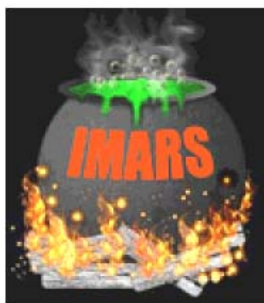


IMARS Highlights



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>

Volume 3, Number 1

January 15, 2008

Editor

Naila Rabbani (UK)

Contributing Editors

John Baynes (USA)
Timo Buetler (CH)
Vincenzo Fogliano (IT)
Josephine Forbes (AU)
Alejandro Gugliucci (USA)
Toshio Miyata (JP)
Teruo Miyazawa (JP)
Monika Pischetsrieder (DE)
Caspar Schalkwijk (NL)
Alan Stitt (NI)
Varoujan Yaylayan (CA)
Rosario Zamora (ES)

CONTENTS

Editorial comment	<i>i</i>
Interplays among hypoxia, oxidative stress, and advanced glycation? <i>Toshio Miyata</i>	1
Seed germination and Maillard reaction <i>Rosario Zamora</i>	2
Oxidative stress and glycation <i>Timo Buetler</i>	3
Dicarbonyls in coffee <i>Timo Buetler</i>	5
The role of histidine in protein crosslinking <i>Rosario Zamora</i>	8
Glyoxalase 1 becomes a vitagene <i>P.J.Thornalley</i>	9
The oxidation step in AGEs production and its relation to mitochondrial leakage. <i>Gerhard Spiteller</i>	10
FEATURES	
Highlights of the glycation literature November – December 2007 <i>Naila Rabbani</i>	13
Corporate Sponsors	14

Editorial comment

Welcome to the January issue of IMARS Highlights. It gives me great pleasure to bring to your attention highlights in glycation research selected by our contributing editors – this month, Toshio Miyata, Timo Buetler, Rosario Zamora and Paul Thornalley. I have also added an article by Gerhard Spitteller for discussion. I will encourage the readers of highlights to add their voice and comments related to Gerhard Spitteller's article. I hope to make these regular features in IMARS highlights. Feedback from readers on these and other features in this issue are very welcome.

I would like to thank Paul Thornalley for providing an article at the last minute on the forthcoming work by Michael Morcos and collaborators on prevention of dicarbonyl glycation and life extension. We would all like to live a long time, so please watch out for those dicarbonyls! I would like to remind our Junior Scientist about the Writing Competition for postgraduate and post-doctoral researchers in Biomedical Sciences and Food Sciences divisions. The winners will be invited to the 10th Maillard Reaction Symposium, Australia, 2009, with a contribution to the cost of attending the meeting.

Naila Rabbani

n.rabbani@warwick.ac.uk

Interplays among hypoxia, oxidative stress, and advanced glycation?

Toshio Miyat, Tohoku University, Graduate School of Medicine, Japan

Hypoxia and oxidative stress are two major abnormalities associated with oxygen metabolisms. Recent studies have opened new avenues in cellular defensive mechanisms against hypoxia and oxidative stress, especially primary regulatory nuclear factors in these processes.

Hypoxia and ‘HIF’: Oxygen supply declines under acute or chronic ischemic conditions in many human diseases. The resulting hypoxia causes functional impairment of cells. There exist however a broad spectrum of cellular defensive mechanisms against hypoxia, including angiogenesis, erythropoiesis, glycolysis, and anti-oxidative enzymes. Hypoxia-inducible factor (HIF) is a crucial intermediate in these defensive mechanisms (1). Under hypoxic conditions, HIF transactivates in the nucleus a host of genes involved in the adaptation to hypoxic stress (e.g., erythropoietin, vascular endothelial growth factor (VEGF), glycolytic enzymes, glucose transporters, heme oxygenase).

Oxidative stress and ‘Nrf2’: Transcriptional factor, Nrf2, regulates the basal and inducible expressions of numerous antioxidant stress genes (2). Upon exposure to oxidative stress and/or electrophiles, Nrf2 translocates into nuclei, heterodimerizes with a small Maf protein, and eventually leading to the transcriptional activation of antioxidant stress genes, including heme oxygenase-1, NAD(P)H-quinone oxidoreductase 1 (NQO1), and glutathione peroxidase-2 (GSH-Px2).

Both HIF and Nrf2 therefore activate “master gene” switches that result in broad and coordinated downstream reactions, protecting tissues against the consequences of hypoxia and oxidative stress, respectively.

Of particular note, the formation of advanced glycation end products (AGEs) is closely linked to hypoxia and oxidative stress. We have several clues in this direction. For example, cerebral ischemic injury is well-known to be associated with an increased genesis of AGEs. In this model, infarct volume is significantly larger in Nrf2 knockout mice than in wild type mice, but it is significantly smaller in knockdown mice of Keap1, a negative regulator of Nrf2 (3). Furthermore, in the same model, HIF activity inducers, e.g., cobalt or prolylhydroxylase inhibitors, reduce cortical damage in parallel with reduction of AGE formation (4, 5).

Respective or coordinate contribution of HIF and/or Nrf2 pathway to the biochemistry or pathophysiology of advanced glycation is therefore a future target of investigation.

