

Acrylamide formation and its impact on the mechanism of the early Maillard reaction

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Summary

The contribution of the acrylamide research to the detailed understanding of the mechanism of the early phase of the Maillard reaction is outlined with emphasis on the formation of oxazolidin-5-one and azomethine ylide. The importance of the type and structure of the saccharide or the carbonyl moiety of the reactants to the selective stabilization of one resonance-contributing form of the azomethine ylide over the other is elaborated. Furthermore, the significance of the formation of two isomeric and decarboxylated Schiff bases from the azomethine ylide to the Maillard and Strecker reactions is discussed.

Keywords

Maillard reaction; acrylamide; azomethine ylide; oxazolidin-5-one; saccharose (sucrose); glucose, fructose, amino acids; hydroxymethylfurfural

None of the thermally generated toxicants identified so far has had the same impact as acrylamide in advancing our knowledge of the mechanism of the early Maillard reaction. Prior to the discovery of acrylamide in processed foods [1], the generation of most of the Maillard reaction products was mainly rationalized based on the decomposition of the Amadori product or the independent degradations of saccharides and amino acids [2]. Furthermore, most of the observed decarboxylation reactions of the amino acids were attributed to their interaction with α -dicarbonyl compounds through the Strecker reaction. However, in an attempt to elucidate the mechanism of formation of acrylamide, some of the previously unrecognized details of the early phase of the Maillard reaction were revealed in the process. It has been shown, for example, that the glycosyl amino acids or the Schiff bases play a far more important role in the Maillard reaction than previously thought, such as in initiating the process of decarboxylation of amino acids and subsequent generation of two isomeric and decarboxylated Schiff bases [3] of which only one can lead to the formation of acrylamide. Furthermore, it has been shown also that decarboxylation of Schiff bases is facilitated through the formation of *N*-protonated azomethine ylide (Fig. 1) which undergoes 1,2-prototropic shift to

generate two isomeric Schiff bases [3]. Although azomethine ylides can be envisaged to be formed by direct decarboxylation of the initial Schiff base formed between the saccharides and amino acids, however, there is overwhelming evidence [4] to support the hypothesis that prior to decarboxylation, the Schiff bases undergo cyclization to oxazolidin-5-one intermediate that is known to undergo facile decarboxylation to form the above mentioned azomethine ylide [5]. Interestingly, one of the decarboxylated isomeric Schiff bases that is able to generate acrylamide can also release the α -aminocarbonyl moiety and the other can release the so called Strecker aldehyde after a hydrolytic step, in effect, generating Strecker reaction products in the total absence of α -dicarbonyl compounds.

Direct precursors of acrylamide

Although styrene was a known degradation product of phenylalanine at the time of the publication of the first report on acrylamide in 2002 [1], however it took more than a year and the efforts of many research groups to identify how its counterpart from asparagine, the acrylamide, can be formed [6–8]. The first indication that acryla-

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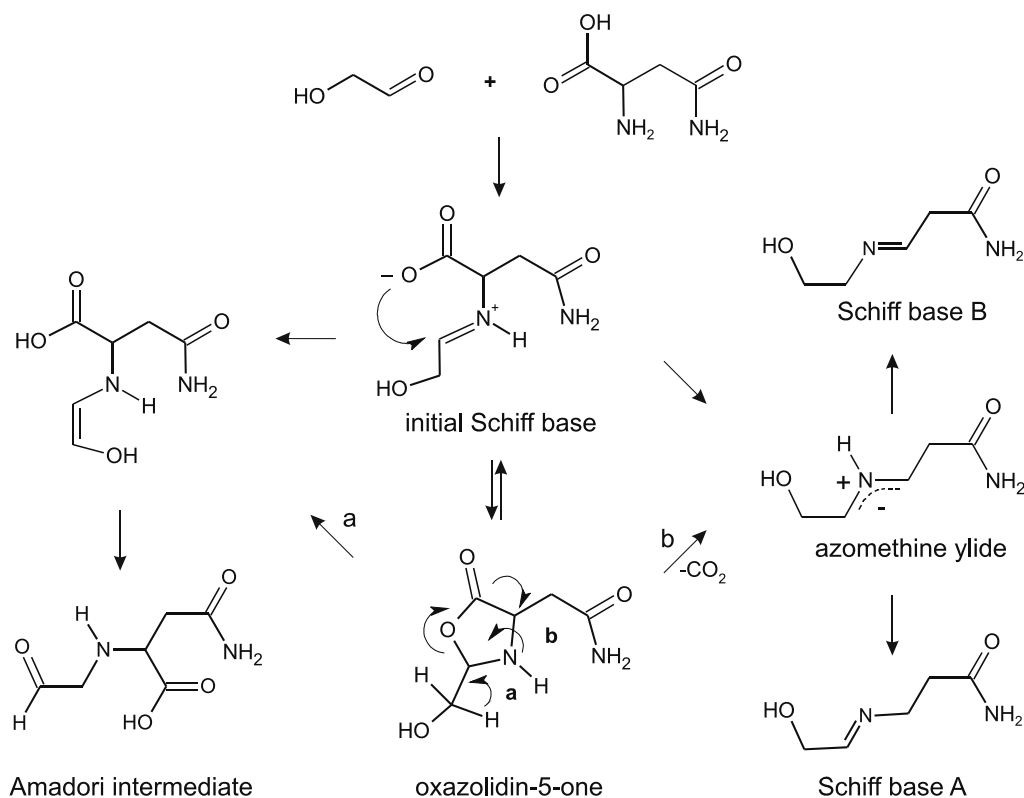


