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Melanoidins and dietary fiber: closer than expected.

by Vincenzo Fogliano

Nutritionists recommend an increase in the intake of dietary fiber, particularly of soluble dietary fiber which is quite low in typical Western diets. Dietary fiber is defined as “edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine” (1). According to this definition melanoidins are not dietary fiber because they are not “naturally present” in foods, but formed as consequence of thermal treatment. However, the importance of dietary fiber is related to its biological functions particularly to prebiotic effects (2), and in the last few years, some reports suggested that the physiological effect of melanoidins in the gastro-intestinal tract could be similar to that of dietary fiber (3).

In a comprehensive review in press in Molecular Nutrition and Food Research (MNF, 2006, 50) Richard Tuohy and coworkers discussed the latest findings on the metabolic fate of dietary melanoidins and AGE modified proteins and the consequence of their interaction with gut microflora. In this respect the gut model approach developed by the Gibson group in Reading, UK, coupled with the new possibilities of molecular technology for microbe identification, has an enormous potential for new discoveries.

As a general and rough rule we can say that the amount of melanoidins and AGE modified proteins reaching the lower gut depends on the extent of thermal treatment: the more severe the treatment the higher the quantity. This is due both to the decrease of protein digestibility caused by Maillard reaction driven crosslinking, and also to the formation of new polymers, particularly in carbohydrate-rich food items such as bread crust or coffee. Unlike carbohydrate, the fermentation of proteins in the lower gut is generally not desirable. It increases the concentration of ammonia and leads to the formation of toxic amines, amides and phenolic compounds. Excessive protein fermentation has been linked with colon cancer and ulcerative colitis. In support of this association, an increase in detrimental bacteria and a decrease of Bifidobacteria and Lactobacilli was observed after administration of AGE-BSA compared to native BSA to healthy subjects and particularly in ulcerative colitis patients (4).

On the other hand, preliminary experiments carried out with melanoidins having a higher content of polysaccharide and a relatively low amount of protein gave different results. An interesting example is the coffee silverskin (CS) which is the external layer of the coffee beans. CS is broken during roasting and it becomes a by product of the roasting plant. CS has 60% dietary fiber (14% soluble fiber), and in practice CS dietary fiber is mainly melanoidins. Static batch culture fermentation experiments showed that CS induces preferential growth of Bifidobacteria rather than Clostridia and Bacteroides (5). In other studies it was reported that anaerobic bacteria, particularly Bifidobacteria strains, are able to use bread melanoidins as a carbon source. The bacterial growth is different for melanoidins extracted from the bread crusts obtained from different starting materials (wheat, durum wheat, whole-meal) or different processing conditions (time and temperature of cooking). These findings suggest that there potential to modulate the prebiotic activity of bread melanoidins (6).

What can be the rationale for the prebiotic effect of different types of melanoidins? Two points should be considered. Firstly, some melanoidins (i.e those from coffee) have a relevant soluble moiety and the fraction of soluble dietary fiber has a major role in determining the prebiotic activity. Secondly the antioxidant compounds linked to some typology of dietary fiber seem beneficial for gut microflora (7-9), but we all know that antioxidant activity is also one of the major features of food melanoidins!
References


Glucose-6-phosphate steps into the limelight of the Maillard arena

By Alejandro Gugliucci

The first intracellular glucose metabolite, glucose-6-phosphate (G-6-P) has been known for many years to be much more aggressive as a glycating agent (mainly in vitro) than glucose itself (1-2). However, no studies had addressed the role of G-6-P in vivo. The recent paper by Juliette Fortpied et al not only underscores the role of G-6-P as an important glycating agent in skeletal muscle (and other tissues) but goes on to discover an enzyme that catalyzes the dephosphorylation of these adducts and propose a putative, novel, 2 step mechanism for protein repair.

Scheme 1. Fructosamine-6-phosphatase: a new deglycating enzyme working in concert with fructosamine-3-kinase

The investigators were intrigued by the fact that the enzyme fructosamine-3-kinase (which catalyzes deglycation of proteins modified by glucose, see scheme above), is present in heart and muscle, tissues in which intracellular glucose is very low. In search for a more logical glycating agent that would justify the need for this de-glycase in these tissues, the authors turned their attention to G-6-P, which can reach 0.3-0.6 mmol/L in these muscles. After confirming a 3-8 fold higher rate of glycation of model proteins by G-6-P (vis-à-vis glucose), the authors produced free and macromolecular fructosamine-6-phosphates which they elegantly use as substrates to purify and characterize a fructosamine-6-phosphatase from rat skeletal muscle, and which can efficiently de-phosphorylate fructosamines in G-6-P-modified proteins. They also show that fructosamine-3-kinase has no activity on the same substrates. The enzyme
was next found to be identical to a known magnesium-dependent phosphatase 1, whose main
known substrate (up to now) is arabinose-5-P, a non physiological sugar.