References

1. Marx J. How cells endure low oxygen. (2004) *Science* 303: 1454-1456.
2. Motohashi H, Yamamoto M. Nrf2-Keap1 defines a physiologically important stress response mechanism. (2004) *Trends Mol Med.* 10:549-557.
3. Shih AY, Li P, Murphy TH. A small-molecule-inducible Nrf2-mediated antioxidant response provides effective prophylaxis against cerebral ischemia in vivo. (2005) *J Neurosci.* 25:10321–10335.
4. Nangaku M, Izuhara Y, Takizawa S, Yamashita T, Fujii-Kuriyama Y, Ohneda O, Yamamoto M, van Ypersele de Strihou C, Hirayama N, Miyata T. A novel class of prolyl hydroxylase inhibitors induces angiogenesis and exerts organ protection against ischemia. (2007) *Arterioscler Thromb Vasc Biol* 27: 2548-2554.
5. Ohtomo S, Nangaku M, Izuhara Y, Takizawa S, van Ypersele C, Miyata T. Cobalt ameliorates renal injury in an obese, hypertensive type 2 diabetes rat model. (2007) *Nephrol Dial Transplant* "in press"

Seed germination and Maillard reaction

Rosario Zamora, Instituto de la Grasa, Seville, Spain

During germination, reserves are mobilized and, as a result, polymers are converted into simpler molecules that can be transported to the embryo and used as energy sources and as substrates for synthesis in the new sprout. Accordingly, proteins and starches undergo enzymatic hydrolysis, raising the concentrations of free amino acids and soluble carbohydrates. These compounds might then react among them, therefore forming Maillard products.

A recent study of Rodríguez et al. (1) has observed an increase of furosine content in pea, bean, and lentil sprouts as a function of the germinating time. Thus, raw peas did not have furosine, and furosine content increased to 16 or 32 mg/16 g of N after germination in the presence or in the absence of light, respectively. Analogous increases were observed during the germination of beans (from 21 to either 28 or 68 mg/16 g of N after germination in the presence or in the absence of light, respectively) and lentils (from 6 to either 84 or 117 mg/16 g of N after germination in the presence or in the absence of light, respectively). Although other hypotheses cannot be discarded at this point, these results are in agreement with the formation of Maillard products following mobilization of seed's reserves. Thus, a direct linear correlation between the amount of furosine produced and the chemically available free lysine was observed.

The relationship between Maillard reaction and germination is not very well-known, although previous studies have shown that compound(s) responsible for germination stimulation can be formed from the amino-carbonyl reactions of a variety of amino-containing compounds with glucose (2). Although both studies have not been related and the compound identified by Light et al. (2) was not studied by Rodríguez et al. (1), the possibility that some Maillard products might be formed during germination and, simultaneously, play a role in the stimulation of the germination process, opens interesting perspectives on a potential role of Maillard reaction in this essential physiological process.

References

1. Rodríguez C, Frías J, Vidal-Valverde C, Hernández A (2007) Total chemically available (free and intrachain) lysine and furosine in pea, bean, and lentil sprouts. *J. Agric. Food Chem.* 55:10275–10280.
2. Light ME, Burger BV, van Staden J (2005) Formation of a seed germination promoter from carbohydrates and amino acids. *J. Agric. Food Chem.* 53:5936–5942.

Oxidative stress and glycation

Timo Buetler, Lausanne, Switzerland.

Oxidative stress and glycation is the classical hen and egg question. Does oxidative stress impinge on AGE formation or do AGEs influence oxidative stress? Although probably both are true which one is more important? The fact is that it is impossible to separate oxidative stress from glycation or lipid peroxidation. Neither can disease be disconnected from oxidative stress. Disease can be regarded as a redox imbalance towards a more oxidized state. What role do AGEs play in this context? Are they agents that can push an organism into redox imbalance and promote disease? It is more likely that they are not causative in promoting redox imbalance because we are constantly exposed to AGEs from endogenous formation and exogenous (dietary) sources. So the question can be asked whether AGEs may then be markers of redox imbalance or oxidative stress?

A series of recent publications by an Indian group around Zachariah Bobby have provided support for the theory that AGEs may be markers of oxidative stress (1). This is, in part, based on the observation that, in different diseases, increased lipid peroxidation, a commonly accepted marker for oxidative stress, is paralleled by increased AGE levels (2-4). *In vitro* studies have shown that the lipid peroxidation product malondialdehyde can increase glucose-mediated hemoglobin glycation (5) which can be inhibited by antioxidants such as lipoic acid or taurine. Already 10 years ago Jain and Palmer (6) have shown that hemoglobin glycation by glucose was increased by hydrogen peroxide, *tert*-butylhydroperoxide and malondialdehyde but inhibited by vitamin E.

Other recent studies have also found that serum AGEs were strongly associated with the lipid peroxidation marker f₈-isoprostane (7, 8). Eating of high-fat meals results in lipid peroxidation (9), oxidative stress (10) and increased AGEs (11). Likewise, eating of an excessively heated (thermolyzed) meal results in oxidative stress and inflammation (12). Thus, it is possible that AGEs found in the circulation after eating such meals may not solely reflect their absorption from the diet but may also be attributed to their *de novo* generation due to postprandial oxidative and carbonyl stress.

On the other hand it is now well documented that antioxidants have the ability to reduce or block AGE formation in parallel with exerting beneficial effects to correct the redox imbalance (13). This was also very nicely discussed by Josephine Forbes at the Maillard Symposium in Munich.

In conclusion, while it is now accepted that AGEs build up under conditions of oxidative stress evidence is accumulating that AGE formation may be a consequence of oxidative stress rather than its cause.