Fig. 1. The formation of azomethine ylide from oxazolidin-5-one intermediate (for clarity, a simple saccharide glycolaldehyde is used as an example).

mide formation represented an unexpected shift from the norm was the finding of STADLER et al. [6] that glycosylamine of asparagine (also known as the Schiff base or the *N*-glycoside) rather than its Amadori product was the main precursor of acrylamide (Fig. 1). This mechanism deviated from the generally accepted pathway of formation of many Maillard reaction products through the Amadori rearrangement and formation of reactive α -dicarbonyl compounds. Initially, MOTTRAM et al. [9] assumed that one of the key steps in the formation of acrylamide, the decarboxylation of asparagine, occurred via the well known Strecker degradation, however, this reaction will also oxidize the amino group into its corresponding aldehyde and therefore will require several steps to achieve the conversion to acrylamide. In 2003, YAYLAYAN et al. [7] followed by ZYZAK et al. [8] indicated that decarboxylation of asparagine can occur via the Schiff base intermediate due to the formation of a relatively stable azomethine ylide (Fig. 1). Furthermore, YAYLAYAN et al. [7] suggested a cycloreversion mechanism from the proposed oxazolidin-5-one intermediate as a means of decarboxylation rather than a direct decarboxylation of the Schiff

base proposed by ZYZAK et al., [8]. Following a 1,2-prototropic shift, the azomethine ylide can generate two isomeric and decarboxylated Schiff bases A and B (Fig. 1), one of which has the tendency to generate acrylamide either directly or through Amadori rearrangement to form a decarboxylated Amadori product, or through hydrolysis to generate free 3-aminopropionamide. As shown in Fig. 2, the three 3-aminopropionamide derivatives can exist in equilibrium with each other in the presence of carbonyl compounds and all can generate acrylamide through 1,2-elimination-type reactions. Initially, YAYLAYAN et al. [7] proposed the decarboxylated Amadori product to be the main precursor and ZYZAK et al. [8] considered the decarboxylated Schiff base as the main precursor. However, GRANVOGL and SCHIEBERLE [10] proposed the 3-aminopropionamide to be another possible direct precursor. STADLER et al. [11] provided further evidence that supported the decarboxylated Amadori product as a viable precursor of acrylamide under pyrolytic conditions. On the other hand, kinetic studies using glucose-asparagine model system indicated that the rate determining step for the formation of acrylamide in phos-

phate buffer (pH 7.6) varied depending on the temperature [12]. At high temperatures (180 °C), formation of Schiff base was rate determining step and, at lower temperatures, decarboxylation became rate determining. The authors have detected 3-aminopropionamide in this system only after significant amount of acrylamide was formed. They concluded that this observation favoured acrylamide formation directly from the decarboxylated Schiff base or Amadori compound and not from 3-aminopropionamide. However, a recent study measuring intrinsic abilities of 3-aminopropionamide and its synthetic Amadori and Schiff base adducts have indicated that, at higher temperatures and under dry conditions, 3-aminopropionamide and its Amadori product had comparable efficiencies of conversion into acrylamide, whereas the corresponding Schiff base had a four-fold higher tendency [13]. At lower temperatures and in the presence of moisture, the Schiff base adduct had 27-fold higher tendency to form acrylamide and the corresponding Amadori adduct had 13-fold higher tendency, both compared with 3-aminopropionamide. These observations indicate that in real food systems the importance and relative contribution of each of these direct precursors may be determined by the environmental conditions at which the decarboxylated Schiff base is initially formed, such as the composition of the food matrix, water content, temperature, pH and the type of saccharide and carbonyl species present. In the presence of excess saccharide

