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ARTICLE

Model Reactions of Acrylamide with Selected Amino Compounds

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The reaction of acrylamide with amines, amino acids, and polypeptides was studied in an attempt to understand the role of amino compounds on acrylamide fate. The obtained results showed that amino compounds are added to acrylamide by means of a Michael addition to produce the corresponding 3-(alkylamino)propionamides. Although 3-(alkylamino)propionamides can also be added to a new molecule of acrylamide to produce a new adduct, this last adduct was not detected under the employed conditions in which the concentration of acrylamide was much lower than the concentration of the amino compounds. The produced 3-(alkylamino)propionamides were not stable, and the addition reaction was easily reversed by heating. Thus, acrylamide was produced from 3-(alkylamino)-propionamides by means of an elimination reaction. However, the activation energies (E_a) of both reactions are not the same. In fact, acrylamide seems to be converted into its Michael adduct with a lower activation energy than the elimination reaction of the Michael adduct. For this reason, when acrylamide was stored in the presence of glycine at 60 °C, acrylamide disappeared after 14 days. However, when these samples were heated again for 20 min at 180 °C, the equilibrium was reestablished and a significant amount of acrylamide was detected. All of these results suggest that amino compounds may play a significant role in the changes observed in acrylamide content in foods upon storage. In addition, they also point to 3-(alkylamino)propionamides as possible compounds in which acrylamide might be potentially hidden.

KEYWORDS: Acrylamide; 3-(alkylamino)propionamides; amines; amino acids; carbonyl−amine reactions; Hoffman elimination; Maillard reaction; Michael addition

INTRODUCTION

Foods are processed for a variety of reasons: to render them edible if they are not; to permit storage; to alter texture and flavor; and to destroy microorganisms, undesirable enzymes, and other toxins (*1*). Although processing can improve foods in many senses, these procedures can occasionally lead to the formation of toxic compounds. Among them, acrylamide has received increased worldwide attention in recent years (*2*). This toxicant is formed upon heating in the course of the Maillard reaction (*3*,*4*). It is produced by reaction of carbohydrates with asparagine (*5*−*9*), and recent studies have also pointed to some lipid oxidation products as potential contributors to acrylamide formation (*10*).

However, once formed, acrylamide concentration is not always constant. Thus, acrylamide losses have been described in different foods, including roasted coffee and cocoa powder, among others (*11*). These losses are believed to be produced by reaction of the acrylamide with some of the other compounds present in those foods. Although, at this time, these compounds have not been clearly identified, the elimination of the acrylamide formed via nucleophilic groups (−SH, $-NH_2$) on amino acid side chains has been proposed (*12*). In fact, acrylamide is typically formed in carbohydrate-rich and protein-low plant commodities, which would be in agreement with the apparent inhibitory effect of proteins in acrylamide formation (*13*). In addition, other studies have suggested that the addition of amino acids is a potential way to decrease acrylamide content in foods (*12*, *14*, *15*).

In an attempt to clarify some of the reaction pathways by which amino acids, and amino acid side chains, affect the acrylamide content determined in foods, this study analyzes the reaction between acrylamide and amino groups. These reactions were carried out, in a first step, with model amines to understand the different reactions pathways involved and, then, extended to amino acids and polypeptides.

MATERIALS AND METHODS

Materials. All chemicals were purchased from Aldrich (Milwakee, WI), Sigma (St. Louis, MO), Fluka (Buchs, Switzerland), or Merck (Darmstadt, Germany) and were of analytical grade. Labeled [1,2,3,-$^{13}C_3$]-acrylamide was obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA). The two polylysines of different molecular weights employed in these studies were obtained from Sigma. According to the certificate of analysis provided by Sigma, the low molecular weight polylysine employed had a molecular weight (determined by viscosity) of 4200. The molecular weight determined by the same procedure of the high molecular weight polylysine employed was 68300.