References

- (1) Selvaraj, N., Bobby, Z., Sridhar, M. G. (2007) Oxidative stress: Does it play a role in the genesis of early glycated proteins? *Med. Hypotheses.*,
- (2) Sathiyapriya, V., Bobby, Z., Vinod Kumar, S., Selvaraj, N., Parthibane, V., Gupta, S. (2006) Evidence for the role of lipid peroxides on glycation of hemoglobin and plasma proteins in non-diabetic asthma patients. *Clin. Chim. Acta* 366:299-303,
- (3) Sathiyapriya, V., Selvaraj, N., Nandeeshha, H., Bobby, Z., Agrawal, A., Sridhar, M. G., Pavithran, P., Rattina Dasse, N. (2007) Increased glycation of hemoglobin and plasma proteins in

- normotensive, non-diabetic obese Indian subjects: putative role of lipid peroxides. *Clin. Chem. Lab. Med.* 45:996-999,
- (4) Sathiyapriya, V., Selvaraj, N., Nandeesha, H., Bobby, Z., Agrawal, A., Pavithran, P. (2007) Enhanced glycation of hemoglobin and plasma proteins is associated with increased lipid peroxide levels in non-diabetic hypertensive subjects. *Arch. Med. Res.* 38:822-826,
 - (5) Selvaraj, N., Bobby, Z., Sathiyapriya, V. (2006) Effect of lipid peroxides and antioxidants on glycation of hemoglobin: an in vitro study on human erythrocytes. *Clin. Chim. Acta* 366:190-195,
 - (6) Jain, S. K., Palmer, M. (1997) The effect of oxygen radicals metabolites and vitamin E on glycosylation of proteins. *Free Radic. Biol. Med.* 22:593-596,
 - (7) Cai, W., He, J. C., Zhu, L., Chen, X., Wallenstein, S., Striker, G. E., Vlassara, H. (2007) Reduced oxidant stress and extended lifespan in mice exposed to a low glycotxin diet. Association with increased AGER1 expression. *Am. J. Pathol.* 170:1893-1902,
 - (8) Uribarri, J., Cai, W., Peppas, M., Goodman, S., Ferrucci, L., Striker, G., Vlassara, H. (2007) Circulating glycotoxins and dietary advanced glycation endproducts: two links to inflammatory response, oxidative stress, and aging. *J. Gerontol. A* 62:427-433,
 - (9) Staprans, I., Pan, X. M., Rapp, J. H., Feingold, K. R. (2005) The role of dietary oxidized cholesterol and oxidized fatty acids in the development of atherosclerosis. *Mol. Nutr. Food Res.* 49:1075-1082,
 - (10) Innis, S. M. (2007) Dietary lipids in early development: relevance to obesity, immune and inflammatory disorders. *Curr. Opin. Endocrinol. Diabetes Obes.* 14:359-364,
 - (11) Li, S. Y., Liu, Y., Sigmon, V. K., Mccort, A., Ren, J. (2005) High-fat diet enhances visceral advanced glycation end products, nuclear O-Glc-Nac modification, p38 mitogen-activated protein kinase activation and apoptosis. *Diabetes Obes. Metab.* 7:448-454,
 - (12) Shangari, N., Depeint, F., Furrer, R., Bruce, W. R., Popovic, M., Zheng, F., O'brien P, J. (2007) A thermolyzed diet increases oxidative stress, plasma alpha-aldehydes and colonic inflammation in the rat. *Chem. Biol. Interact.* 169:100-109,
 - (13) Monnier, V. M. (2003) Intervention against the Maillard reaction in vivo. *Arch. Biochem. Biophys.* 419:1-15,

Dicarbonyls in coffee

Timo Buetler, Lausanne, Switzerland.

Coffee is one of the most consumed beverages worldwide and is now considered to promote health. Coffee is also the Maillard beverage *par excellence*. The roasting process, carried out at 200-250°C for several minutes (5-15 minutes), is responsible for the development of the brown colour as well as the rich flavour and aroma of coffee. A second thermal treatment is performed during aqueous extraction, either at 100°C in home use or at up to 170°C in the industrial processing of instant coffee, further adding to the Maillard reaction in coffee.

Coffee is a rich brew of more than a thousand different molecules, many of which are formed during roasting. The probably largest class of molecules formed during roasting are the melanoidins. They make up 25-30% of the coffee dry matter. Although the term is not well defined, melanoidins are formed during the Maillard reaction and include AGEs. Some of the known Maillard reaction products in roasted coffee are 5-hydroxymethylfurfural (1) pentosidine (2) and acrylamide (3).

Recently, Daglia *et al.* (4) have reported that roasting also increased the formation of dicarbonyls in coffee. Both glyoxal and methylglyoxal are present at low levels in green coffee and their levels increase early during roasting while diacetyl is formed to lesser degrees late in the roasting process. Both glyoxal and methylglyoxal levels decrease with longer roasting times probably indicating their participation in the advanced stages of the Maillard reaction. Dicarbonyls are reactive molecules and were suggested to contribute to adverse health effects (5). It should, however, be noted that dicarbonyls are ubiquitous and are found in many food stuffs, beverages (6), commensal bacteria, water, rain, and the urban atmosphere (reviewed in 7). Furthermore, dicarbonyls may also be able to stimulate protective mechanisms (8).

The fact is that coffee has a long history of safe use and moderate coffee consumption is considered safe. During the last decade or so new evidence has emerged demonstrating a positive association of coffee consumption with better health as well as protection against various diseases (9, 10). The beneficial effects of coffee include protection certain against cancers (*e.g.* liver, 10, colon, 11), type 2 diabetes (12) and Parkinson's disease (9). Then, how can we explain the discrepancy between the presence of potentially toxic molecules in roasted coffee and its general beneficial effects on human health?

