathogenic activation of RAGE.

1. Kislinger, T., Fu, C., Huber, B., Qu, W., Taguchi, A., Du Yan, S., Hofmann, M., Yan, S. F., Pischetsrieder, M., Stern, D., and Schmidt, A. M. (1999) N(epsilon)-(carboxymethyl)lysine adducts of proteins are ligands for receptor for advanced glycation end products that activate cell signaling pathways and modulate gene expression. *J Biol Chem* 274, 31740-31749.
2. Goldin, A., Beckman, J. A., Schmidt, A. M., and Creager, M. A. (2006) Advanced glycation end products: sparking the development of diabetic vascular injury. *Circulation* 114, 597-605.
3. Wautier, J. L., and Schmidt, A. M. (2004) Protein glycation: a firm link to endothelial cell dysfunction. *Circ Res* 95, 233-238.
4. Zen, K., Chen, C. X., Chen, Y. T., Wilton, R., and Liu, Y. (2007) Receptor for advanced glycation endproducts mediates neutrophil migration across intestinal epithelium. *J Immunol* 178, 2483-2490.
5. Chavakis, T., Bierhaus, A., Al-Fakhri, N., Schneider, D., Witte, S., Linn, T., Nagashima, M., Morser, J., Arnold, B., Preissner, K. T., and Nawroth, P. P. (2003) The pattern recognition receptor (RAGE) is a counterreceptor for leukocyte integrins: a novel pathway for inflammatory cell recruitment. *J Exp Med* 198, 1507-1515.
6. Henle, T., and Miyata, T. (2003) Advanced glycation end products in uremia. *Adv Ren Replace Ther* 10, 321-331.
7. Uribarri, J., Cai, W., Sandu, O., Peppas, M., Goldberg, T., and Vlassara, H. (2005) Diet-derived advanced glycation end products are major contributors to the body's AGE pool and induce inflammation in healthy subjects. *Ann N Y Acad Sci* 1043, 461-466.
8. Faist, V., and Erbersdobler, H. F. (2001) Metabolic transit and in vivo effects of melanoidins and precursor compounds deriving from the Maillard reaction. *Ann Nutr Metab* 45, 1-12

## A NEW ADDITION TO THE FAMILY OF $\alpha$ -DICARBONYLS: 3,4-DIDEOXYPENTOSULOSE (3,4-DDPS).

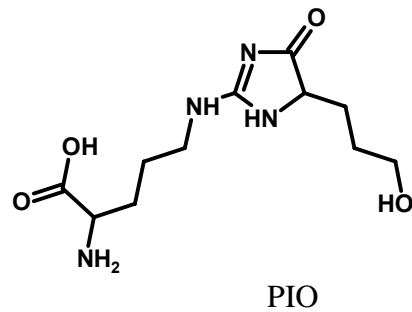
by Varoujan A. Yaylayan

The formation of reactive 1,2-dicarbonyl compounds both in food and biological systems constitute a significant milestone in the time-scale of the Maillard reaction both qualitatively and quantitatively. Important amino acid/protein initiated cross-links and formation of many heterocyclic compounds are dependant on the amount and type of reactive 1,2-dicarbonyl compounds present. Knowledge of the origin and structure of such dicarbonyls can further accelerate our understanding and discovery of end products resulting from their interactions. The chemistry of Maillard reaction in fact is the chemistry of formation and interaction of various dicarbonyls. In general, monodeoxy-dicarbonyls such as 1-deoxy and 3-deoxyglucosones can be formed mainly from monosaccharides and dideoxy-dicarbonyls such as 1,4-dideoxyhexosulose can be formed mainly from oligosaccharides and disaccharides (see tscheme below).



A recent contribution by Mavric and Henle (2006) have disclosed the identification and structural elucidation of another oligosaccharide-specific dicarbonyl namely 3,4-dideoxypentosulose (see the Scheme above). The authors have confirmed that the newly discovered dicarbonyl can arise only from disaccharides containing 1,4-glycosidic linkages such as lactose, maltose and I

lactulose. The proposed mechanism suggests the initial formation of 3-deoxy-erythro-pentulose from the disaccharide followed by dehydration and  $\beta$ -elimination. The authors conclude that 3,4-DDPS is involved in the formation of an arginine initiated cross link known as (N- $\delta$ -[5-(3'-hydroxypropyl)-4-oxo-imidazolone-2-yl]-L-ornithin (PIO) that they have identified in different heated foods such as milk, bakery products, malt, beer and coffee.



## **Reference**

1. Mavric E, Henle T. (2006) Isolation and identification of 3,4-dideoxypentosulose as specific degradation product of oligosaccharides with 1,4-glycosidic linkages. *Eur Food Res. Technol.* 223: 803-810.



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