



IMARS Highlights

Research Commentaries for Members of The International Maillard Reaction Society

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

Some of the finest recipes are those that require a long cooking time and are thus enriched in the most flavorful Maillard reaction products. We are happy to say that *IMARS Highlights* is now ready to be served.

After each of the International Maillard Symposia attendees have expressed a desire to promote the continued exchange of information among researchers in diverse disciplines. This need for a venue for communication led to the founding of IMARS - a forum to foster on-going inter-disciplinary communication among Maillard researchers. As part of that effort, *IMARS Highlights* will begin to appear in your mailbox on a bimonthly basis. During our first year, we will publish brief reports from contributing editors providing summaries and critiques of papers from the current literature that are selected because of their novelty or interest across a range of disciplines from food technology to health and disease. The articles in this inaugural issue cover such a spectrum of issues, where underlying non-enzymatic chemistry may be of relevance in both food and biomedical sciences. For example, new studies suggest that melanoidins formed during food processing now appear to be potential sources of reactive oxygen species, while an analogous process may be happening in the human lens with age and diabetes.

Our long-term goal is to develop a full-function web journal, including both original research articles and reviews. The publication committee has opted to begin with the Highlights format. *IMARS Highlights* may best be viewed as a stepping stone to the future for an important journal for interdisciplinary research at the intersection of food and biomedical sciences. The journal welcomes contributions in the form of letters, short reports and reviews from IMARS members. We also encourage submission of news from members, including news of research awards as well as public policy and advocacy information for inclusion in sections on "Members in the News" and "Public Policy Forum." Letters and or short articles should be sent by email to the attention of the editor at IMARS@case.edu

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Increased glycated hemoglobin in non-diabetic patients. What does it mean?

By John W. Baynes

Two recent papers from the Department of Biochemistry, Jawaharlal Institute, Pondicherry, India, report on the increase in glycated hemoglobin (GlcHb) in non-diabetic patients with chronic renal failure or asthma (1, 2). The increases were significant – 30-50% over controls, without a change in blood glucose. The authors suggest that the level of GlcHb is sensitive to oxidative stress and demonstrate that the increase in GlcHb, measured by the Bio-Rad micro-column assay, is correlated the plasma malondialdehyde, assayed by the thiobarbituric acid assay. In the asthmatic patients, they also report a significant decrease in erythrocyte reduced glutathione (GSH) (65%) and a mild, but significant (10%) decrease in plasma ascorbic acid (AA). However, in neither study were levels of GSH or AA correlated with the increase in GlcHb.

What's happening? Why should GlcHb be increase in patients in good glycemic control? First, the above work confirms earlier studies by Jain and Palmer (3) demonstrating an increase in GlcHb in red cells exposed to *t*-butylhydroperoxide *in vitro*, and also showing that this increase in GlcHb was inhibited by vitamin E. Second, all three of the studies use ion exchange affinity micro-columns for measuring GlcHb. Unlike phenylboronate columns which bind Amadori adducts on Hb, these columns are designed to retain anionic forms of Hb, primarily HbA_{1c}. Thus, it is possible that oxidative stress and lipid peroxidation reactions associated with pro-inflammatory renal and pulmonary disease are producing reactive oxygen species that generate an anionic form of hemoglobin – not GlcHb, but oxidized Hb (HbOx). Identification of the anionic modification on oxidatively damaged Hb might lead to a useful assay to integrate long-term oxidative stress. Using modern methods for LC/MS/MS analysis of hemoglobin, it should be possible to identify this modification, and then to search for this modification on plasma proteins. Assay of plasma proteins would provide an intermediate-term integration of oxidative stress in the vascular compartment.

There is another possibility. Szwergold and colleagues (4) suggest that GSH, through a transglycosylation reaction with Schiff bases, inhibits glycation of protein. Thus, a decrease in mean GSH concentration – which may not be readily detected by acute measurements of GSH in red cells - may also lead to an increase in glycation of protein in inflammatory disease. Indeed, Donde *et al.* (5) reported nearly 20 years ago that GlcHb was increased in persons with glucose-6-phosphate deficiency.