Coffee, being a mix of chemicals, contains both potentially deleterious as well as protective compounds with the beneficial ingredients apparently being more important. Among the beneficial ingredients in coffee are polyphenols (*e.g.* chlorogenic acids) and (again) the melanoidins that possess antioxidant and metal chelating properties (1, 13, 14). In addition to these antioxidant activities melanoidins have been shown to inhibit angiotensin-I converting enzyme activity *in vitro* (15) and to have the capacity to bind carcinogens resulting in anti-mutagenic properties (16). It is now time to introduce and accept the concept that low levels of toxins are probably good for our health because they stimulate our defense systems. This phenomenon is known as hormesis (17). One of the known hormetic effects induced by coffee is the Nrf2-mediated induction of protective phase II drug metabolizing enzymes (18, 19).

Thus, the example of coffee nicely illustrates that one chemical present in a complex mixture cannot be looked at separately. The toxicity of one compound isolated from food cannot be taken as argument that this food is unsafe for human consumption. The Maillard reaction results in the formation of numerous products and the net effect of these compounds on

health cannot be established by looking at each compound individually. In analogy to our need to be exposed to dirt and bacteria to challenge and prime our immune system, we may also need to be exposed to low doses of some potentially toxic products present in our food for better health.

References

- (1) Yen, W. J., Wang, B. S., Chang, L. W., Duh, P. D. (2005) Antioxidant properties of roasted coffee residues. *J. Agric. Food Chem.* 53:2658-2663
- (2) Foerster, A., Kühne, Y., Henle, T. (2005) Studies on absorption and elimination of dietary Maillard reaction products. *Ann. N. Y. Acad. Sci.* 1043:474-481
- (3) Parzefall, W. (2007) Minireview on the toxicity of dietary acrylamide. *Food Chem. Toxicol.*
- (4) Daglia, M., Papetti, A., Aceti, C., Sordelli, B., Spini, V., Gazzani, G. (2007) Isolation and determination of alpha-dicarbonyl compounds by RP-HPLC-DAD in green and roasted coffee. *J. Agric. Food Chem.*
- (5) Kalapos, M. P. (1999) Methylglyoxal in living organisms: chemistry, biochemistry, toxicology and biological implications. *Toxicol. Lett.* 110:145-175
- (6) Lo, C.-Y., Li, S., Wang, Y., Tan, D., Pan, M.-H., Sang, S., Ho, C.-T. (Reactive dicarbonyl compounds and 5-(hydroxymethyl)-2-furfural in carbonated beverages containing high fructose corn syrup. *Food Chem.* In Press, Corrected Proof
- (7) Nemet, I., Varga-Defterdarovic, L., Turk, Z. (2006) Methylglyoxal in food and living organisms. *Mol. Nutr. Food Res.* 50:1105-1117
- (8) Buetler, T. M., Latado, H., Baumeyer, A., Delatour, T. (2007) Dicarbonyls stimulate cellular protection systems in primary rat hepatocytes and show anti-inflammatory properties. *Ann. NY Acad. Sci.* In press
- (9) Higdon, J. V., Frei, B. (2006) Coffee and health: a review of recent human research. *Crit. Rev. Food Sci. Nutr.* 46:101-123
- (10) Larsson, S. C., Wolk, A. (2007) Coffee consumption and risk of liver cancer: a meta-analysis. *Gastroenterology* 132:1740-1745
- (11) Lee, J. E., Hunter, D. J., Spiegelman, D., Adami, H. O., Bernstein, L., Van Den Brandt, P. A., Buring, J. E., Cho, E., English, D., Folsom, A. R., Freudenheim, J. L., Gile, G. G., Giovannucci, E., Horn-Ross, P. L., Leitzmann, M., Marshall, J. R., Mannisto, S., McCullough, M. L., Miller, A. B., Parker, A. S., Pietinen, P., Rodriguez, C., Rohan, T. E., Schatzkin, A., Schouten, L. J., Willett, W. C., Wolk, A., Zhang, S. M., Smith-Warner, S. A. (2007) Intakes of coffee, tea, milk, soda and juice and renal cell cancer in a pooled analysis of 13 prospective studies. *Int. J. Cancer* 121:2246-2253
- (12) Van Dam, R. M., Willett, W. C., Manson, J. E., Hu, F. B. (2006) Coffee, caffeine, and risk of type 2 diabetes: a prospective cohort study in younger and middle-aged U.S. women. *Diabetes Care* 29:398-403
- (13) Daglia, M., Racchi, M., Papetti, A., Lanni, C., Govoni, S., Gazzani, G. (2004) *In vitro* and *ex vivo* antihydroxyl radical activity of green and roasted coffee. *J. Agric. Food Chem.* 52:1700-1704
- (14) Takenaka, M., Sato, N., Asakawa, H., Wen, X., Murata, M., Homma, S. (2005) Characterization of a metal-chelating substance in coffee. *Biosci. Biotechnol. Biochem.* 69:26-30
- (15) Rufian-Henares, J. A., Morales, F. J. (2007) Angiotensin-I converting enzyme inhibitory activity of coffee melanoidins. *J. Agric. Food Chem.* 55:1480-1485
- (16) Powrie, W. D., Wu, C. H., Molund, V. P. (1986) Browning reaction systems as sources of mutagens and antimutagens. *Environ. Health Perspect.* 67:47-54
- (17) Mattson, M. P. (2007) Dietary factors, hormesis and health. *Ageing Res. Rev.*

