Further Insight into Thermally and pH-Induced Generation of Acrylamide from Glucose/Asparagine Model Systems

CAROLINA PEREZ LOCAS AND VAROUJAN A. YAYLAYAN*

Department of Food Science and Agricultural Chemistry, McGill University, 21,111 Lakeshore, Ste. Anne de Bellevue, Quebec, Canada H9X 3V9

On the basis of numerous studies on the mechanism of formation of acrylamide (AA) from asparagine and reducing sugars, the decarboxylated Schiff base [*N*-(D-glucos-1-yl)-3′-aminopropionamide] and its corresponding Amadori product [*N*-(1-deoxy-D-fructos-1-yl)-3′-aminopropionamide) are considered to be possible direct precursors in addition to 3-aminopropionamide (AP). Furthermore, the mechanism of decarboxylation of the initially formed *N*-(D-glucos-1-yl)asparagine to generate the above-mentioned precursors also remains to be confirmed. To identify the relative importance of AA precursors, the decarboxylated Amadori product (AP ARP) and the corresponding Schiff base were synthesized and their relative abilities to generate AA under dry and wet heating conditions were studied. Under both conditions, the *N*-(D-glucos-1-yl)-3′-aminopropionamide had the highest intrinsic ability to be converted into AA. In the dry model system, the increase was almost 4-fold higher than the corresponding AP ARP or AP; however, in the wet system, the increase was 2-fold higher relative to AP ARP but >20-fold higher relative to AP. In addition, to gain further insight into the decarboxylation step, the amino acid/sugar reactions were analyzed by FTIR to monitor the formation of the previously proposed 5-oxazolidinone intermediate known to exhibit a peak in the range of 1770−1810 cm^{-1}. Spectroscopic studies clearly indicated the formation of an intense peak in the indicated range, the precise wavelength being dependent on the amino acid and the sugar used. The identity of the peak was verified by observing a 40 cm^{-1} shift when [^{13}C-1]-labeled amino acid was used.

KEYWORDS: 3-Aminopropionamide; oxazolidinone; acrylamide; *N*-(D-glucos-1-yl)-3′-aminopropionamide; Hofmann elimination; *N*-(1-deoxy-D-fructos-1-yl)-3′-aminopropionamide; Py-GC/MS analysis; ion chromatography; FTIR

INTRODUCTION

Although the general features of the mechanism of formation of acrylamide (AA) from asparagine and reducing sugars have been confirmed through different studies (*1–5*), detailed knowledge regarding the relative importance of the different precursors of acrylamide such as the decarboxylated glycosyl amine, its corresponding Amadori product, and 3-aminopropionamide or the mechanism of decarboxylation of the asparagine is still lacking (**Figure 1**). It is well documented that in a glucose/asparagine system, the glucos-1-yl-asparagine (**1**) undergoes decarboxylation prior to its rearrangement into Amadori product, to generate *N*-(D-glucos-1-yl)-3′-aminopropionamide (**2**); this intermediate in turn can undergo Amadori rearrangement to produce *N*-(1-deoxy-D-fructos-1-yl)-3′-aminopropionamide (**3**). Both aminopropionamide intermediates (**2** and **3**) are capable of generating acrylamide directly or through formation of free 3-aminopropionamide (**4**). The relative importance of these three possible routes to acrylamide is not well understood. This fact constitutes an important hindrance toward a complete understanding of the mechanistic details of AA formation. In addition, no direct evidence has been presented for the mechanism of decarboxylation of glucos-1-yl-asparagine (**1**) through the 5-oxazolidinone intermediate **1′** (*4*). Decarboxylation through such an intermediate generates azomethine ylides (*6*), the stability of which can determine the ease of decarboxylation and hence the ease of acrylamide formation and, consequently, different carbonyl sources or sugars can provide azomethine ylides with differing stabilities and hence different abilities to generate AA. In this study, for the first time, we provide evidence for the formation of the 5-oxazolidinone intermediate in amino acid/carbohydrate systems. In addition, the relative abilities of the precursors **2**, **3**, and **4** shown in **Figure 1** to generate acrylamide was studied under dry and wet conditions to evaluate their contribution to the total acrylamide formation.