or reactive dicarbonyls, the efficiencies of both 3-aminopropionamide and its Amadori product to generate acrylamide increased due to the formation of quaternary imminium ions (Fig. 2) that can facilitate Hofmann-type 1,2-elimination reactions [13, 14]. The enhancement of acrylamide formation by the reactive carbonyl compounds was also demonstrated by AMRIEN et al. [15]. The extent of hydrolysis or the conversion of Schiff base A into Amadori compound will depend therefore on the temperature, type of saccharide, pH and water content. Although the Schiff base A will be in equilibrium with the Amadori product as soon as it forms, however, the extent of their interconversion with the 3-aminopropionamide will depend on the presence of free reactive carbonyls or dicarbonyls, which at the same time will catalyse their elimination into acrylamide. Consequently, all the three direct precursors can be considered to be important contributors to the formation of acrylamide, most probably their relative importance in generating acrylamide will not remain the same and will change over time with the changing of the composition of food matrix during processing.

Oxazolidin-5-one and azomethine ylide formation

Although it can be argued that the Schiff bases formed between saccharides and amino acids can undergo decarboxylation and form azomethine

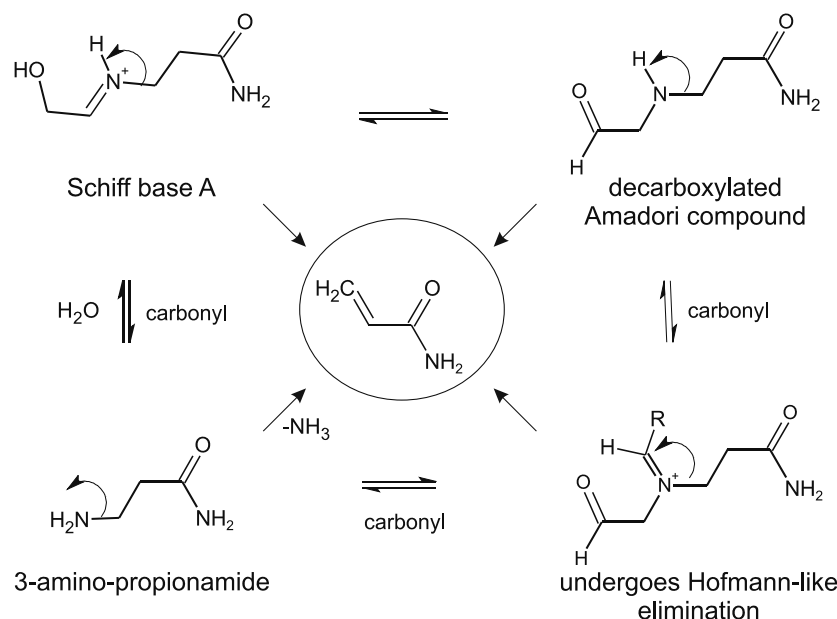


Fig. 2. Direct precursors of acrylamide.

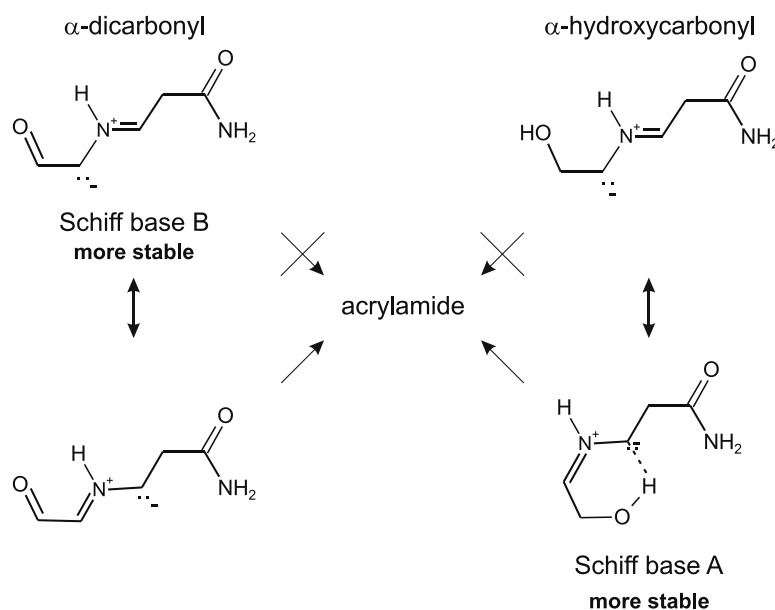


Fig. 3. Selective stabilization of resonance contributing forms of azomethine ylide by α -dicarbonyl and α -hydroxycarbonyl species.