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3-(Butylamino)propanamide (**3**) was prepared by reaction of acrylamide with butylamine. Briefly, acrylamide (14.5 mmol) was dissolved in methanol (29 mL) and treated with the butylamine (14.5 mmol). The obtained solution was heated at 60 °C for 24 h and, then, the solvent was evaporated. The residue was treated with toluene/hexane (1:1) to crystallize compound **3** and recrystallized using the same solvent. ^{1}H NMR (CD_3OD) δ 0.95 t (3H, J = 7.3 Hz, $C\underline{H}_3$), 1.37 sx (2H, J = 7.3 Hz, $CH_3C\underline{H}_2$), 1.50 qu (2H, J = 7.3 Hz, $CH_3CH_2C\underline{H}_2$), 2.43 *t* (2H, J = 6.9 Hz, $C\underline{H}_2CONH_2$), 2.61 t (2H, J = 7.3 Hz, $CH_3CH_2CH_2C\underline{H}_2$), and 2.84 t (2H, J = 6.9 Hz, $CH_2C\underline{H}_2NH$); ^{13}C NMR (CD_3OD) δ 14.28 (CH_3), 21.46 ($CH_3\underline{C}H_2$), 32.44 ($CH_3CH_2\underline{C}H_2$), 35.33 ($\underline{C}H_2CONH_2$), 46.38 ($CH_2\underline{C}H_2NH$), 50.06 ($CH_3CH_2CH_2\underline{C}H_2$), and 177.22 ($CONH_2$); MS (relative intensity, ion structure) m/z 144 (1, M^+), 101 (55, $M^+ - CH_3CH_2CH_2$), 84 (65, $M^+ - CH_3CH_2CH_2 - NH_3$), 56 (22, $M^+ - CH_3CH_2CH_2 - NH_3 - CO$), 44 (100), and 42 (77).

2-(Bis(3-amino-2-oxopropyl)amino)acetic acid (**4**) was prepared by reaction of acrylamide with glycine. Briefly, acrylamide (3.75 mmol) and glycine (7.5 mmol) were dissolved in water (14.5 mL) and treated with 2 M potassium hydroxide until basic pH. The obtained solution was heated at 60 °C for 24 h and, then, treated with 2 M acetic acid until acid pH. The obtained compound **4** was recrystallized using water. ^{1}H NMR (D_2O) δ 2.65 t (4H, $C\underline{H}_2CONH_2$), 3.37 t (4H, $C\underline{H}_2CH_2CONH_2$), and 3.63 s (2H, $C\underline{H}_2COOH$); ^{13}C NMR (D_2O) δ 31.16 ($\underline{C}H_2CONH_2$), 53.72 ($\underline{C}H_2CH_2CONH_2$), 58.82 ($\underline{C}H_2COOH$), 172.65 ($\underline{C}ONH_2$), and δ 176.81 ($\underline{C}O$-OH); MS (relative intensity, ion structure) of the trimethylsilyl derivative m/z 433 (3, M^+), 416 (1, $M^+ - CH_3$), 343 (5, $M^+ - HOSi(CH_3)_3$), 316 (28, $M^+ - COOSi(CH_3)_3$), 305 (15), 185 (100), 173 (74), 144 (62, $CH_2CH_2CONHSi(CH_3)_3$), 128 (15), and 73 (67, $Si(CH_3)_3$).

Acrylamide/Amino Compound Reactions. Model reactions were carried out analogously to the method of Granvogl and Schieberle (*7*), with the modifications described by Zamora and Hidalgo (*16*). Briefly, mixtures of the acrylamide (0.2 μmol) and the amino compound (0−50 μmol) were singly homogenized with 0.063−0.200 mm silica gel 60 (300 mg) (Macherey-Nagel, Düren, Germany), 30 μL of 0.3 M buffer (sodium citrate for pH 3−6 and sodium phosphate for pH 6−8), and 180 μL of water in closed test tubes and heated in a heater block under nitrogen at 180 °C for 10 min, unless otherwise indicated. The water activity (a_w) of the samples was determined with a Pawkit Decagon analyzer (Pullman, WA) as described previously (*17*). The a_w of the assayed systems was 0.95. The amino compounds assayed were butylamine, glycine, lysine, and two poly-L-lysine hydrobromides of different molecular weights. The reaction pH was maintained upon heating.

