SPECIAL GUEST EDITOR SECTION

Acrylamide Formation in Food: A Mechanistic Perspective

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Earliest reports on the origin of acrylamide in food have confirmed asparagine as the main amino acid responsible for its formation. Available evidence suggests that sugars and other carbonyl compounds play a specific role in the decarboxylation process of asparagine, a necessary step in the generation of acrylamide. It has been proposed that Schiff base intermediate formed between asparagine and the sugar provides a low energy alternative to the decarboxylation from the intact Amadori product through generation and decomposition of oxazolidin-5-one intermediate, leading to the formation of a relatively stable azomethine ylide. Literature data indicate the propensity of such protonated ylides to undergo irreversible 1,2-prototropic shift and produce, in this case, decarboxylated Schiff bases which can easily rearrange into corresponding Amadori products. Decarboxylated Amadori products can either undergo the well known β-elimination process initiated by the sugar moiety to produce 3-aminopropanamide and 1-deoxyglucosone or undergo 1,2-elimination initiated by the amino acid moiety to directly generate acrylamide. On the other hand, the Schiff intermediate can either hydrolyze and release 3-aminopropanamide or similarly undergo amino acid initiated 1,2-elimination to directly form acrylamide. Other thermolytic pathways to acrylamide—considered marginal at this stage—via the Strecker aldehyde, acrolein, and acrylic acid, are also addressed. Despite significant progress in the understanding of the mechanistic aspects of acrylamide formation, concrete evidence for the role of the different proposed intermediates in foods is still lacking.

During thermal processing of food, heat-induced chemical changes can have both beneficial and deleterious effects. Thermal degradation of major food components not only can cause caramelization and lead to the formation of desirable browning, aromas, and flavors but also can generate foodborne toxicants that may exhibit mutagenic and carcinogenic properties (1). For example, the nongenotoxic carcinogen 3-monochloropropane-1,2-diol (3-MCPD) was first identified in acid hydrolyzed proteins (2), and recently in toasted bread at levels proportional to the degree of toasting (3). One possible origin of 3-MCPD can be traced back to interaction of glycerol with chloride ions (2, 4). Heterocyclic amines (HA), specifically the class belonging to aminoimidazoaazarenes, were first discovered (5) on the charred surfaces of fish and meat and later found to require creatine as one of the precursors. Polycyclic aromatic hydrocarbons (PAH) are another class of contaminants that can be formed in food subjected to extreme temperatures, or can be incorporated into the food from the environment (6). Another example is the N-nitrosamines that can be formed in the presence of some food additives such as nitrates/nitrites (7). More recently, acrylamide, classified as a type 2A carcinogen, was identified in most heat-treated foods (8), with a direct link to the amount of the amide amino acid asparagine (9). The detailed mechanisms of formation of these heat-induced toxicants in foods are still widely unknown, as exemplified very recently by the discovery of formation of the parent furan from ascorbic acid and polyunsaturated fatty acids (10). However, the chemical reactions responsible for the majority of thermally induced changes can be linked to thermal oxidations, hydrolysis of triglycerides, proteins and carbohydrates, sugar caramelization and, most importantly, to the Maillard reaction (1). Furthermore, the formation of HAs and acrylamide has been associated specifically with both the Maillard reaction, which in turn is intimately connected with heating and sterilization processes, and with the production of microbiologically safe and organoleptically desirable food products (9).

This paper describes the progress made to date in understanding the fundamental mechanistic aspects of the formation of acrylamide. Essentially all experiments were conducted in a laboratory environment, and their validity in food remains to be assessed.

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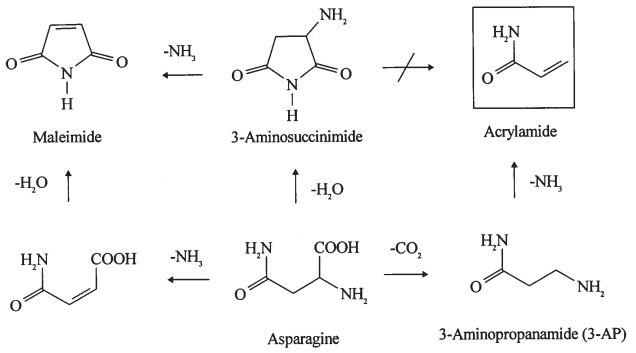

Figure 1. Thermal degradation of asparagine.