- (18) Steinkellner, H., Hoelzl, C., Uhl, M., Cavin, C., Haidinger, G., Gsur, A., Schmid, R., Kundi, M., Bichler, J., Knasmuller, S. (2005) Coffee consumption induces GSTP in plasma and protects lymphocytes against (+/-)-anti-benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide induced DNA-damage: Results of controlled human intervention trials. *Mutat. Res.* 591:264-275
- (19) Cavin, C., Marin-Kuan, M., Langouet, S., Bezencon, C., Guignard, G., Verguet, C., Piguat, D., Holzhauser, D., Cornaz, R., Schilter, B. (2007) Induction of Nrf2-mediated cellular defenses and alteration of phase I activities as mechanisms of chemoprotective effects of coffee in the liver. *Food Chem. Toxicol.*

The role of histidine in protein crosslinking

Rosario Zamora, Instituto de la Grasa, Seville, Spain

Protein crosslink is a well-known consequence of Maillard reaction. In fact, different mechanisms of protein crosslinking have been proposed and several crosslinks characterized. These crosslinks mostly involve lysine or arginine residues and the formation of imidazolic structures, such as in MOLD or GOLD, is not strange. However, the involvement of the imidazolic ring of histidine in these crosslinks was unknown until a recent study of Dai et al. (1).

When authors incubated a mixture of lysine, histidine, and threose, they isolated a novel acid-labile yellow chromophore which was identified as 2-amino-5-(3-((4-(2-amino-2-carboxyethyl)-1H-imidazol-1-yl)methyl)-4-(1,2-dihydroxyethyl)-2-formyl-1H-pyrrol-1-yl)pentanoic acid, a crosslink between lysine and histidine with addition of two threose molecules. The trivial name histidino-threosidine has been proposed for this compound.

Histidino-threosidine was detected in bovine lens proteins incubated with >10 mM threose, but not with 2 mM threose, leading to the conclusion that the formation of this crosslink requires saturation with sugars, i.e. conditions that are unlikely healthy physiological conditions. However, histidino-threosidine is the first Maillard product described to involve histidine in a crosslink.

Different studies have pointed out to the beneficial effects and utilization of histidine as an anti-cataract and anti-crosslinking agent (2). In fact, histidine has been proposed as the representative structure for an anti-crosslinking agent, containing the necessary functional groups for optimal protection against crosslinking agents. Thus, the imidazolium group of histidine or carnosine would stabilize adducts formed at the primary amino group. However, the results obtained in the study of Dai et al. provide experimental evidence that histidine can also be involved in the formation of stable structures with primary amino groups.

References

1. Dai Z, Nemet I, Shen W, Monnier VM (2007) Isolation, purification and characterization of histidino-threosidine, a novel Maillard reaction protein crosslink from threose, lysine and histidine. *Arch. Biochem. Biophys.* 463:78–88.
2. Hobart LJ, Seibel I, Yeagans GS, Seidler NW (2004) Anti-crosslinking properties of carnosine: significance of histidine. *Life Sci.* 75:1379–1389.

Glyoxalase 1 becomes a vitagene

Paul J. Thornalley, Warwick Medical School, Coventry, UK

The tiny worm or nematode *Caenorhabditis elegans* is a well-established experimental model for studying the ageing process (1). The normal lifespan is 15 – 17 days and so studies of *C. elegans* on the ageing process are practicable and great advances on the understanding of ageing have been made. Recent research indicates that increased expression of enzymes linked to detoxification is associated with life extension (2). Glyoxalase 1 (Glo1) metabolizes glyoxal and methylglyoxal (MG) thereby preventing the glycation of cellular and extracellular protein. Glyoxal and MG are major precursors of advanced glycation endproducts (AGEs). In several years of time-consuming and difficult work, Michael Morcos and co-workers at the University of Heidelberg, Heidelberg, Germany, identified the gene for Glo1 in *C. elegans* and studied the effect of overexpression and silencing on lifespan. The glyoxalase 1 homologue in the *C. elegans* genome data bank was identified, CeGly. Overexpression of CeGly increased lifespan by *ca.* 40% and RNAi silencing of CeGly decreased lifespan by *ca.* 40%. Overexpression of CeGly decreased the concentration of glyoxal and MG-derived AGEs and surprisingly also decreased the concentrations of markers of oxidative and nitrosative damage, methionine sulphoxide and 3-nitrotyrosine (3). Further studies showed that MG-derived AGE accumulation in ageing was localized to mitochondria and were associated with increased mitochondrial superoxide formation. This study is now to be published in the journal “Aging cell”. These data suggest a causative role of age-dependent mitochondria modification by AGEs in the aging process. Additionally, it suggests a role of Glo1 in the aging process. The network of genes that influence longevity have been termed “vitagenes” (4). Possibly the overexpression of Glo1 in many human tumours – also exhibiting multidrug resistance (5) – is a case of too much longevity for tumour tissue. There is now great interest on the critical proteins and sites within them that suffer functional impairment when glycated by dicarbonyls – the “dicarbonyl proteome”. Glycation researchers now have a stake in the vitagene debate.