Overall, the increase in GlcHb in patients with inflammatory disease or decreased antioxidant defenses appears to lead to an increase in either GlcHb or HbOx. The tools are available to address this question and provide new insights into the role of oxidative stress in glycation of protein.

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Melanoidins: More evidence for a dark side

By Monika Pischetstrieder

Reports documenting the anti-oxidative activity of Maillard products and melanoidins are readily abundant. A refreshingly new study by Mariana Argirova from the Medical University in Plovdiv, Bulgaria now sheds light on the other side of the coin (1). Irradiation of melanoidins with UV light or simple exposure to sun light led to bleaching of the brown color. More importantly, the production of reactive oxidative species was observed during treatment with UV light. The experiments point out that the melanoidins serve as type II photosensitizers leading to the formation of $^1\text{O}_2$. Furthermore, the generation of hydrogen peroxide and superoxide anion radicals was observed in high concentrations.

The study expands upon earlier findings from Ortwerth's group and others who observed singlet oxygen production from advanced glycation end-products (AGEs) after irradiation with UVA light (2,3). If present in vivo, the photosensitizing effect of AGEs would promote cataract development in human lenses or skin ageing: when aged human lens proteins are exposed to UV light, $^1\text{O}_2$, hydrogen peroxide and superoxide anion radicals are generated. These reactive oxidative species could then lead to oxidative damage of the lens, which is characteristic for cataract development. Linetsky and Ortwerth were able to mimic this effect with ascorbylated lens proteins. Thus, AGEs, which are abundant in aged human lenses, may be the culprits responsible for photo-oxidation. Masaki et al. irradiated human dermal fibroblasts and AGE-BSA with UV light and observed cytotoxicity, which was not present when unglycated BSA was added. The cytotoxic effect was also accompanied by the formation of reactive oxygen species. Therefore, they concluded that AGEs, which accumulate in skin collagen, promote skin ageing by photo-oxidation.

The photosensitizing activity of food melanoidins may have diverse consequences. Food stuffs rich in melanoidins, which are generally considered as good antioxidants, may have the opposite effects under certain conditions, for example under exposure to sun light. Thus, melanoidins may promote oxidative food spoilage instead of preventing it. Furthermore, reactive oxidative species may be consumed with the food leading to adverse health effects. The studies from Argirova suggest that more work is needed to clarify the complex role of Maillard products in redox reactions.

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The Maillard reaction lives long in the memory

By Alan Stitt

The recent report from the DCCT skin collagen ancillary study group (1) has added considerable weight to the assertion that glycation and advanced glycation endproduct (AGE)-modifications on long-lived proteins could be associated with progression of microvascular complications in type 1 diabetes. It will be no surprise to many in IMARS that glycated collagen (fructose-lysine modification) and carboxymethyl-lysine (CML) correlate significantly with the degree of retinopathy and nephropathy in type 1 diabetic patients (2). However, the recent study is especially compelling because it has followed over 200 patients from the original DCCT cohort for a further 10 years under the auspices of the Epidemiology of Diabetes Interventions and Complications (EDIC) study.

DCCT-EDIC has shown that levels of diabetic complications were significantly less in the group which was initially maintained under tight glycaemic control and that these benefits extended far beyond the original period of intensive insulin therapy (3). The patients under standard or conventional control for the first 10 years maintained a so-called “hyperglycaemic” or “metabolic memory” – that is a strong association with progression of complications. Interestingly, the same memory phenomenon was also shown for retinopathy in diabetic dogs nearly 20 years previously by Kern and Engerman (4).

The key question was whether fructose-lysine and CML on long-lived extracellular proteins from skin biopsies correlated with the risk of progression of diabetic microvascular disease a decade later? The answer from the current study is a resounding yes. Using multivariate analysis of the clinical data, glycated and CML-modified collagen significantly predicted the progression of retinopathy and nephropathy even after initiation of intensive insulin therapy. Furthermore, the predictive effect of glycated hemoglobin (HbA1c) vanished after adjustment for fructose-lysine and CML, suggesting that accumulation of these adducts is an excellent predictive marker and could offer a basis for the metabolic memory phenomenon.