Precursors of Acrylamide

Initial investigations that followed the discovery of acrylamide in cooked food have led not only to the unambiguous identification of asparagine (11–14) as the main amino acid precursor of acrylamide, but has also confirmed that the 3 carbon backbone of acrylamide and the amide nitrogen originate from corresponding locations in asparagine in model system studies using ^{15}N and ^{13}C-labeled precursors (12, 15). Although thermally allowed decarboxylation and deamination reactions (16) of asparagine alone, in principle, can produce acrylamide (Figure 1), the presence of sugars was necessary to effect the conversion of asparagine into acrylamide. Subsequent studies (14, 15) have indicated that any carbonyl-containing moiety can perform a similar transformation and that asparagine alone prefers to undergo intramolecular cyclization (16) and form an imide (Figure 1) rather than decarboxylate and form acrylamide. These observations have raised the intriguing question: can carbonyl compounds in general and sugars in particular assist in the nonoxidative decarboxylation of amino acids?

Major Pathways of Acrylamide Formation

The first 2 reports (11, 12) published on the origin of acrylamide in food had entirely different perspectives on the mode of sugar-assisted decarboxylation of asparagine. Mottram et al. (11) proposed a known oxidative decarboxylation process known as Strecker degradation (17), which is a well established process in thermally generated aroma formation in food (18); however, this process will not only decarboxylate asparagine but also at the same time oxidize it into Strecker aldehyde that needs further reduction and dehydration steps to be converted into acrylamide. On the other hand, Stadler et al. (12) reported the unexpected discovery of asparagine *N*-glycoside or Schiff base (Figure 2), the initial interaction product between glucose and asparagine, as the direct precursor of acrylamide, producing higher yields than any of the corresponding more stable Amadori product (Figure 2) or the sugar and asparagine mixtures. This critical finding provided the initial clues for the first detailed mechanism that linked decarboxylation to the presence of open form of asparagine *N*-glycoside (Schiff base) as proposed by Yaylayan et al. (16; Figure 2).

The proposed mechanism was based on the known ability of imines (or Schiff betaines) formed between amino acids and aldehydes (19) to undergo intramolecular cyclization (Figure 2, pathway A) initiated by the carboxylate anion and formation of oxazolidin-5-one intermediate (Figure 2). Manini et al. (20) observed the formation of such an intermediate and its facile decarboxylation at room temperature in D-glucose/L-DOPA model system. This facile decarboxylation of the oxazolidin-5-one intermediate affords a stable azomethine ylide (19, 21, 22) which is prone to undergo an irreversible 1,2-prototropic shift (19) to produce decarboxylated Schiff base and, eventually, decarboxylated Amadori product (Figure 3). Although the decarboxylated intermediate can be formed under mild conditions, it may require higher temperatures to cleave the carbon-nitrogen covalent bond and produce acrylamide (Figure 2). Consequently, Zyzak et al. (15) proposed a similar mechanism (Figure 2, pathway B) depicting direct decarboxylation of the Schiff betaine without passing through oxazolidin-5-one intermediate. However, literature evidence indicates that amino acids in the presence of carbonyl compounds such as aromatic aldehydes or ninhydrin undergo decarboxylation through the formation of oxazolidin-5-one intermediate (19). On the other hand, if the asparagine *N*-glycoside (Schiff base) is allowed to undergo Amadori rearrangement without decarboxylation, it can still undergo this step, but at much higher temperatures because the developing negative charge on the Amadori carbanion intermediate (Figures 2 and 3) is not stabilized similar to azomethine ylide. However, at this high temperature, competition with intramolecular cyclization

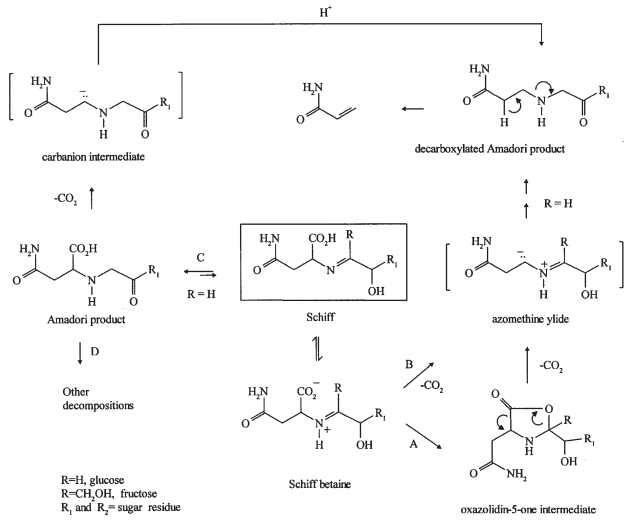

Figure 2. Proposed mechanisms of formation of acrylamide by Yaylayan et al. (pathway A) and by Zyzak et al. (pathway B).