After cooling (15 min at −20 °C), 10 μL of internal standard solution (1 mg/mL of labeled [1,2,3-$^{13}C_3$]acrylamide in methanol) and 2 mL of 0.3 M sodium citrate buffer, pH 2.2, were added. Suspensions were stirred for 1 min, the supernatant was then filtered, and its acrylamide content was determined. In addition, other heated samples were cooled (15 min at −20 °C) and extracted with methanol (2 × 2 mL). The extracts were studied by GC-MS.

Additionally, the formation of acrylamide in the thermal degradation of compounds **3** and **4** was also studied. This reaction was carried out analogously to acrylamide/amino compound reactions. Thus, the Michael adduct **3** or **4** (0.2 μmol) was singly homogenized with 0.063−0.200 mm silica gel 60 (300 mg), 30 μL of 0.3 M buffer (sodium citrate for pH 3−6 and sodium phosphate for pH 6−8), and 180 μL of water and heated under nitrogen at 180 °C in closed test tubes for 10 min, unless otherwise indicated. After cooling, acrylamide was extracted as described above.

Analysis of Acrylamide. Acrylamide was analyzed as the stable 2-bromopropenamide by gas chromatography−mass spectrometry (GC-MS) using the method of Castle et al. (*18*) with the modifications of Andrawes et al. (*19*). Briefly, 1 mL of the supernatant was treated with 0.3 g of potassium bromide and 400 μL of saturated bromine solution in water. After 1 h in the dark at 0 °C, the excess of bromine was removed by the addition of 1 M sodium thiosulfate until the solution became colorless, and the solution was extracted with 1 mL of ethyl acetate/hexane (4:1). The organic layer was finally dried with sodium sulfate, evaporated until a volume of ~50 μL, treated with 50 μL of triethylamine, and analyzed by GC-MS.

The ions monitored for the identification of the analyte, 2-bromopropenamide, were $[C_3H_4NO]^+$ = 70, $[C_3H_4{}^{79}BrNO]^+$ = 149, and $[C_3H_4{}^{81}BrNO]^+$ = 151, using m/z 149 for quantitation. The ions monitored for identification of the internal standard (2-bromo[$^{13}C_3$]propenamide) were $[^{13}C_2H_3{}^{81}Br]^+$ = 110 and $[^{13}C_3H_4{}^{81}BrNO]^+$ = 154, using m/z 154 for quantitation. The separation of acrylamide analyte after derivatization was performed on GC capillary columns of middle to high polarity. GC-MS analyses were conducted with a Hewlett-Packard 6890 GC Plus coupled with an Agilent 5973 MSD (Mass Selective Detector-Quadrupole type). In most experiments, a 30 m × 0.25 mm i.d. × 0.25 μm HP5-MS capillary column was used. Working conditions were as follows: carrier gas, helium (1 mL/min at constant flow); injector, 250 °C; oven temperature, from 50 °C (1 min) to 240 °C at 5 °C/min and then to 325 °C at 10 °C/min; transfer line to MSD, 280 °C; and ionization EI, 70 eV.

Quantification of acrylamide was carried out by preparing standard curves of this compound in the 300 mg of silica gel and following the whole procedure described above. For each curve, 15 different concentration levels of acrylamide (0−200 μg) were used. Acrylamide content was directly proportional to the acrylamide/internal standard area ratio (r = 0.999, $p < 0.0001$). The coefficients of variation at the different concentrations were < 10%.