MATERIALS AND METHODS

All reagents, chemicals, and deuterated NMR solvent (CH$_3$OD) were purchased from Aldrich Chemical Co. (Milwaukee, WI) and used

* Corresponding author [telephone (514) 398-7918; fax (514) 398-7977; e-mail varoujan.yaylayan@mcgill.ca].
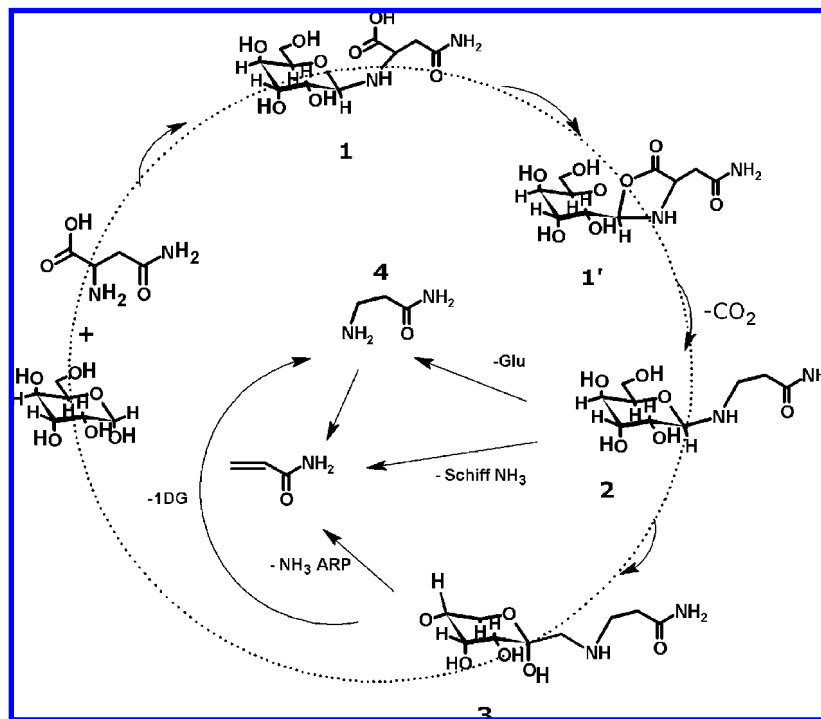
Figure 1. Proposed reactive intermediates involved in the generation of acrylamide. Glu, glucose; ARP, Amadori rearrangement product; 1DG, 1-deoxyglucosone.

without further purification. The 3-aminopropionamide hydrochloride salt was purchased from Chem Impex International (Wood Dale, IL). The ^{13}C-1- and ^{15}N-labeled phenylalanines were purchased from Cambridge Isotope Laboratories (Andover, MA). ^{13}C NMR spectra were acquired on a 500 MHz Varian Unity spectrometer. *Caution: Acrylamide (CAS 79-06-1) is classified as toxic and may cause cancer. Wear suitable protective clothing, gloves, and eye/face protection when handling this chemical.*

Synthesis of *N*-(D-Glucos-1-yl)-3′-aminopropionamide (2). A modified procedure for the synthesis of *N*-glycosides of amino acids as reported by Stadler et al. (*3*) was followed. The 3-aminopropionamide hydrochloride (0.2 g) was dissolved in methanol (8 mL), and excess KOH (0.12 g) was added; the solution was stirred for 10 min and filtered to remove the precipitated KCl. A slight excess of glucose (0.32 g) was then added to the filtrate and heated in an open beaker at 110 °C for 30 min to help remove the water, replenishing evaporated methanol when needed. The solution was transferred into a round-bottom flask and refluxed for another 30 min. The methanol was evaporated in vacuo, and the resulting oil was analyzed by NMR, FTIR, Py-GC/MS, and ion chromatography, which indicated a purity of >80%, the residue being unreacted excess glucose. Py-GC/MS generated mainly acrylamide as a major volatile product (85% of the total area). FTIR (cm^{-1}) 3300 s (OH), 2939m, 2834m (alkyl), 1665 s (amide I), 1612 m (amide II), 1077s 1013 s (sugar C-OH); ^{13}C NMR (500 MHz, CH$_3$OD) δ 175.4 (CONH$_2$, C$_{1'}$), 34.2 (CH$_2$CONH$_2$, C$_{2'}$), 40.6 (NH$_1$CH$_2$, C$_{3'}$), 89.1 (C$_1$), 69.2 (C$_2$), 72.1 (C$_3$), 76.2 (C$_4$), 75.4 (C$_5$), 60.3 (C$_6$).

Synthesis of *N*-(1-Deoxy-D-fructos-1-yl)-3′-aminopropionamide (3). The 3-aminopropionamide hydrochloride (0.2 g) was dissolved in methanol (8 mL), and KOH (0.03 g) was added to adjust the pH to around 6; the solution was stirred for 10 min and filtered to remove any precipitated KCl. A slight excess of glucose (0.30 g) was added to the filtrate and heated in an open beaker at 110 °C for 30 min to help remove the water, replenishing evaporated methanol when needed. The solution was transferred into a round-bottom flask and refluxed for another 40 min. The methanol was evaporated, and the resulting oil was analyzed by NMR, FTIR, MS, Py-GC/MS, and ion chromatography, which indicated a purity of >85%, the residue being mainly excess glucose. Py-GC/MS generated mainly acrylamide as a major volatile product (75%). FTIR (cm^{-1}) 3313 s (OH), 2929m, 2838m (alkyl), 1728w (keto sugar), 1669 s (amide I), 1618 m (amide II), 1073s, 1015s