For AGE researchers this is an important outcome. It re-affirms the validity of the AGE-hypothesis and Maillard chemistry’s contribution to pathogenesis of microvascular disease in diabetic patients. It can also serve to focus our research and the model systems we use. Glycation and AGE-modification of long-lived proteins in the extracellular matrix (whether derived directly from glucose oxidative reactions, or -oxoaldehydes) has been widely studied in vitro and in vivo, but can this help to explain metabolic memory?

We know that the extracellular matrix (ECM) is highly dynamic and cells are constantly interacting with component proteins via integrin and non-integrin receptors and this cross-talk maintains function and survival characteristics of many cell-types, including microvascular cells. The ECM is also active in immobilisation/presentation of heparin-binding growth factors that act as paracrine signals to determine cell functionality and survival. AGE-modifications (often resulting in ECM cross-links) severely disrupt these interactions with clear pathogenic potential, long-after blood glucose has returned to normal levels. Could this also be true for ECM in the bone marrow that has a recognised role in immobilisation / differentiation of progenitor stem cells and their systemic vasoreparative capacity?

The link between Maillard chemistry and metabolic memory in diabetes is an important one. While intracellular AGEs remain important, we should also not forget about cross-links and other modifications on structural proteins. The clinical evidence presented by Genuth et al.

(1) provides the jumping-off point for innovative studies that can dissect key biochemical and cellular mechanisms to link diabetes and age-related collagen modifications to pathophysiology.

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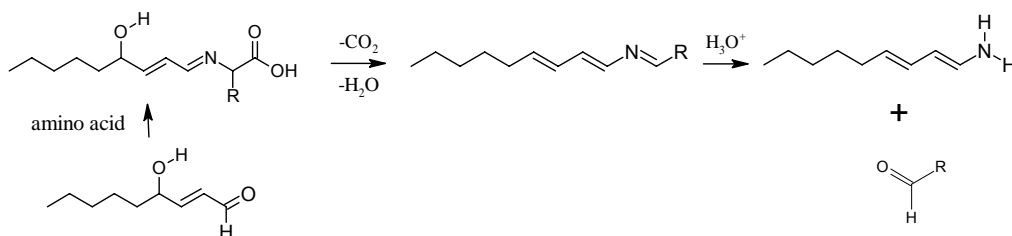
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Expanding the horizons of Strecker reaction

By Varoujan Yaylayan

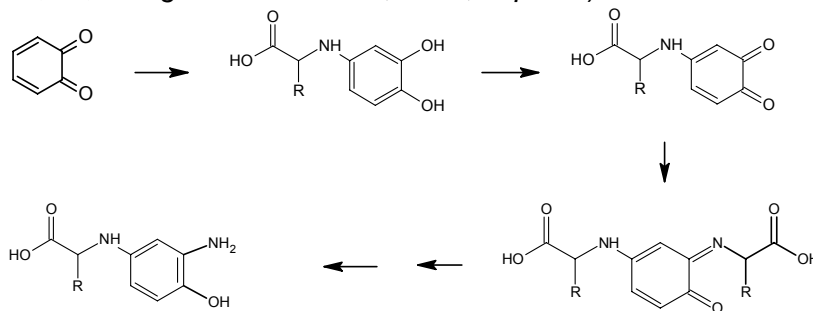
Recent publications (1 & 2) in the *Journal of Agricultural and Food Chemistry* suggest that precursors other than sugars may also be included in the list of reactants able to generate reactive intermediates during Maillard reactions that can interact with amino acids, and perhaps with proteins, in a manner similar to sugar-derived α -dicarbonyls. Lipid oxidation by-products such as 4-hydroxy-alkenals can be produced both in food and in biological systems, and have been shown by Hidalgo et al. (1) to react with amines and amino acids alike, to produce pyrrole derivatives. However, the initial Schiff base of amino acid adducts has been shown to undergo decarboxylation in a similar fashion to the α -dicarbonyl adduct during Strecker degradation, producing Strecker aldehyde as shown below:



(based on Hidalgo et al., *J. Agric. Food Chem.*, **2005**, 53, 10254)

In another publication, George Rizzi (2) demonstrates that amino acids can undergo Strecker-type reactions even with *o*-quinones, however with a twist. They first react in a Michael-type addition reaction followed by oxidation to regenerate an *o*-quinone moiety which subsequently reacts with a second mole of amino acid that then undergoes the classical Strecker decarboxylation sequence, with the formation of a cyclic enaminol and the Strecker aldehyde as shown below:

(based on Rizzi, G., *J. Agric. Food Chem.*, **2006**, in press)



These examples indicate the presence of multiple sources of Strecker aldehyde in food. In addition they raise the question of the fate of the products formed during these reactions, other than Strecker aldehydes, specifically their further reactions and their safety.