References

1. Partridge,L, Gems,D: Mechanism of ageing: public or private. *Nature Reviews in Genetics* 3:165-175, 2002
2. Gems,D, McElwee,JJ: Broad spectrum detoxification: the major longevity assurance process regulated by insulin/IGF-1 signalling. *Mech.Ageing Dev.* 126:381-387, 2005
3. Morcos, M., Du, X., Sayed, A. A. R., Hutter, H., Pfisterer, F., Thornalley, P. J., Baynes, J. W., Thorpe, S. R., El Baki, R. A., Ahmed, N., Miftari, N., Stern, D., Schlotterer, A., Mohrlen, F., Hamman, A., Becker, C., Humpet, P., Hammes, H.-P., Buchler, M., Bierhaus, A., Brownlee, M., and Nawroth, P. Glyoxalase-I reduces mitochondrial reactive oxygen production and enhances life span in *C. elegans*. *Aging Cell*, 2008, in press
4. Calabrese,V, Guagliano,E, Sapienza,M, Panebianco,M, Calafato,S, Puleo,E, Pennisi,G, Mancuso,C, Butterfield,AD, Stella,A: Redox Regulation of Cellular Stress Response in Aging and Neurodegenerative Disorders: Role of Vitagenes. *Neurochemical Research* 32:757-773, 2007
5. Thornalley,PJ: Protecting the genome: defence against nucleotide glycation and emerging role of glyoxalase I over expression in multidrug resistance in cancer chemotherapy. *Biochem.Soc.Trans.* 31:1372-1377, 2003

The oxidation step in AGEs production and its relation to mitochondrial leakage.

Gerhard Spitteller, University of Bayreuth, Germany

“I want to induce a discussion about the main steps in the production of AGEs, I assume it would be important that our colleagues know the tremendous differences between generation of lipidhydroperoxide molecules within enzymes and the generation of peroxy radicals and their reaction with all types of secondary alcohols by release of hydroperoxy radicals”- Gerd

In the IMARS highlights as well as in many papers often the term “ROS” (reactive oxygen species) is used. “ROS” comprises a mixture of molecules (e.g. H_2O_2 , lipid hydroperoxides (LOOH) and radicals (e. g. $HOO\bullet$, $LOO\bullet$)¹. Because there are fundamental differences between the reactivity of molecules and radicals² a more precise definition is required. Free radicals are often several orders of magnitude more reactive compared to other molecules.

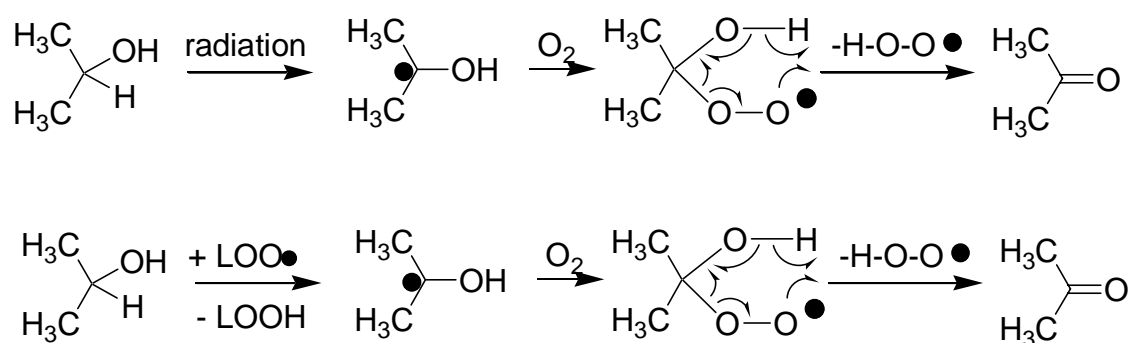
Generation of free radicals: Free radicals are produced in plants by radiation and in food by heat. Therefore, free radical formation cannot be avoided by baking or grilling or by preparing potato chips. In contrast, generation of free radicals in biological systems is often a two step process. Changes in cell membrane structure^{Error! Bookmark not defined.}, e.g. by growth or aging and especially by injury as occurs during attack by bacteria or fungi, induces influx of Ca^{2+} ions into the affected cells. The Ca^{2+} ions activate phospholipases. Phospholipases A_2 ³ cleave phospholipids to produce lysolipids and free polyunsaturated fatty acids (PUFAs). Free PUFAs are substrates for lipoxygenases (LOX)⁴ also activated like many other degrading enzymes, by Ca^{2+} ions influx. LOX generate LOOH molecules. These – and their enzymatically altered products - serve as ligands for proteins¹ which induce an adequate gene response. Thus the incorporation of PUFAs - belonging to the most oxygen sensitive molecules in nature – as constituents in cell membranes does not only serve to give the cell flexibility - as assumed previously - but are necessary to provide an appropriate response to inside or outside events. Nature has selected PUFAs for this purpose because their oxidation in a sequence of electron transfer reactions within the enzyme complex by formation of radicals as intermediates requires an extreme low amount of energy: The electron transfer reactions within LOX⁶ are catalyzed by iron ions. These induce the cleavage of the activated a C-H bond of a CH_2 group located between two double bonds of a PUFA. The thus generated hydrogen atom, $H\bullet$, transfers its electron to the Fe^{3+} iron, converting it to Fe^{2+} . The remaining radical reacts instantly to a lipid peroxy radical ($LOO\bullet$). Now Fe^{2+} transfers an electron to the $LOO\bullet$ radical to an LOO^- anion which combines with the generated proton to an LOOH molecule. The radicals formed as intermediates within LOX never leave the complex; the enzyme releases only molecules.

When the amount of free PUFAs reaches a critical threshold level, the free radicals formed as intermediates within LOX attack the bonds which keep the iron ions in the complex. As a consequence, iron ions are released which react with the already generated LOOH molecules still in close vicinity to the enzyme to produce $LO\bullet$ radicals. This event represents a switch from physiological reactions to pathologic ones. $LO\bullet$ radicals are of extreme high reactivity, exemplified by their ability to attack not only activated CH_2 groups of free PUFAs but also those in still intact phospholipids by hydrogen abstraction. The thus formed carbon-centered radicals produce peroxy radicals of phospholipids ($POO\bullet$). These are stabilize by hydrogen abstraction nearly from all types of biological molecules and by reactions not regarded sufficiently in the past.