(sugar C-OH); ^{13}C NMR (500 MHz, CH$_3$OD) δ 172.3 (CONH$_2$, C$_{1'}$), 34.4 (CH$_2$CONH$_2$, C$_{2'}$), 29.4 (NH$_1$CH$_2$, C$_{3'}$), 52.9 (C$_1$), 95.5 (C$_2$), 69.1 (C$_3$), 70.4 (C$_4$), 71.2 (C$_5$), 62.5 (C$_6$).

Pyrolytic Generation of Acrylamide (Dry Heating). A Hewlett-Packard GC with a mass selective detector (5890 GC/5971B MSD) interfaced to a CDS pyroprobe 2000 unit was used for the Py-GC/MS analysis. One milligram samples of reactants were introduced inside a quartz tube (0.3 mm thickness), plugged with quartz wool, and inserted inside the coil probe with a total heating time of 20 s. The column was a fused silica DB-5 column (50 m length × 0.2 mm i.d. × 0.33 μm film thickness; J&W Scientific). The pyroprobe interface temperature was set at 250 °C. The capillary direct MS interface temperature was 280 °C, and the ion source temperature was 180 °C. The ionization voltage was 70 eV, and the electron multiplier was 2471 V. All injections were in splitless mode. The mass range analyzed was 33−650 amu. The initial temperature of the column was set at −5 °C for 2 min and was increased to 50 °C at a rate of 30 °C/min; immediately the temperature was further increased to 250 °C at a rate of 8 °C/min and kept at 250 °C for 5 min. The identity and purity of the chromatographic peaks were determined using NIST AMDIS version 2.1. The reported percent label incorporation values (corrected for natural abundance and for percent enrichment) are the average of duplicate analyses and are rounded off to the nearest multiple of 5%.

Thermal Generation of Acrylamide in the Presence of Water. Acrylamide precursors (10 mg) were mixed with water and alumina (50% w/w moisture) in a 10 mL flame sealed glass ampule and heated in an oven at 170 °C for 20 min. The samples were diluted with water (100 mL) and analyzed by ion chromatography as described below.

Quantification of Acrylamide by Ion Chromatography. A Metrohm MIC-8 modular IC system (Herisau, Switzerland) consisting of a pulsed amperometric detector (E_1 = 0.15 V, t_1 = 400 ms, E_2 = 0.75 V, t_2 = 200 ms; E_3 = −0.15 V, t_3 = 400 ms), a pump, and a sample injection unit connected to Metrosep Carb1-150 anion exchange column thermostated at 31 °C was used for the analysis of AA and its precursors. The mobile phase was 0.1 N NaOH and the flow rate, 1 mL/min. The calibration curve for acrylamide was constructed by injecting serially diluted solutions in the range of 1−20 ppm concentrations.

Generation and FTIR Analysis of 5-Oxazolidinone. An equimolar mixture (10 mg) of sugar and the amino acid was heated in toluene (2

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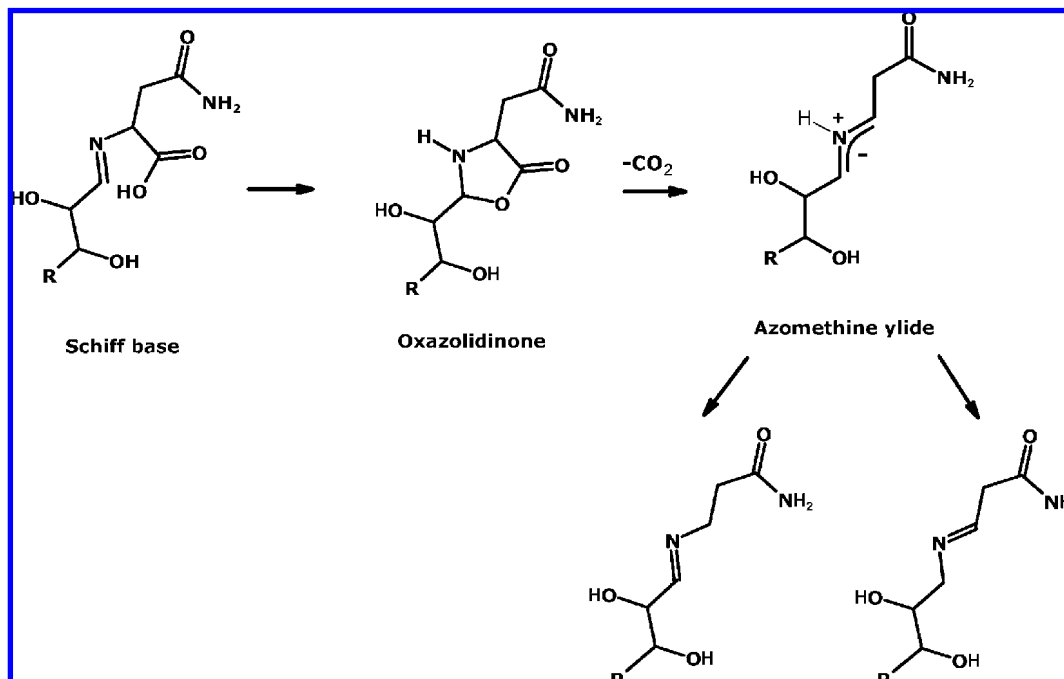*J. Agric. Food Chem.,* Vol. 56, No. 15, 2008 **6071**