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Chelation Therapy – Is it really alternative medicine?

By John Baynes

Despite numerous website claims, chelation therapy is not a mainstream approach for treatment of diabetic complications. Regardless, there are about a dozen papers in Pub Med that describe the efficacy of the chelator trientine for treatment of diabetic complications in animal models. These papers, which deal with renal, neural and cardiac complications, are often cited as evidence that oxidative stress contributes to pathology in diabetes. Hamada *et al.* (1) now report that trientine inhibits the increase in the AGE precursors, methylglyoxal (MGO) and 3-deoxyglucosone, and the AGE argpyrimidine in lenses of STZ diabetic rats, without an effect on polyol pathway metabolites. Trientine also inhibited the increase in semicarbazide-sensitive amine oxidase (SSAO) in the lens; this enzyme is a source of MGO from aminoacetone, and also generates aldehydes from other amines. SSAO is a copper enzyme and one wonders whether trientine enters the lens and, if so, whether it inhibits the synthesis or the activity of SSAO through binding of copper. This work expands on recent studies of Cooper and colleagues in New Zealand (2, 3) who demonstrated beneficial effects of trientine on diabetic cardiomyopathy - in humans as well as in rats (2) - and showed that trientine restores imbalances in Cu^{++} homeostasis in diabetic subjects (3).

Trientine, with only carboxyl and tertiary amino groups [N,N'-bis(2-aminoethyl)-1,2-ethanediamine], does not have the carbonyl scavenging activity characteristic of other AGE inhibitors, so it is likely that chelation is its primary mechanism of action. However, the work of Hamada *et al.* raises interesting questions about the role of chelation in the activity of other AGE inhibitors. Aminoguanidine and pyridoxamine are weak chelators, but are typically administered at high doses; the hydrolysis products of AGE-breakers are also potent chelators, which may explain their cardioprotective effects (4). The LR compounds, a new generation of AGE inhibitors from Samuel Rahbar's laboratory, also lack direct carbonyl scavenging activity, but appear to be potent chelators (5). It will be interesting to see whether trientine will also prove useful for the treatment of diabetic renal and retinal disease and, if so, whether this well-tolerated, long-tested drug that is approved for treatment of Wilson's disease, will become a standard rather than alternative therapy for treatment of diabetes and its complications.

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Brown Maillard products: Effective inhibitors of enzymatic browning

By Monika Pischetrieder

It is a long and well established concept in biochemistry that glycation leads to inactivation of enzymatic activity (1). Some recent studies indicate that this concept is now successfully exploited for applications in food technology. Enzymatic browning, catalyzed by polyphenol oxidases, is a major problem in processed plant derived foods. Up to now, the most effective agent to prevent unwanted discoloration by polyphenol oxidation has been sulfite. However, major toxicological concerns regarding sulfites have necessitated the development of alternative polyphenol oxidase inhibitors. A recent paper from Billaud et al. (2) from the Conservatoire National des Arts et Métiers in Paris now offers the almost ironic conclusion that brown Maillard reaction products serve as potent inhibitors of enzymatic browning. Reaction mixtures of cysteine or glutathione with sugars inhibited polyphenol oxidases with a similar activity as sulfite. Further, the Maillard mixtures could be diluted so that their own color did not interfere with the visual appearance of the products. The technological application is impressively demonstrated for several food stuffs such as apples, eggplants and mushrooms.