Recombinant magnesium-dependent phosphatase 1 was then shown to share the same
properties as the enzyme this group purified. More striking was the finding that this enzyme
prefers protein-fructosamine-6-phosphates ten times better than its next known substrate,
strongly suggesting a physiological role behind this property. The Scheme above summarizes
these findings and illustrates the role the authors propose for this new enzyme in the
deglycating armamentarium. A 2 step mechanism, in essence acting like a mutase would be
necessary to remove fructosamine-6-P from proteins.

More research needs to be done to definitely establish the former as a physiological pathway,
by confirming the actual presence of fructosamine-6-P in cells and by inactivating the gene for
the enzyme, but this work provides sound support for that to be true. One can hypothesize that
an increased intracellular fructosamine-6-P (protein bound) could occur in patients with G-6-P
DH deficiency (or a compensatory increase in magnesium-dependent phosphatase 1). The ER
and Golgi are rich in G-6-P, which participates as a donor in several glycosylation reactions.
Could proteins come out from the Golgi, already glycated by G-6-P? Could G-6-P glycation
occur in proteins in the circulation, as a consequence of G-6-P leaking as a byproduct of cell
turnover, especially in chemotherapy treatments or hemolytic episodes? In any case, for ever
and a day G-6-P is back to stay!!

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Evaluation method of aging of human lens using LC/MS analysis

by Teruo Miyazawa

In recent papers by Cheng et al (1), new evidence supporting the chemical identity of the ascorbic acid-modified amino acids in lens proteins glycated by ascorbic acid was collected using a newly developed two-dimensional LC-MS mapping technique, supported by tandem mass analysis of the major species.

The aging of the human lens is characterized by an increase in water-insoluble proteins associated with high levels of yellow chromophores and non-tryptophan fluorescence (2). These protein modifications are all enhanced in senile and brunescent cataractous lenses. The glycation of lens proteins has been suggested to be one of the major protein modifications present in older lenses, and indeed diabetic patients are at higher risk for cataract formation (3). Due to the possibly lifelong stability of the lens crystallins, they can accumulate damaging modifications continuously. In time, these may be responsible for protein aggregation and crosslinking and eventually to age-onset cataract.

Ascorbic acid is present in the human lens at relatively high concentrations (up to 2 mM), which is higher than the normal glucose levels in lens (<1 mM) (4). This is potentially significant, because ascorbic acid has been shown to participate in both glycation reactions and the metal-dependent generation of oxygen free radicals in vitro (5). In vitro incubation experiments have revealed that the oxidation products of ascorbic acid can rapidly react with lens proteins through glycation reactions to form cross-linked proteins with characteristic browning and fluorescence (6). These changes resemble those occurring in the lens during normal aging and cataract formation. The structures of several advanced glycation endproducts (AGEs), such as pentosidine, carboxymethyllysine, LM-1 (vesperlysine A), glucosepane and K2P are known, and have been identified in human lens proteins. Similar compounds can be formed by the reaction of lens proteins with ascorbic acid in vitro, however, the exact glycating agent responsible for forming these AGEs in vivo has yet to be identified with certainty.

In order to support a role for ascorbate glycation in lens aging and cataract formation, it will be necessary to isolate the AGEs from ascorbylated proteins, and show that several of these are identical to the yellow, fluorescent compounds isolated from both aged human and brunescent cataract lenses. In previous papers by Cheng et al, it was reported that the yellow chromophores and fluorophores in human cataractous lenses are chromatographically nearly identical to those formed from in vitro ascorbylation of calf lens proteins (7) and all these yellow chromophores increased quantitatively with age in the normal human lens water-insoluble sonicate supernatant proteins (8). Recently, they developed a new 2-dimensional LC-MS technique, which allows them to analyze the total modified amino acids in cataract lens protein digests and in digests of lens proteins glycated by ascorbic acid in vitro. Furthermore, this technique permits an LC-MS/MS analysis of the major modifications present in these two preparations. Based upon the data obtained by this method, they conclude that the majority of the major modified amino acids present in early stage brunescent Indian cataract lens proteins appear to arise as a result of ascorbic acid modification, and are presumably advanced glycation end-products.

Hopefully, this work provides a general method, which can be employed in the future, to identify individual modified amino acids which develop with aging in vivo and during glycation reactions in vitro. An LC/MS analysis in this manner can be used to obtain a complete picture of
the Maillard reaction products obtained with different carbonyl compounds, as well as the protein modifications that accumulate during aging.