Figure 2. Intramolecular cyclization of the Schiff base of asparagine into 5-oxazolidinone intermediate and its subsequent decarboxylation into azomethine ylide.

mL; methanol and/or *p*-toluenesulfonic acid could be added to help dissolve insoluble models) for 10 min or until most reactants dissolved at 115 °C in an open vial. The solution was passed immediately through glass wool, and the sample (5 μL) was applied on the ATR crystal and scanned after evaporation of the solvent. Infrared spectra were recorded on a Nicolet 380 FTIR spectrometer (Thermo Electron Corp., Madison, WI) equipped with a single-bounce ATR sampling unit. A total of 64 scans at 4 cm^{-1} resolution were coadded. Processing of the FTIR data was performed using GRAMS/32 AI (ThermoGalactic). Second-order derivatization was performed using Savitsky–Golay function (30 points) to enhance closely absorbing peaks.

Browning Measurement by UV–Vis. An equimolar solution of reactants (0.03 M each) in dimethyl sulfoxide was stirred in the presence and absence of dimethyl fumarate (0.03M) at 80 °C for 30 min. The solution was cooled, and browning was measured by scanning between 360 and 830 nm using an Evolution 300 scanning spectrophotometer from Thermo Electron Corp. CIE chromaticity coordinates were calculated using ACD/Laboratories SpecManager version 8.2 (Toronto, Canada).

Effect of pH on Acrylamide Generation from *N*-(1-Deoxy-D-fructos-1-yl)-3′-aminopropionamide. Alumina (50 mg) was mixed with 3-AP-ARP (20 mg), glucose (30 mg), and water (9 μL). The mixture was divided into four portions and heated in separate 10 mL flame-sealed glass ampules in an oven at 165 °C for 20 min. Two samples were diluted with water (2.5 mL), and the remaining two with an equal volume of NaOH solution (pH 12) and incubated at room temperature overnight and analyzed by ion chromatography as described above.

RESULTS AND DISCUSSION

Decarboxylation of the initially formed Schiff base (**1**) of glucose with asparagine is one of the critical steps in the pathway of conversion of asparagine into acrylamide (**Figure 1**). Although amino acids can be decarboxylated at high temperatures into their corresponding free amines, in the presence of aldehydes and ketones and in low-moisture systems, this process is facilitated due to the formation of a relatively stable azomethine ylide (*6*) after the loss of CO$_2$ from 5-oxazolidinone intermediate (**Figure 2**). In dry systems, the open-form Schiff bases are prone to undergo intramolecular cyclization to form either 5-oxazolidinone or glycosylamines, unlike in high-moisture systems where they tend to undergo Amadori rearrangement (*3*), the more stable isomer. In fact, the efficiency of decarboxylation of phenylalanine, for example, as measured by the amount of phenethylamine produced during Py-GC/MS analysis, increased around 300-fold in the presence of phenylacetaldehyde and other carbonyl-containing compounds. However, the formation of 5-oxazolidinone and subsequent generation of azomethine ylides have so far been verified only in model systems consisting of amino acids and simple aldehydes (*6*). In this study we provide evidence for their formation in amino acid/carbohydrate systems. Furthermore, to ascertain the relative abilities of the immediate precursors **2**, **3**, and **4** shown in **Figure 1** to generate AA under dry and wet (50% moisture) conditions, the proposed intermediates were either pyrolyzed at 250 °C/20 s or heated with moisture at 170 °C for 20 min as described under Materials and Methods.