First evidence that Maillard products inhibit apple polyphenol oxidase was obtained more than ten years ago by Tan and Harris (3). These results were recently confirmed for potato polyphenol oxidase by Lee and Park from the Dong-A University in Korea (4), whose results further indicate that the inhibition is non-competitive. The active components as well as the mechanism of inhibition, however, still remain unclear. Reactive intermediates, for example dicarbonyl compounds, which are formed in the Maillard mixtures, may covalently bind to the enzyme leading to a loss of its activity. Alternatively, non-covalent allosteric hindrance can be considered. Reduction or addition to the quinone products seems to be unlikely, since Billaud et al. (2) used oxygen consumption to measure enzyme activity. Knowledge of both the inhibitory mechanism and of the active components in the heterogeneous Maillard mixture, however, are crucial for industrial application. Otherwise it will be difficult to standardize and toxicologically evaluate active mixtures.

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Hyperglycemia and hypoxia – a double whammy for the diabetic retina?

By Alan Stitt

It is widely acknowledged that AGEs accumulate in tissues during diabetes where they may play an important role in pathophysiology. The retina is no exception, although despite retinopathy being the most common complication suffered by diabetic patients, there has been comparatively little attention devoted to this highly specialised neural tissue from the perspective of Maillard chemistry. While recognizing that AGE formation has been demonstrated in the retina by many groups, it is still important to ask if there is anything about this tissue that makes it different when evaluating the Maillard reaction and the potential pathogenic role of AGEs?

It may be interesting for IMARS members to learn that in periods of hyperglycaemia excess glucose enters the retina, a response which contrasts with the brain, where glucose levels are more tightly regulated (1, 2). In the context of diabetic retinopathy, this often occurs in combination with retinal hypoxia (decreased oxygen), a coincidental metabolic state resulting from progressive vascular insufficiency.

A paper by Nyengaard et al. (3) is pertinent because it suggests that the combination of high glucose (30mmol/l) and hypoxia (36 torr PO₂) produce an additive effect on glycolytic activity as evidenced by increased levels of cytosolic NADH leading to accumulation of triose phosphates such as glyceraldehyde-3-phosphate (G-3-P) (3). It is now known that hypoxia may serve to exacerbate glycolytic metabolism since cells often respond by drawing remaining intracellular oxygen away from mitochondria-associated oxidative phosphorylation. This drives further flux through glycolytic pathways. This is significant because Brownlee's group have shown that accumulation of G-3-P, fructose-6-phosphate (F-6-P) and excessive intracellular methylglyoxal can lead to rapid AGE formation (4). Transketolase activating compounds (such as thiamine and benfothiamine) have the potential to shunt G-3-P and F-6-P into the pentose phosphate pathway thereby reducing hyperglycaemia-mediated damage (4); these agents have shown efficacy in prevention of diabetic retinopathy in a pre-clinical model (5).

The retina is served by two vascular beds, the high-flow through choroidal circulation and the tightly regulated intra-retinal circulation and while there is variation within the retinal layers, this tissue is remarkably hypoxic overall. This is especially true at night (dark adaptation) when rod photoreceptors consume up to 4-fold more oxygen in the dark when compared to light illumination. Consequently, hypoxia may serve to exacerbate the effects of hyperglycemia and greatly impact upon the generation of tissue damaging AGEs in the diabetic retina and further contribute to tissue pathology and disease progression.

So what is known about Maillard chemistry in the diabetic retina? Methylglyoxal-derived hydroimidazolone increased by nearly 300% in diabetic rat retina (6) and it is established that a range of AGE-modifications are associated with neurons and the intraretinal microvasculature. The derivation of these AGEs is important to establish especially in the unique microenvironments of the neural retina. Closer study of this tissue can tell us much about Maillard chemistry and, in turn, we may uncover urgently needed diagnostic / therapeutic approaches that limit vision loss.