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Reduced protein digestibility of Maillard Reaction product rich food: an emerging nutritional problem?

by Vincenzo Fogliano

Studies on potential health hazards related to the dietary intake of thermally treated foods have traditionally focused on the presence of specific Maillard reaction (MR) products such as acrylamide, heterocyclic amines and HMF (1). During the seventies and the eighties another area of concern to food scientists was the decrease in protein nutritional quality resulting from the decrease of available lysine caused by the MR (2). However, for the populations of Western countries, where there is an abundance of protein in the diet, the decrease of available lysine is a marginal problem, with the important exceptions of infant formulas and preparations used for enteral nutrition.

The decrease in protein digestibility as a result of protein structural changes induced by the MR has been less investigated. Food scientists working in this field know that one of the major effects of thermal treatment is a major increase in protein crosslinking, leading to the formation of “melanoprotein,” the term coined by Thomas Hofmann in 1998 to describe this phenomenon (3). It is quite impressive to observe that proteins extracted from severely heated foods are hardly able to enter a 10% SDS-PAGE gel! As a consequence, it is quite easy to imagine that human digestion and absorption of this protein network could be very different from that of the original protein. Actually, it can be hypothesized that moderate thermal treatment increases digestibility by denaturing the proteins, particularly those of vegetable origin. On the other hand, the massive covalent aggregation of protein caused by more severe treatment could affect digestibility. Some studies have been carried out using both in vitro (4) and animal (5) models, but in vivo intervention trials to address this issue are still in their infancy.

One in vivo study to evaluate the effect of MR on protein digestibility was carried out on adolescent males at the University of Granada by Isabel Seiquer and co-workers and the results were recently published (Am. J. Clin. Nutr., 2006, 83, 1082-1088). A cross over experimental plan with 2 weeks of MR product rich “Brown diet” and a white diet was designed. The results clearly show that during the brown diet period subjects have a higher fecal nitrogen excretion and lower values of nitrogen absorption and digestibility which is related to the greater MR product content in the diet. The efficiency of the utilization of ingested nitrogen was 11% lower during the brown diet consumption, but the difference did not reach statistical significance. As expected, urine color was also darker during the brown diet due to the higher urinary excretion of premelanoidins and other MR products (6-7).

On the other hand the data on the distribution of nitrogen absorbed and remaining in the feces indicate that the reduction of protein digestibility in the brown diet, although significant, was only 6% of that of the white diet, and the retention of nitrogen was not significantly lower during the brown diet. Therefore, the brown diet, which is quite similar to the standard diet of a western population and particularly of young people eating many snacks and fast foods, do not appear to afford any significant health risk with respect to digestive and metabolic protein utilization. However, the authors stressed that long term effects should be investigated considering the negative effect of brown diet on protein digestibility.

The other side of the coin is the metabolic fate of the “melanoproteins” which reach the lower gut and become substrates for microflora fermentation. A 47% increase in fecal nitrogen was found during brown diet: is that beneficial or negative for the equilibrium of gut microflora? Nobody knows….
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A two-hit, AGE-calgranulin ‘shock’ treatment of RAGE greatly increases inflammation

By Alejandro Gugliucci

The Receptor for Advanced Glycation End Products (RAGE) interacts with AGEs, products of the Maillard reaction, that accumulate in various conditions, such as diabetes, inflammation, renal failure, pro-oxidant states and natural aging (1). Research in both cell culture and in vivo shows that interaction of RAGE ligands with the receptor, activates vital signal transduction pathways that modulate key cellular events, leading to vascular and inflammatory cell disorders (2). Recent studies have shown that RAGE also interacts with pro-inflammatory molecules, including S100/calgranulins (3). The S100/calgranulins are known to be released from activated inflammatory cells, including mononuclear phagocytes as well as polymorphonuclear leukocytes and lymphocytes. What would be the result of a double stimulation of RAGE by AGEs and S100/calgranulins? Would there be synergy? Ehlermann et al have recently posed these questions and conducted experiments to address the hypothesis (4).

In a simple model (see diagram below) they stimulated RAGE on human endothelial cells with AGE-albumin for a few days as a pre-activation step. This was followed by incubation with heterodimeric recombinant calgranulins S100A8 and A9 (see diagram).

A8/A9 dimers greatly enhanced (3 - 8 fold) the MAP kinase-NF kappaB-mediated secretion of several proinflammatory mediators, including IL-6, ICAM-1, VCAM-1, and MCPI. No effects

Figure. The endothelial cell RAGE response to calgranulins is enhanced by previous exposure to AGEs: a two hit model that would help explain enhanced inflammation in diabetics.
could be elicited with several homodimeric forms of these calgranulins. It is important to note here, that the authors used physiologically relevant concentrations of AGEs; 1-2 orders of magnitude lower than those used in some other studies (5). Because the first hit was low, it was a true pre-activation, and the results thus acquire greater physiological importance, since the AGE concentrations are far closer to those found in patients.