Spectroscopic Evidence for the Formation of 5-Oxazolidinone Intermediate in Sugar/Asparagine Model Systems. Indirect evidence for the involvement of Schiff bases in assisting the decarboxylation process was obtained earlier (*7*) when [^{13}C-4]-aspartic acid was pyrolyzed alone and in the presence of glucose and label incorporation in the resulting decarboxylation product the acrylic acid was calculated. Analysis of the data showed the formation of 65% of labeled acrylic acid and 35% of unlabeled product when [^{13}C-4]-aspartic acid was pyrolyzed alone, and when [^{13}C-4]-aspartic acid was pyrolyzed in the presence of glucose, 100% labeled acrylic acid was observed (for details see ref *7*), indicating preferential decarboxylation of the C-1 carboxylate moiety that is able to cyclize and form the 5-oxazolidinone intermediate, rather then the C-4 acid group unable to cyclize in the same fashion, consistent with the mechanism of sugar-assisted decarboxylation shown in **Figure 2** However, to provide direct evidence for the formation of 5-oxazolidinone, the amino acid/sugar reactions were analyzed by FTIR to monitor the formation of a peak in the range between 1780 and 1800 cm^{-1}, where 5-oxazolidinones are known to exhibit a strong absorption band (*8*). Spectroscopic studies using
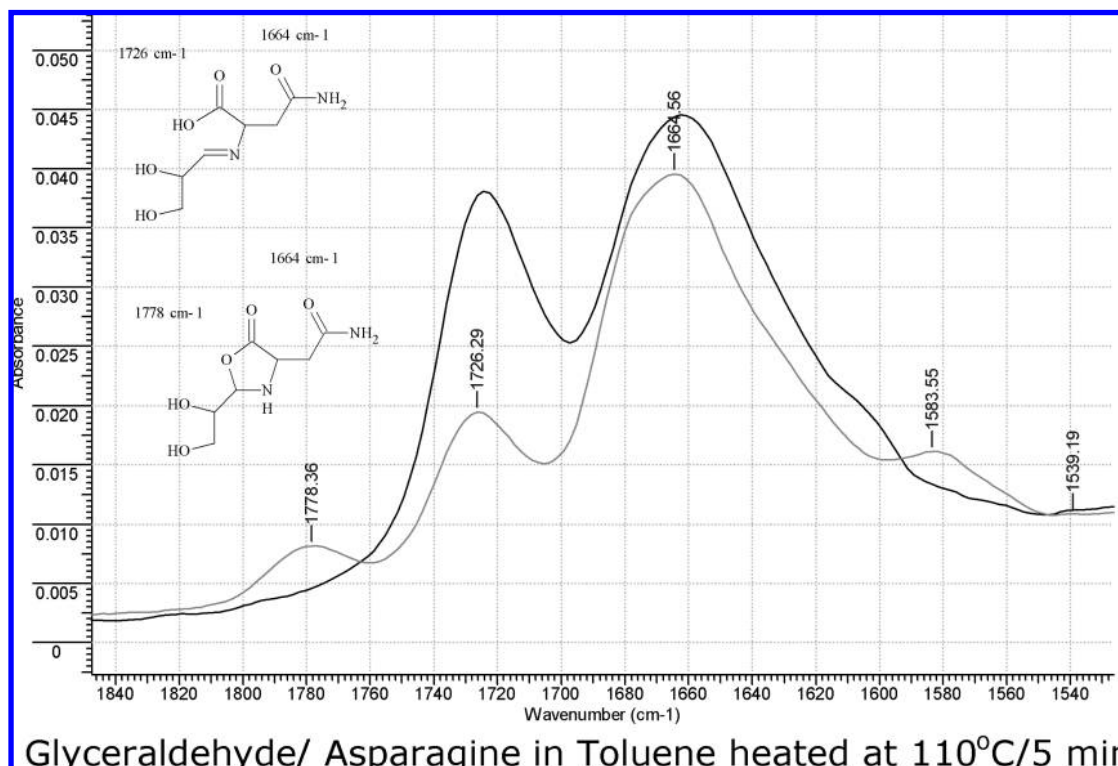
Figure 3. FTIR spectrum of asparagine (black trace) and heated glyceraldehyde/asparagine mixture (gray trace) showing the buildup of the absorption peak of 5-oxazolidinone intermediate centered at 1778 cm^{-1}.

Table 1. Effect of Dimethyl Fumarate on the Intensity of Absorption at 450 nm in Different Model Systems[a]

model system	450 nm
glyceraldehyde/phenylalanine[b]	0.698
glyceraldehyde/phenylalanine/dimethyl fumarate	0.344
% difference	**−50**
mannose/phenylalanine	0.095
mannose/phenylalanine/dimethyl fumarate	0.074
% difference	**−22**
control	
1-aminopropanediol/phenylacetaldehyde	0.060
1-aminopropanediol/phenylacetaldehyde/fumarate	0.063
% difference	**0**

[a] Solutions (0.03 M) heated in DMSO at 80 °C for 30 min. Average of two replicates with coefficient of variation < 10%. [b] Heated for 8 min only due to intense color formation.