ylide directly without necessarily passing through oxazolidin-5-one intermediate as suggested by YAYLAYAN et al. [7], however there is convincing spectroscopic evidence for its formation in different model systems containing saccharides and amino acids [3, 13]. Recently, further evidence in support of oxazolidin-5-one formation and decomposition was provided through monitoring the strong and characteristic absorption band of oxazolidin-5-one intermediate at 1778 cm^{-1} in the infrared region after its extraction with toluene from a heated model system composed from phenylalanine and glycolaldehyde [4]. The identity of this peak was confirmed by observing a shift to 1736 cm^{-1} when $[^{13}\text{C}-1]$ phenylalanine was used. The intensity of this peak was decreased over time with simultaneous increase of new bands in the carbonyl and imine absorption regions that were identified through the use of isotope labeling technique and standard precursors. The authors were able to confirm the reversion of oxazolidin-5-one into Amadori product or its decomposition into decarboxylated imines. Furthermore, evidence for the formation of the resulting azomethine ylide was also provided using their specific ability to undergo 1,3-dipolar cycloaddition reaction with 1,3-dipolarophiles [3]. The addition of dipolarophiles, such as dimethyl fumarate, to the heated model systems has lead to a significant drop in the intensity of the Maillard browning indicating the importance of the resulting imines to the genera-

tion of color and to the progress of the Maillard reaction in general.

Research into acrylamide has therefore contributed to the identification of an important process that leads to the decarboxylation of Schiff bases and formation of nitrogen-containing reactive intermediates.

Type of carbonyl compound and the role of saccharose

Carbonyl compounds that can contribute to the propagation of the Maillard reaction can be divided into *keto* and *aldo* saccharides, short chain α -hydroxy carbonyls and α -dicarbonyl compounds in addition to disaccharides. Literature indicates that all types of carbonyl compounds play an important role in the generation of acrylamide. According to STADLER et al [11] and YAYLAYAN et al. [7], *keto* saccharides generate more acrylamide than *aldo* saccharides under dry conditions. In addition, short chain α -hydroxy carbonyls such as 2-hydroxy-1-butanal and acetol are orders of magnitude more effective than aldoses and ketoses [16] in the generation of acrylamide. On the other hand, α -dicarbonyl compounds are far less effective in generating acrylamide than saccharides. According to the accepted mechanism of acrylamide formation shown in Figures 1 to 3, the azomethine ylides formed through the reaction of α -dicarbonyl

compounds can preferentially be converted into Schiff base B due to the stabilization of the negative charge of the ylide on the carbon next to the carbonyl [17] (Fig. 3). This additional delocalization of the charge leads to the preferential formation of Schiff base B which, if hydrolysed, can generate Strecker aldehyde and α -amino carbonyl compound. This pathway is known as Strecker reaction [17]. On the other hand, the azomethine ylide formed through the reaction with α -hydroxy-carbonyl compound can preferentially be converted into Schiff base A due to the stabilization of the negative charge through the formation of a six-member ring stabilized hydrogen bond with the α -hydroxyl group of the saccharide. With *keto* saccharides, two such hydrogen bondings are possible [18, 19] due to the presence of two α -hydroxyl groups further stabilizing the azomethine ylide and enhancing acrylamide formation as confirmed by many groups. The higher reactivity of the short chain α -hydroxy carbonyl compounds relative to hexoses can be explained therefore by their inability to mutarotate and hence furnish a much higher concentration of the reactive carbonyl moiety in the reaction mixture. Interestingly, the acrylamide precursor Schiff base A formed from α -hydroxy carbonyls (Fig. 3) can also generate α -amino carbonyl compound after Amadori rearrangement and acrylamide elimination steps. In the past, such compounds which are important precursors of

pyrazines, were thought to be formed only through Strecker reaction and in the presence of α -dicarbonyl compounds.