The diagram on the previous page (this columnist’s interpretation) graphically summarizes the 2 hit hypothesis (1-3), and further illustrates the proposed model of enhanced procoagulant-proinflammatory effects of RAGE activation by calgranulin heterodimers, against a continuous background of AGE pre-engagement of the receptor.

Further studies are warranted to clearly establish this model as a pathophysiologically relevant pathway. However, the magnitude of the effect observed in the present experiments, together with the presence of co-morbid infection so characteristic of the diabetic state, suggests that this endothelial activation might be taking place and feed-forward in a deleterious cycle. Together with the ongoing research on strategies to block RAGE, this work opens new avenues for exploration, namely, how to block either calgranulin secretion from monocytes or their interaction with RAGE. Accelerated atherosclerosis in chronic human diseases with a high AGE burden (diabetes and renal failure) could benefit significantly from these putative therapeutic modalities.

References

The ‘match’ for the protective effects of anti-glycation compounds ended in a victory for vitamin E!

by Teruo Miyazawa

The chronic hyperglycemia of diabetes mellitus is believed to have an important role in the pathogenesis of long-term complications (1). Hyperglycemia may cause defective angiogenesis with reduced endothelial cell proliferation, migration and capillary tube formation, and this may underlie diabetic complications such as impaired wound healing, stroke and heart disease (2). It is believed that an increase in tissue and serum advanced glycation endproducts (AGEs) are responsible for the vascular damage observed in diabetes (1). This damage is compounded by production of free radicals from the autoxidation of glucose and Amadori products and from interaction of AGEs with their cellular receptors often abbreviated as RAGE (3).

The location and longevity of endothelial cells means they are prime targets for hyperglycemia-induced cell toxicity. Therefore, endothelial dysfunction may underlie the pathogenesis of diabetic vascular complications. Hyperglycemia is known to reduce the in vitro proliferation of endothelial cells obtained from a number of sources, including human umbilical vein, bovine retina and skin (4). Hyperglycemia has also been shown to reduce migration of cultured endothelial cells in vitro (5).

During hyperglycemia, endothelial cells may be exposed to circulating AGEs that have accumulated on small proteins or peptides. Circulating serum AGEs increase in patients with renal failure and have been implicated in the high incidence of vascular disease in such patients (6). AGE-modified bovine serum albumin (BSA-AGE) has been used to study AGE toxicity on a number of cell types and shown to be toxic to mesangial cells, bovine aortic endothelial cells and retinal pericytes (7). This toxicity is mediated via production of free radicals, particularly when AGEs interact with their receptors on the cell surface (3).

There is considerable interest in compounds that can protect against glucose-induced cellular damage because of their therapeutic potential. Such compounds may act by (i) protecting protein amino groups available for glycation; (ii) blocking carbonyl groups on Amadori products and 3-deoxyglucosone (3-DG) thus reducing AGE formation or (iii) protecting against glucose and AGE-derived free radicals. Accordingly, Zhang et al (8) investigated and compared the protective effects of three different anti-glycation compounds, aspirin, D-penicillamine and vitamin E, against high glucose and advanced glycation endproduct (AGE) mediated toxicity in cultured bovine endothelial cells using two approaches. Cell proliferation was assessed in culture in different concentrations of glucose (5.5–100 mmol/l) with and without these inhibitors. A monolayer of cultured endothelial cells was wounded and recovery at the wound site was measured following exposure to different concentrations of glucose with and without inhibitors. The ability of these compounds to protect cultured endothelial cells following exposure to bovine serum albumin-derived advanced glycation endproducts (BSA-AGE) was also studied. Addition of glucose to cultured endothelial cells inhibited their proliferation in a dose dependent manner. All three compounds protected against the anti-proliferative effects of high glucose, with vitamin E being the most effective. The migration of cultured endothelial cells following wounding was inhibited by increasing concentrations of glucose but was maintained in the presence of all three anti-glycation compounds with vitamin E, again giving the greatest protection. Vitamin E was also the most effective at protecting against the anti-proliferative effects of BSA-AGE. D-Penicillamine was not as effective as vitamin E whereas aspirin offered no significant
protection against AGE-induced cellular toxicity. Hence, these studies suggest that compounds, such as vitamin E, with combined antiglycation and antioxidant properties offer maximum therapeutic potential in protection against high glucose and AGE-mediated cellular toxicity.

Hopefully, natural compounds, such as vitamin E, which have combined anti-glycation and antioxidant properties are likely to offer maximum therapeutic potential.

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