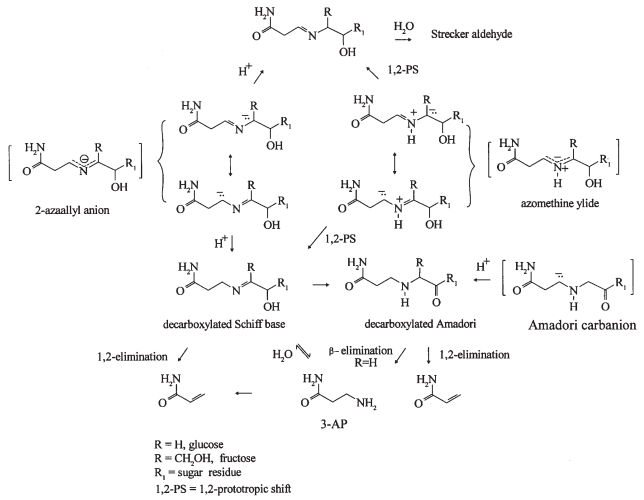

Figure 3. Proposed role of reactive intermediates 2-azaallyl anion, azomethine ylide, and Amadori carbanion in the generation of acrylamide.

to form N-substituted succinimide Amadori product might hinder acrylamide formation even further (16). According to Figure 3, both decarboxylated species, the Schiff base and Amadori products, are capable of generating acrylamide either directly or indirectly through release of 3-aminopropanamide (3-AP). Decarboxylated Amadori products can undergo the well known β-elimination process initiated by the sugar moiety to produce 3-aminopropanamide and 1-deoxyglucosone or undergo 1,2-elimination initiated by the amino acid moiety to directly generate acrylamide. On the other hand, the Schiff intermediate can either hydrolyze and release 3-AP or similarly undergo amino acid-initiated 1,2-elimination to directly generate acrylamide. There is compelling evidence that decarboxylated species are indeed formed in heated aspargine/sugar mixtures, as evidenced by the detection of ion at *m/z* 251 (molecular ion of decarboxylated Amadori or Schiff intermediates; 15). However, their relative importance in generating acrylamide is still to be determined.

Effect of Keto Sugars versus Aldehydo Sugars

Numerous studies on the effect of sugar type on the yield of acrylamide have determined that *keto* sugars such as fructose are more efficient than *aldehydo* sugars such as glucose in generating acrylamide in model systems. Fructose seems to generate more acrylamide at a relatively lower temperature under low-moisture conditions (12). A mechanistic explanation of this observation can be formulated based on the ability of fructose Schiff intermediate to stabilize the azomethine ylide further, through the formation of a stable 6-member ring hydrogen bond (Figure 4). This extra stabilization of the azomethine ylide can increase the rate of its formation and, hence, the rate of acrylamide generation relative to glucose systems that are unable to stabilize azomethine ylide through H-bonding. The fact that the reactivity order is reversed in aprotic solvents such as dimethyl sulfoxide (DMSO; 23) supports the H-bonding hypothesis. DMSO is able to solvate more strongly the azomethine ylides than water, preventing easy exchange from taking place between solvent and intramolecular H-bonding, thus eliminating the advantages gained by the extra stabilization offered by fructose. This leaves the intrinsic reactivities of the sugars to determine the yields. Based on these observations, Blank et al. (23) offer an alternate explanation using differential scanning colorimetry (DSC) experiments, in which they studied the importance of moisture and molecular mobility (melting point) of the reducing sugars in the essentially dry reaction mixtures. These studies show that the higher reactivity of fructose is due to its lower melting point (about 126°C), thereby promoting faster reaction with the amine to form the Schiff base (23). More detailed aspects of this work will be presented elsewhere.