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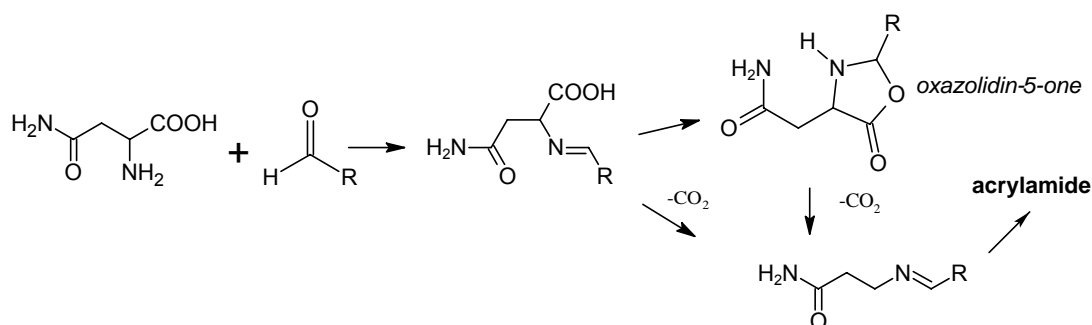
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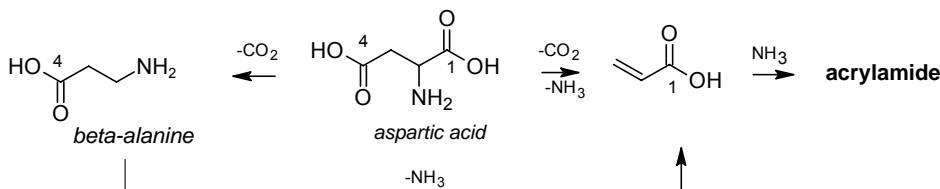
More acrylamide stories...a new look at an old reaction

By Varoujan Yaylayan

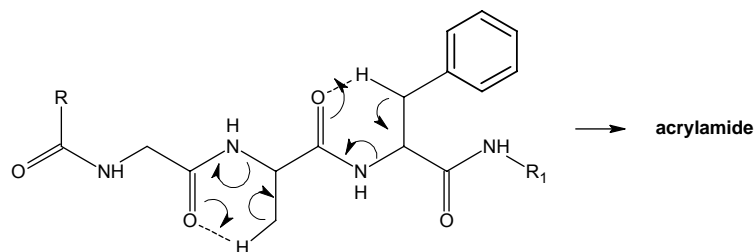
According to ISI Web of Knowledge, since 2003 nearly 17 % of all publications related to the Maillard reaction were on acrylamide. Discovery of acrylamide in thermally processed potatoes provided researchers in the field of Maillard chemistry a fresh look at an old reaction. It also initiated a whole set of projects that generated new information both at the practical level in terms of prevention and at the theoretical level in terms of our understanding the chemistry at the interface of the Schiff base and Amadori rearrangement. Fragmentation of the sugar amino acid Schiff bases under basic pH was the only major pathway known to operate prior to the Amadori rearrangement (Namiki pathway). After identification of asparagine (1,2) as the principle amino acid responsible for the generation of acrylamide, attempts to identify its mechanism of formation led to the discovery that Schiff bases can also undergo decarboxylation prior to Amadori rearrangement (3,4), and that this step is crucial for the formation of acrylamide from asparagine. This decarboxylation is most likely facilitated by the ability of the Schiff base to form oxazolidin-5-one intermediate (3) as shown below:



Consequently, other amino acids such as aspartic acid and β -alanine and peptides containing β -alanine such as carnosine, were also identified as precursors of acrylamide (5). However, these amino acids did not require the presence of reducing sugars to generate acrylamide, as shown below:



Now, researchers (6) at Hohenheim University in Stuttgart, Germany, have discovered that even α -alanine which in its free form is unable to generate acrylamide, but as part of a peptide or protein structure such as in wheat gluten is able to do so at high temperatures as shown below.



The structural requirement in this case is the presence of an amino acid next to the α -alanine, possessing a proton at its β -position, such as phenylalanine. As expected, the researchers also identified the formation of cinnamic acid amide as a side product. The electrocyclic reaction shown above, allows the abstraction of the non acidic β -proton from α -alanine due to the formation of a stable six-membered transition state. In the case of the phenylalanine the β -proton is intrinsically acidic due to its location on a benzylic carbon.

In summary, while asparagine remains the principle source of acrylamide in food, other amino acids are able to generate acrylamide, but structural constraints suggest that they will make relatively minor contributions.

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