GC-MS Analyses. GC-MS analyses of extracted samples with methanol were carried out to identify the reaction products. A Hewlett-Pachard 6890 GC Plus coupled with an Agilent 5973 MSD (Mass Selective Detector Quadrupole type) and a 30 m × 0.25 mm i.d. × 0.25 μm HP5-MS capillary column was used in these experiments. Working conditions were as follows: carrier gas, helium (1 mL/min at constant flow); injector, 250 °C; oven temperature, from 40 °C (1 min) to 240 °C at 5 °C/min and then to 300 °C at 10 °C/min; transfer line to MSD, 280 °C; and ionization EI, 70 eV.

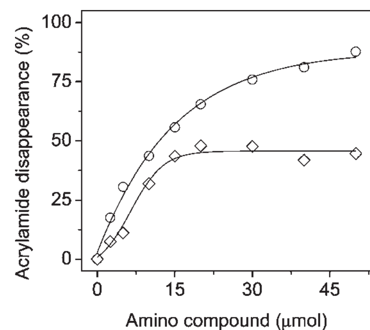

Figure 1. Effect of the concentration of the amino compound on the acrylamide determined after heating an acrylamide/amino compound model system for 10 min at 180 °C. The amount of acrylamide in the model system was 0.2 μmol. Amino compounds assayed were butylamine (○) and glycine (◇).

RESULTS

Acrylamide Disappearance in Acrylamide/Butylamine Reaction Mixtures. When acrylamide was heated in the presence of butylamine, the acrylamide disappeared and the appearance of a new compound was observed. This compound was identified as 3-(butylamino)propanamide (**3**) on the basis of its retention index and mass spectrum determined by GC-MS using the synthesized compound **3** as standard. Although the Michael addition of this adduct to a second molecule of acrylamide is possible, the formation of the corresponding 3,3′-(butylazanediyl)dipropanamide was not observed under the conditions employed.

The disappearance of the acrylamide depended on the reaction conditions, and the presence of the amine was responsible for the decreases observed in acrylamide content. **Figure 1** shows that the acrylamide remained unchanged when the amine was not present, but acrylamide disappeared as a function of the amount of butylamine added. These reactions were carried out with 0.2 μmol of acrylamide and, under the assayed conditions, an amount of ~12 μmol of butylamine was needed to reduce acrylamide content to half. Although acrylamide and butylamine react equimolecularly, the much higher concentration of butylamine than acrylamide

Table 1. Effect of pH on Acrylamide Determined after Heating either an Acrylamide/Amino Compound Model System or Compound **3**[a]

	model system		
pH	acrylamide/butylamine	compound **3**	acrylamide/glycine
2.15[b]	64.8	8.8	30.5
3[b]	69.1	14.7	28.0
4[b]	71.1	37.1	40.2
5[b]	73.1	50.0	52.7
6[b]	74.5	60.0	52.9
6[c]	70.0	27.3	53.5
6.5[c]	63.0	24.3	nd[d]
7[c]	61.9	24.4	49.4
8[c]	64.4	30.0	55.1

[a] Values are acrylamide disappearance (%) in acrylamide/amino compound (0.2 μmol/20 μmol) model systems and acrylamide formation (%) in the thermal decomposition of 3-(butylamino)propanamide (**3**). Samples were heated for 10 min at 180 °C. [b] Sodium citrate buffers were employed. [c] Sodium phosphate buffers were employed. [d] Not determined.