Effect of pH

Various attempts to reduce acrylamide content through manipulation of pH have appeared in the literature. It is important, therefore, to assess the ability of the Schiff base to form azomethine ylide under various pH conditions. At neutral or slightly acidic pH, the zwitterionic form of the Schiff base (Schiff betaine) mainly undergoes Amadori rearrangement and a small proportion forms oxazolidin-5-one intermediate to eventually afford a stable azomethine ylide (Figure 5, pathways A and B) and produce acrylamide. Under basic conditions, however, the Schiff intermediate will exist mainly in its anionic form (Figure 5, pathway C). Decarboxylation of this species will afford a stable 2-azaallyl anion (24) isoelectronic with azomethine ylide (21, 22), the only difference being the occupancy of the nonbonding electron pair on the nitrogen by a proton (Figures 3 and 5). Ruhemann's purple generated from ninhydrin reaction with amino acids is such a stable 2-azaallyl anion. As shown in

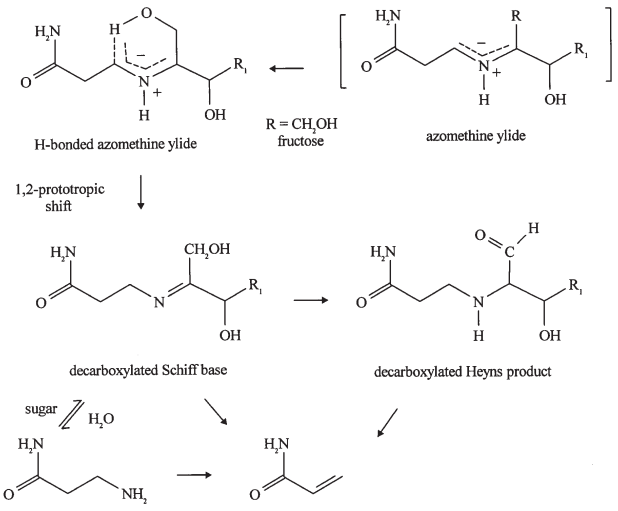

Figure 4. Proposed role of fructose in enhancing the stability of azomethine ylide through H-bonding.

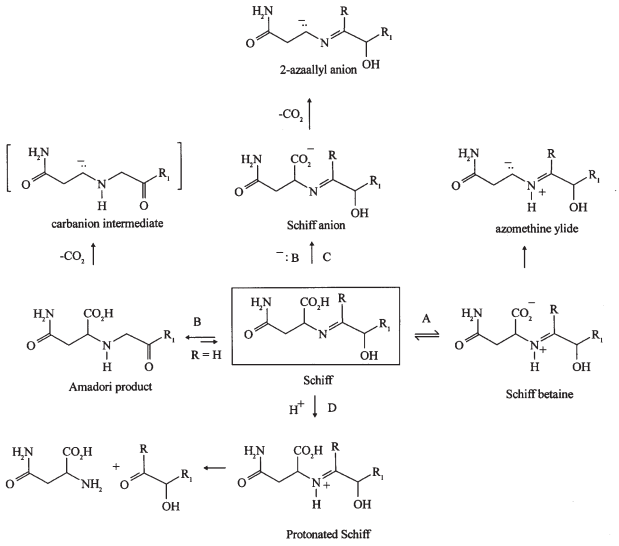

Figure 5. The fate of Schiff base under neutral/mildly acidic (pathways A and B), basic (pathway C), and strongly acidic (pathway D) conditions.

Figure 3, the 2-azaallyl anion generated (Figure 5, pathway C) from the Schiff base can abstract a proton and form decarboxylated Schiff base and generate acrylamide similar to azomethine ylide. Such 2-azaallyl anions have also been postulated (24) as intermediates in the transamination reaction between β-keto acids and amino alcohols to generate Amadori products. Finally, under highly acidic conditions (Figure 5, pathway D), the protonated Schiff base can only undergo hydrolysis and regenerate asparagine and the starting sugar.

Other Pathways of Acrylamide Formation

Several groups have addressed the possible intermediacy of acrolein (14, 25) and acrylic acid (26, 27) in the formation of acrylamide. Acrolein can be formed by different pathways, including the oxidative degradation of lipids, and could react further via acrylic acid to form acrylamide. A recent report (25) also illustrates that, under certain conditions, acrolein together with asparagine may generate appreciable amounts of acrylamide. Acrylic acid can react with ammonia to furnish acrylamide by aminodehydroxylation, which is a well known reaction of acids leading to amides (28). The acrylic acid route via the Maillard reaction has been intensively studied in model systems (26) and involves an analogous reaction as described for acrylamide. In this case, aspartic acid provides the backbone of the vinyl acid, and comparable yields of acrylic acid were recorded over time and temperature, reaching up to 14 mmol of acrylic acid per mol of aspartic acid after 5 min at 230°C (26). To afford acrylamide, the acid moiety must react with ammonia to furnish the corresponding amide. Ammonia can be released during the thermolysis of amino acids (29). In model test tube systems, cocktails composed of amino acids that release ammonia readily at higher temperatures have led to the formation of up to 75 μmol acrylamide/mol amino acid (10 min, 200°C), that represents about 5% of the yield obtained by the asparagine route (26).