Table 2. Relative Efficiencies (Peak Area per Mole) of Different Precursor Systems in Acrylamide (AA) Generation under Dry and Wet Conditions

precursor system[a]	AA in dry system[b]	AA in wet system[c]
AP ARP	0.9	13
AP ARP/glucose[d]	1.2	20
AP	**1**	**1**
AP/glucose[d]	1.6	6
AP/vanillin[d]	2.2	nd
AP glucosylamine	4	27

[a] AP, 3-aminopropionamide; ARP, Amadori rearrangement product. [b] As determined by Py-GC/MS (1 mg samples pyrolyzed at 250 °C for 20 s); values represent average of two replicates with a coefficient of variation of <10%. [c] As determined by ion chromatography (10 mg samples diluted with alumina in 50% moisture to a total weight of 50 mg, heated at 170 °C for 20 min); values represent average of two replicates with a coefficient of variation of <30%. [d] Three-fold molar excess relative to the primary precursor.

glyceraldehyde/amino acid models in toluene heated at 110 °C clearly indicated the formation of an intense peak in the range of 1780−1810 cm^{-1}, depending on the amino acid. The identity of the peak was verified by observing the expected 40 cm^{-1} shift when ^{13}C-1-labeled amino acids were used. **Figure 3** shows the carbonyl absorption peak centered at 1778 cm^{-1} for the glyceraldehydes/asparagine model system. Furthermore, evidence for the formation of the resulting azomethine ylide was also provided using their specific ability to undergo 1,3-dipolar cycloadditions with dipolarophiles (*6*). The addition of dipolarophiles, such as dimethyl fumarate, to the heated model systems has led to a significant drop in intensity of the Maillard browning (**Table 1**), indicating the importance of the resulting imines shown in **Figure 2** to the generation of color.

Relative Efficiency of Different Precursors in the Generation of Acrylamide under Dry and Wet Conditions. Different precursors (**2**, **3**, and **4**) listed in **Table 2** and shown in **Figure**

4 were either pyrolyzed and analyzed by GC/MS or heated in sealed glass ampules in the presence of wet alumina and analyzed by ion chromatography as detailed under Materials and Methods. In both dry and wet systems, the relative amount of AA was estimated from the calculated value of the area of the acrylamide peak per mole of the precursor. The relative efficiencies reported in **Table 2** and in **Figure 4** are normalized values relative to the ability of the free 3-aminopropionamide (AP) to generate acrylamide taken as unity. Inspection of **Table 2** indicates that the most efficient precursor of AA in both dry and wet systems is the *N*-(D-glucos-1-yl)-3′-aminopropionamide (**2**, AP glycosylamine). In the dry system there is no significant difference between free AP (**4**) and AP ARP (**3**) in their abilities to be converted into AA; however, in the wet system there was a significant increase in the ability of AP ARP (**3**) relative to AP in acrylamide generation. Furthermore, the data also indicate the importance of even a small amount of water in enhancing the efficiencies of both AP glycosylamine (6-fold increase) and AP ARP (13-fold increase) in generating AA. The role of water
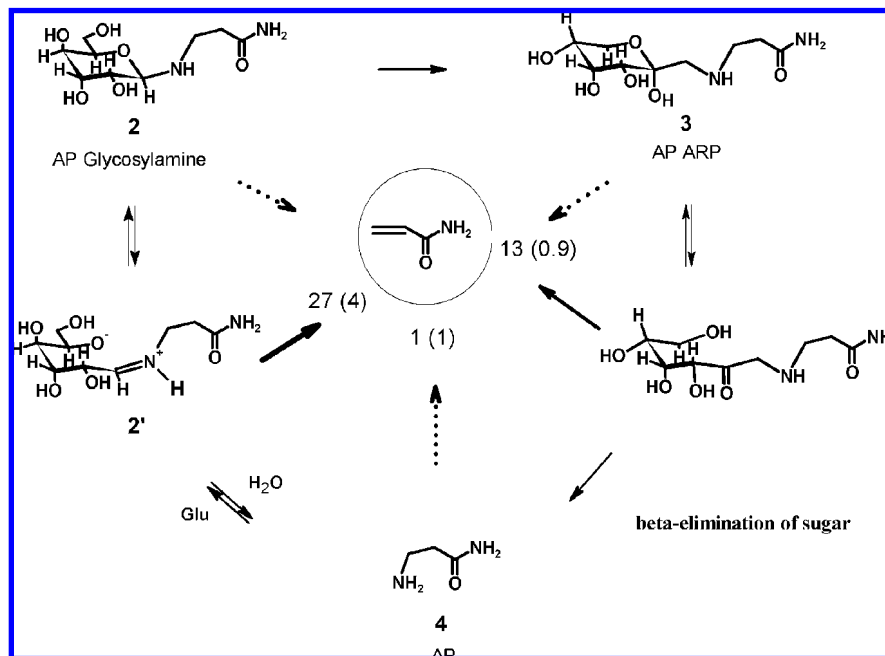
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Figure 4. Reactive precursors and their relative abilities to generate acrylamide in wet and dry (values in parentheses) model systems (see also **Table 2**). Glu, glucose; AP, 3-aminopropionamide; ARP, Amadori rearrangement product.
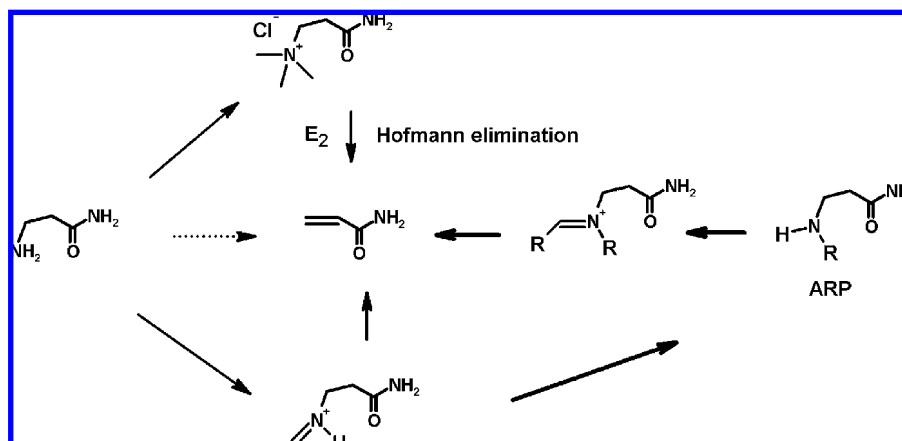