Reactions of peroxy radicals^{Error! Bookmark not defined.}: It must be emphasized that usually any hydrogen abstraction induced by a free radical generates a molecule and a new free radical which carries a chain reaction on and on until it is stopped by producing a free radical with a

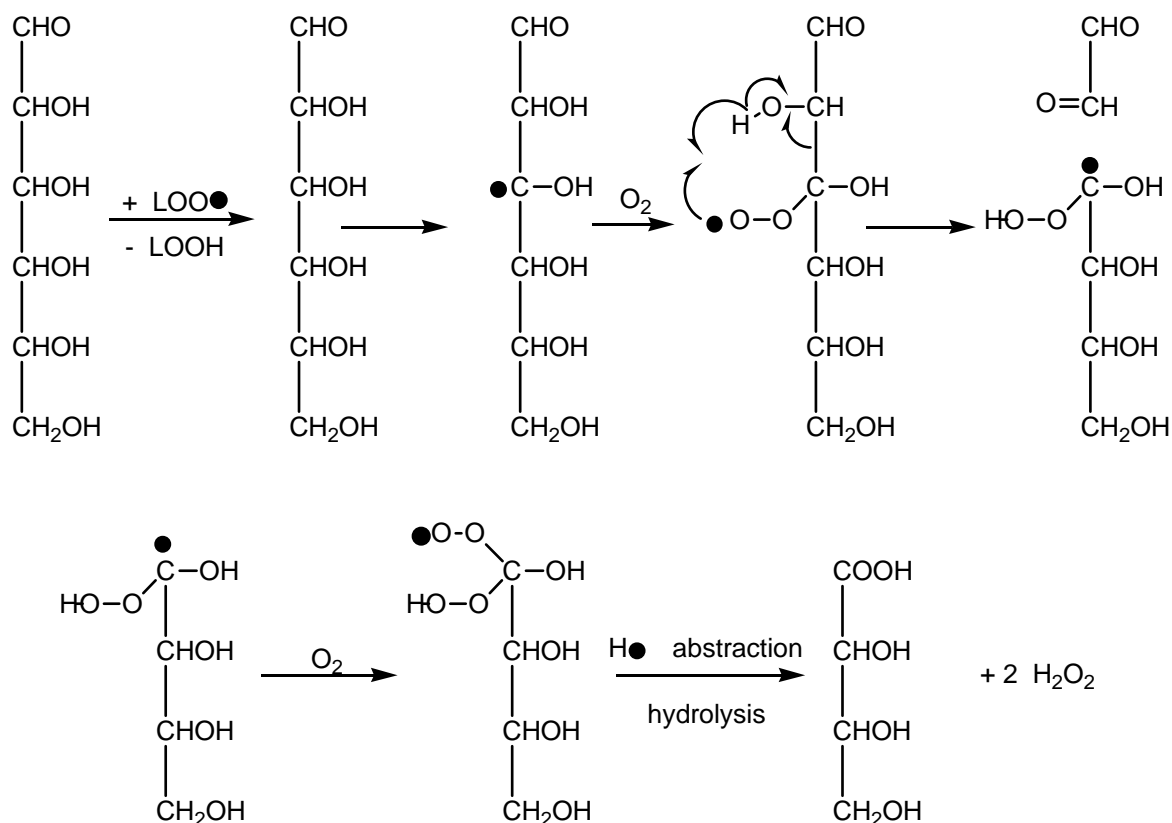
sufficient long life time to combine with a second free radical. Therefore each formed free radical is able to produce modifications of a large number of other molecules.

One of the most important reactions of free radicals is based on the oxidation of secondary alcohols. The mechanism of their oxidation was clarified by the investigation of the transformation of isopropanol to acetone⁷ by radiation: radiation cleaves the C-H bond connected to the alcoholic group to produce a carbon-centered radical which adds instantly oxygen. The thus generated peroxy radical decomposes to acetone by release of a HOO• radical in a five membered intramolecular hydrogen shift reaction.



The HOO• radical removes a hydrogen atom from the next isopropanol molecule and generate H₂O₂. Identical reactions are induced by peroxy radicals and identical reactions occur with sugar molecules or lysophospholipids as outlined above. The important and previously not sufficiently considered event in these reactions is the generation of HOO• radicals. Considering that mitochondria is comprises of membrane components, and stimulation of mitochondria by, for example, addition of an ionophore⁸ generates superoxide and also H₂O₂, it might be hypothesized that the postulated leakage process of mitochondriaⁱⁱ often regarded as the origin of free radical formation is in fact caused by the alteration of mitochondria cell walls by the addition of ionophores which induce an enzymatic lipid peroxidation reaction switching when an excess of free PUFAs is reached to a radical reaction. Moreover, many redox enzymes, e.g. NADPH-dependent reductases and catalase, were found to induce lipid peroxidation reactions. They all contain bivalent metal ions which catalyze electron transfer reactions. In all these reactions H₂O₂ is generated. Therefore it might well be that they induce similar as lipoxygenases the generation of free radicals when their substrates exceed a certain limit.

The release of HOO• radicals from secondary alcohols and sugars in the course of a 5-membered hydrogen transfer^{Error! Bookmark not defined.} is not the only degradation reaction of sugars. Sugar molecules have also the opportunity to be cleaved in a 6-membered hydrogen shift.