Aspartic acid can also release acrylic acid without the involvement of sugars or a carbonyl source following a concerted decarboxylation/deamination pathway (30).

In addition to asparagine, other amino acids have been implicated in the formation of acrylamide (Figure 6). Preliminary studies have shown that other amino acids such as L-alanine and L-arginine are also capable of releasing acrylic acid at temperatures above 180°C, with yields within the same order of magnitude as aspartic acid (unpublished data). Carnosine in meat products can release β-alanine through hydrolysis (30) and form acrylic acid and, eventually, acrylamide or its derivatives (31). Even though several potential indirect routes to acrylamide have been identified, the acrylic acid pathway seems only of marginal importance in foods, probably because of the limitation of free ammonia and necessity of relatively high temperatures for the reaction to proceed efficiently. Other amino acids such as serine and cysteine can be thermally converted into lactic acid via pyruvic acid, and in the presence of ammonia generate lactamide and acrylamide (30). Granvogl et al. (32) suggest the intriguing possibility of enzymatic decarboxylation of asparagine in vivo in potatoes and its conversion into 3-AP prior to thermal processing (Figure 6). During processing of the potato, 3-AP can be converted into acrylamide by deamination, a reaction which gives good yields (approximately 60 mol%) and can be performed in an aqueous environment. Traces (parts-per-million) of 3-AP have been detected in potatoes (33), and it would be interesting to determine if acrylamide could be formed from 3-AP under pressure-cooking conditions. However, 3-AP may also be produced through hydrolysis of decarboxylated Schiff base (Figure 3) under potato storage conditions.

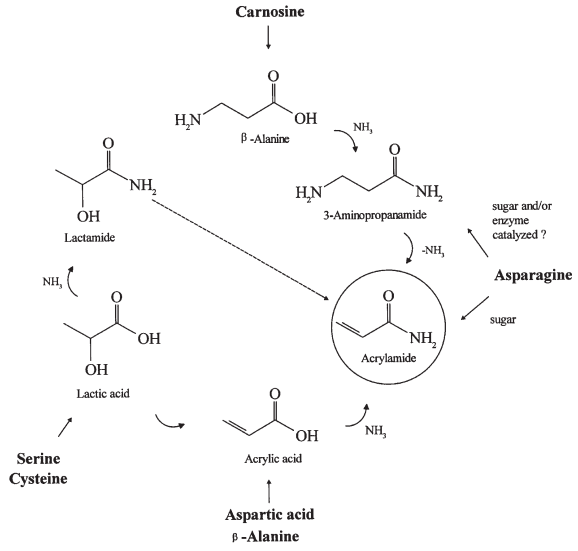

Figure 6. Proposed role of different amino acids in generating acrylamide.

Conclusions

Significant progress has been made over the past 18 months in understanding how acrylamide is formed in foods. Several pathways have been proposed, and there is overall consensus (34, 35) that the major route involves asparagine within the framework of the Maillard reaction. Certain mechanistic aspects have been clarified, e.g., through synthesis of model compounds and intermediates. However, concrete evidence, in particular for the formation of the key intermediates in food products, is still lacking. An important aspect not covered in this review is the kinetic studies, which would allow determination of the rates of the limiting steps in the reaction and thus generate potential avenues of control. In this context, more detailed studies are warranted to investigate the stability/reactivity of acrylamide in food, as preliminary work has shown that the food matrix (e.g., coffee) may play a role in catalyzing the loss of acrylamide (36). Thus, the food matrix adds to the complexity of elucidating reaction pathways in food, and confirms earlier observations that each food category needs careful and individual study. Furthermore, and owing to the importance of the Maillard reaction to the overall quality of manufactured foods, the process of decoupling aromagenesis from reactions leading to the formation of undesirable chemicals, therefore, becomes one of the main challenges that we face today. However, if such a control was possible, it could only be achieved through an in-depth understanding of the mechanistic pathways underlying thermal interactions that occur in food. The discovery of acrylamide in cooked foods and hitherto unknown pathways in the Maillard reaction under low-moisture conditions shows that there is a paucity of knowledge in describing basic chemical pathways, necessitating deeper investigations at the molecular level in food. However, care must be taken in the interpretation of the data and assessing the true health risks associated with trace levels of processing compounds. Rather, a balanced and holistic view must be taken, considering also the positive effect of health-beneficial constituents procured during the heating of food. Perhaps there is an evolutionary significance in the thermal generation of pleasing aromas in tandem with the reduction of microbial hazards.

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