Figure 5. Hofmann elimination of 3-aminopriopionamide and AP ARP through successive glycation reactions. R, sugar residue; ARP, Amadori rearrangement product.

can be explained by its effect on ring opening and on chemical mobility in general. In addition, the common step of acrylamide formation from all three precursors is the elimination of either ammonia, as in the case of AP (**4**), or amines, as in the case of AP ARP (**4**) or AP glycosylamine (**3**). However, deamination of amines whether primary, secondary, or tertiary, is an energetically difficult process due to the basicity of the leaving groups. The common method of deamination of amines is through their conversion into quaternary ammonium salts or quaternary imminium ions (*9*), known as Hofmann elimination (**Figure 5**). Consequently, the enhanced ability of AP glycosylamine (**2**) to undergo deamination can be attributed to its open form, the imminum ion (**2′** in **Figure 4**). This form is capable of undergoing Hofmann-type elimination. The process of ring opening to form the imminium ion is greatly enhanced in the presence of moisture. A higher content of moisture, of course, will degrade the imminium ion and produce free AP and glucose. Similarly, an increased concentration of the open form of AP ARP in the presence of moisture can increase the formation of reactive carbonyl compounds through the known degradation pathways of ARP and consequently form imminium

ions similar to that of AP glycosylamine (**Figures 4** and **5**). When AP or AP ARP was heated in the presence of excess sugar or vanillin (**Table 2**), increased formation of AA was observed under both dry and wet conditions, supporting the proposed mechanism of deamination through a Hofmann-type E2 mechanism after reaction with carbonyl compounds or available sugars.

Role of Basic pH in Poststorage Acrylamide Release. The above-proposed mechanism of base-catalyzed Hofmann-type elimination shown in **Figure 5** can also provide a possible explanation for the observed increase in AA content of food samples if a subsequent extraction is performed under high-pH conditions, indicating the presence of a water-soluble, base-sensitive precursor capable of releasing AA (*10*). On the basis of the above findings it can be assumed that among the known precursors, the AP ARP (**3**) is the most likely candidate to undergo incomplete reaction and partially accumulate in food products due to its lower reactivity. During storage and as depicted in **Figure 5**, AP ARP can react with glucose or other carbonyl compounds and be converted into a more reactive form to undergo base-catalyzed Hofmann-type elimination (*9*) and

Table 3. Acrylamide Content in Heated *N*-(D-Glucos-1-yl)-3′-aminopropionamide (**2**) Model System after Incubation under Acidic or Alkaline pH

model system[a]	acrylamide[b] (mmol/mol)
AP ARP + excess glucose[c] incubated overnight in water (pH 6.5)	2.7
AP ARP + excess glucose[c] incubated overnight in NaOH solution (pH 12)	11.7

[a] Model systems (10 mg samples diluted with alumina in 50% water (w/w) to a total weight of 50 mg) were heated with alumina at 165 °C for 20 min in sealed vials and incubated overnight as shown in the table. AP, 3-aminopropionamide; ARP, Amadori rearrangement product. [b] Based on average of two measurements with coefficient of variance of <15%. [c] Glucose was in 3-fold molar excess.

generate AA. To test this hypothesis, AP ARP samples with excess glucose were heated with alumina at 165 °C for 20 min in sealed ampules containing 50% water (w/w) and incubated overnight; one sample was kept in distilled water and the other in NaOH solution (pH 12). As shown in **Table 3**, the sample incubated in basic solution showed a >4-fold increase in AA.