The resulting dicarbonyl compounds, e. g. glyoxal contribute to the formation of advanced glycation end products (AGEs). Peroxyl radicals have the unique property to react with molecules containing single double bonds, e. g. oleic acid, cholesterol or plasmalogens^{Error!} **Bookmark not defined.** to corresponding toxic epoxides. In addition, peroxyl radicals induce the oxidation of functional groups in side chains of amino acids incorporated in proteins.^{Error!} **Bookmark not defined.**

Due to the extreme reactivity of free radicals, their action is not under genetic control and an extreme large amount of products may be generated. Typical marker compounds of the Maillard reaction and AGE production represent therefore only a tiny fraction of all the deleterious compounds generated in non enzymatic lipid peroxidation reactions. Nature has learned apparently to respond to some of the generated products. As a consequence also a large number of genes are recognized which are activated or deactivated in aging and age related diseased such as atherosclerosis, Alzheimer's disease and diabetes.

Reference

- ¹ Sies, H. *Ang. Chem.* **98**, 1061-1076 (1986).
- ² Spitteller, G. *Free Radic. Biol. Med.* **41**, 362-387 (2006).
- ³ Lambert, I. H., Pedersen, S. F., Poulsen, K. A. *Acta Physiol.* **187**, 75-85 (2006).
- ⁴ Yamamoto, S., Suzuki, H., Ueda, N. *et al.* *Eicosanoids*, 53-9 (2004), John Wiley & Sons, Chicester (UK).
- ⁵ Nagy, L., Szanto, A. *Mol. Nutr. Food Res.* **49**, 1072-1074 (2005).
- ⁶ De Groot, J. J. M. C. *et al.* *Biochim. Biophys. Acta* **377**, 71-79 (1975).
- ⁷ Bothe, E., Behrends G., D. Schulte -Frohlinde, D. *Z. Naturforsch.* **32 B**, 886-889 (1977).
- ⁸ Kleineke, J., Söling H.D. *J. Biol. Chem.* **260**, 1040-1045 (1985); Baritt, G. J. *Cell Calcium* **2**, 53-63 (1981).
- ⁹ Halliwell, B., Gutteridge, J. M. C. *Trends Neurosci.* **8**, 22-26 (1985).

Highlights of the glycation literature November – December 2007

Cohen,G, Glorieux,G, Thornalley,P, Schepers,E, Meert,N, Jankowski,J, Jankowski,V, Argiles,A, Anderstam,B, Brunet,P, Cerini,C, Dou,L, Deppisch,R, Marescau,B, Massy,Z, Perna,A, Raupachova,J, Rodriguez,M, Stegmayr,B, VanHolder,R, Horl,WH: Review on uraemic toxins III: recommendations for handling uraemic retention solutes in vitro - towards a standardized approach for research on uraemia. *Nephrology Dialysis Transplantation* 22:3381-3390, 2007

Takeuchi,M, Sato,T, Takino,JI, Kobayashi,Y, Furuno,S, Kikuchi,S, Yamagishi,SI: Diagnostic utility of serum or cerebrospinal fluid levels of toxic advanced glycation end-products (TAGE) in early detection of Alzheimer's disease. *Medical Hypotheses* 69:1358-1366, 2007

Bhattacharyya,J, Shipova,EV, Santhoshkumar,P, Sharma,KK, Ortwerth,BJ: Effect of a single AGE modification on the structure and chaperone activity of human alpha B-crystallin. *Biochemistry* 46:14682-14692, 2007

Kumar,PA, Kumar,MS, Reddy,GB: Effect of glycation on alpha-crystallin structure and chaperone-like function. *Biochem J* 408:251-258, 2007

Diamanti-Kandarakis,E, Piperi,C, Korkolopoulou,P, Kandaraki,E, Levidou,G, Papalois,A, Patsouris,E, Papavassiliou,AG: Accumulation of dietary glycotoxins in the reproductive system of normal female rats. *Journal of Molecular Medicine-Imm* 85:1413-1420, 2007

den Hollander,NC, Mulder,DJ, Graaff,R, Thorpe,SR, Baynes,JW, Smit,GPA, Smit,AJ: Advanced glycation end products and the absence of premature atherosclerosis in glycogen storage disease Ia. *Journal of Inherited Metabolic Disease* 30:916-923, 2007

Pichiule,P, Chavez,JC, Schmidt,AM, Vannucci,SJ: Hypoxia-inducible factor-1 mediates neuronal expression of the receptor for advanced glycation end products following hypoxia/ischemia. *Journal of Biological Chemistry* 282:36330-36340, 2007

Yamagishi,S, Adachi,H, Takeuchi,M, Enomoto,M, Furuki,K, Matsui,T, Nakamura,K, Imaizumi,T: Serum level of advanced glycation end-products (AGEs) is an independent determinant of plasminogen activator inhibitor-1 (PAI-1) in nondiabetic general population. *Hormone and Metabolic Research* 39:845-848, 2007

Bhattacharyya,J, Shipova,EV, Santhoshkumar,P, Sharma,KK, Ortwerth,BJ: Effect of a single AGE modification on the structure and chaperone activity of human alpha B-crystallin. *Biochemistry* 46:14682-14692, 2007












INTERNATIONAL MAILLARD REACTION SOCIETY

plstein Building
03 Cornell Road, Room 5127
Cleveland, Ohio 44106. USA.

Phone: 216-368-6613
Fax: 216-368-1357
E-mail: imars@case.edu



THANK YOU TO OUR GENEROUS SPONSORS

	 Good Food, Good Life	
 RenaScience Renaissance in kidney disease		
		 <i>Kowa Company, Ltd.</i>
		
		
A DIVISION OF		
 THE ESTÉE LAUDER COMPANIES INC. Bringing the best to everyone we touch.		