As mentioned above, the relative abilities of glucose, fructose and saccharose to generate acrylamide depended on the temperature and the water content of the system. Under dry heating conditions, fructose and saccharose were more reactive than glucose in the generation of acrylamide [7]. However, in the presence of water, the relative abilities of saccharides depended on the temperature and the moisture content. In low moisture systems and at temperatures ranging between 150–210 °C, fructose generated the highest amount of acrylamide but at 240 °C, saccharose was the most efficient [20]. According to SCHIEBERLE et al. [21], at 10% moisture content, glucose was the most efficient saccharide in generating acrylamide relative to fructose and saccharose. Furthermore, a recent kinetic study conducted at pH 6 [22] has indicated that activation energy (E_a) for the formation of acrylamide from saccharose (49 KJ·mol⁻¹) was much lower than that of glucose (169 KJ·mol⁻¹) and fructose (140 KJ·mol⁻¹), which indicates lower temperature dependence for saccharose to generate acrylamide in the presence of asparagine. This behaviour of the *keto* saccharides in generating acrylamide very much parallels their enhanced abilities to form hydroxymethylfurfural (HMF) under similar conditions. The results ob-

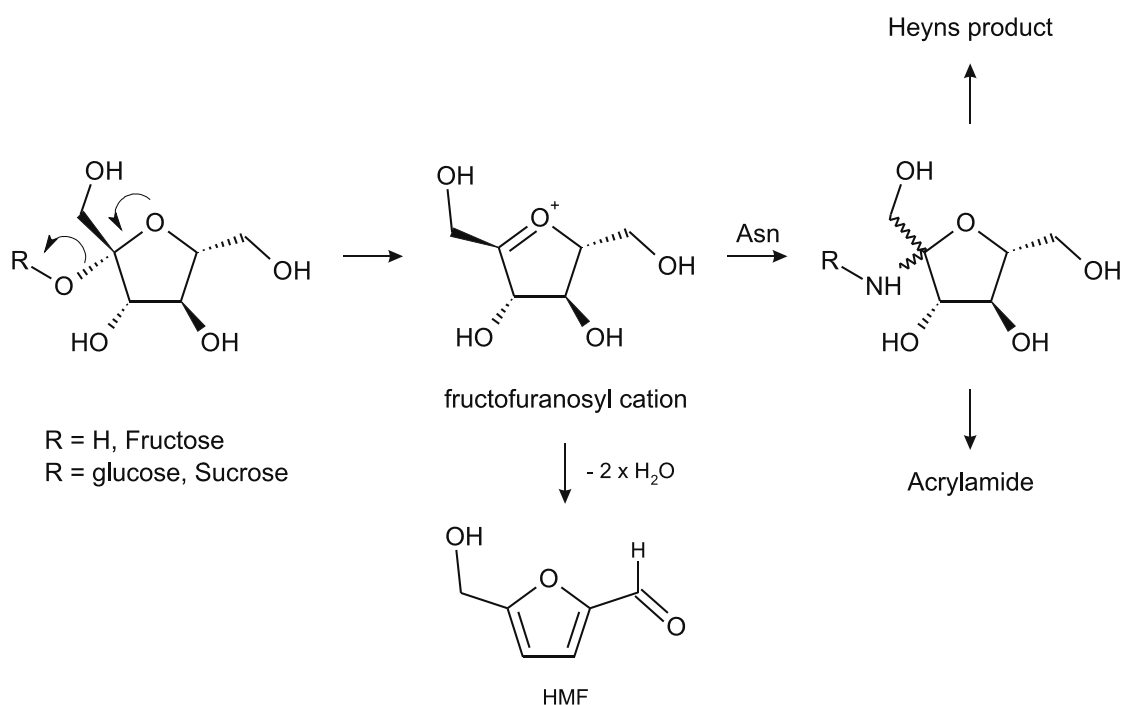


Fig. 4. Formation of fructofuranosyl cation from fructose and saccharose.

tained from a recent study designed to explore this phenomena [23] can offer a theoretical explanation for the observed Maillard reactivity of such saccharides in general and for saccharose in particular. According to this study [23], under dry heating conditions, the glycosidic bond of saccharose can be thermally cleaved to produce glucose and a fructofuranosyl cation (Fig. 4) instead of fructose. In the presence of high moisture, this reactive fructofuranosyl cation is deactivated and easily converted into fructose, however under dry conditions, it can dehydrate directly into HMF or, in the presence of asparagine, can be converted more effectively than glucose into fructofuranosyl asparagine and eventually generate acrylamide. In this pathway, the reaction of asparagine with fructofuranosyl cation represents a mechanistic shift in the process of formation of Heyns product and is therefore expected to affect the rate of the reaction due to the enhanced reactivity of the fructofuranosyl cation relative to the carbonyl moiety, as was indicated above by the observed differences in the activation energies for the formation of acrylamide from saccharose relative to glucose [22].

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