The initial interaction between asparagine and glucose can generate, therefore, a sequence of precursors such as *N*-(D-glucos-1-yl)-3′-aminopropionamide (**2**) and *N*-(1-deoxy-D-fructos-1-yl)-3′-aminopropionamide (**3**) capable of generating acrylamide either directly or through the formation of 3-aminopropionamide (**4**). The relative importance of each of these intermediates in food systems can be determined only by their kinetic parameters. These parameters can change depending on the food matrix and moisture content. In this study only the **intrinsic abilities** of these precursors to generate acrylamide in **model systems** were estimated under dry and wet conditions. Under both conditions the *N*-(D-glucos-1-yl)-3′-aminopropiona-mide (**2**, AP glycosylamine) had the highest intrinsic ability to be converted into AA. In the dry model system the increase was almost 4-fold higher than AP ARP (**3**) or AP (**4**); however, in the wet system, the increase was 2-fold higher than AP ARP but >20-fold higher relative to AP. Furthermore, spectroscopic studies also indicated an increased likelihood of decarboxylation through the 5-oxazolidinone intermediate with decreasing moisture content of the model system.

ABBREVIATIONS USED

AP, 3-aminopropionamide; ARP, Amadori rearrangement product; AA, acrylamide.

LITERATURE CITED

(1) Stadler, R. H.; Blank, I.; Varga, N.; Robert, F.; Hau, J.; Guy, P.; Robert, M.-C.; Riediker, S. Acrylamide from Maillard reaction products. *Nature* **2002**, *419*, 449–450.

(2) Granvogl, M.; Schieberle, P. Thermally generated 3-aminopropionamide as a transient intermediate in the formation of acrylamide. *J. Agric. Food Chem.* **2006**, *54*, 5933–5938.

(3) Stadler, R. H.; Robert, F.; Riediker, S.; Varga, N.; Davidek, T.; Devaud, S.; Goldmann, T.; Hau, J.; Blank, I. In-depth mechanistic study on the formation of acrylamide and other vinylogous compounds by the Maillard reaction. *J. Agric. Food Chem.* **2004**, *52*, 5550–5558.

(4) Yaylayan, V. A.; Wnorowski, A.; Perez Locas, C. Why asparagine needs carbohydrates to generate acrylamide. *J. Agric. Food Chem.* **2003**, *51*, 1753–1757.

(5) Zyzak, D.; Sanders, R. A.; Stojanovic, M.; Tallmade, D. H.; Eberhart, B. L.; Ewald, D. K.; Gruber, D. C.; Morsch, T. R.; Strothers, M. A.; Rizzi, G. P.; Villagran, M. D. Acrylamide formation mechanism in heated foods. *J. Agric. Food Chem.* **2003**, *51*, 4782–4787.

(6) Tsuge, O.; Kanemasa, S.; Ohe, M.; Takenaka, S. Simple generation of nonstabilized azomethine ylides through decarboxylative condensation of α-amino acids with carbonyl compounds via 5-oxazolidinone intermediate. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 4079–4089.

(7) Yaylayan, V.; Perez, L. C.; Wnorowski, A.; O'Brien, J. Role of creatine in the generation of *N*-methylacrylamide: a new toxicant in cooked meat. *J. Agric. Food Chem.* **2004**, *52*, 5559–5565.

(8) Aurelio, L.; Box, J. S.; Brownlee, T. C.; Hughes, A. B.; Sleebs, M. M. An efficient synthesis of *N*-methyl amino acids by way of intermediate 5-oxazolidinone. *J. Org. Chem.* **2003**, *68*, 2652–2667.

(9) Katritzky, A. R.; El-Mouafy, M. A. Pyrylium-mediated conversion of primary amines into olefins via tetrahydrobenzoacrydiniums: a mild alternative to Hofmann elimination. *J. Org. Chem.* **1982**, *47*, 3506–3511.

(10) Goldmann, T.; Perisset, A.; Bertholet, M.-C.; Stadler, R. H.; Petersson, E. V.; Hellenäs, K.-E. Impact of extraction conditions on the content of acrylamide in model systems and food. *Food Addit. Contam.* **2006**, *23*, 437–445.