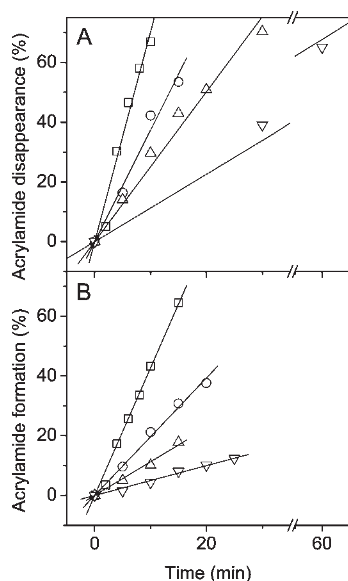

Figure 2. Effect of time and temperature on the acrylamide determined after heating (**A**) an acrylamide/butylamine (0.2 μmol/20 μmol) model system or (**B**) 3-(butylamino)propanamide (**3**) at 180 (□), 160 (○), 140 (△), and 120 (▽) °C and pH 7.

needed may be a consequence of both the low concentration of acrylamide employed in the model reactions and a low reaction yield between acrylamide and butylamine. Nevertheless, acrylamide is always produced in foods in very low amounts, and amino compounds are expected to be present in much higher amounts than acrylamide.

Differently from the concentration of butylamine, the pH did not seem to play a major role in acrylamide/butylamine reactions, and similar amounts of acrylamide were recovered in the range of pH 2.15−8 (**Table 1**).

The disappearance of acrylamide also depended on the heating time and temperature. **Figure 2A** shows the time course of acrylamide disappearance at 120−180 °C. Acrylamide disappearance increased linearly as a function of time at the four assayed temperatures, and this disappearance was faster when a higher temperature was assayed. By employing the disappearance rates obtained from the slopes of the lines in **Figure 2A**, it was possible to determine the activation energy (E_a) of the reaction by means of an Arrhenius plot (**Figure 3A**). The activation energy (E_a) for the disappearance of acrylamide in the presence of butylamine was 44 kJ/mol.

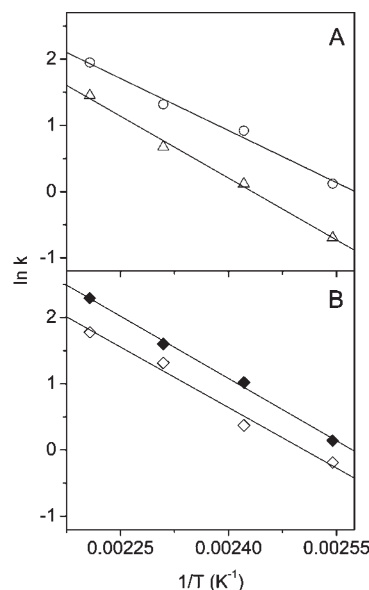

Figure 3. Arrhenius plot for (**A**) acrylamide disappearance in an acrylamide/butylamine (0.2 μmol/20 μmol) model system (○) and acrylamide formation from 3-(butylamino)propanamide (**3**) (△) and (**B**) acrylamide disappearance in both an acrylamide/glycine (0.2 μmol/20 μmol) model system (◇) and an acrylamide/glycine (0.2 μmol/50 μmol) model system (◆).

Acrylamide Formation by Thermal Decomposition of 3-(Butylamino)propionamide (3). Although compound **3** is produced in acrylamide/butylamine reaction mixtures, it is not a stable final reaction product. In fact, it suffers a deamination reaction upon heating to produce acrylamide.

Compound **3** decomposition also depended on the reaction conditions. Thus, the acrylamide produced increased with pH in the range of 2.15−6, when using citrate buffer, but it was less pH dependent with phosphate buffer in the range of 6−8 (**Table 1**). In addition, acrylamide formation increased as a function of time and temperature (**Figure 2B**). From these reaction rates it was also possible to determine the activation energy (E_a) of the process by means of an Arrhenius plot (**Figure 3A**). The activation energy of compound **3** decomposition was 52 kJ/mol.

Acrylamide Disappearance in Acrylamide/Glycine Reaction Mixtures. When butylamine was substituted by glycine in acrylamide reaction mixtures, the above reactions were also produced and both the disappearance of acrylamide in the presence of glycine and the dependence of this disappearance on the reaction conditions were observed. In addition, slight differences were observed between glycine and butylamine. Thus, for example, the amount of acrylamide loss was dependent on the amount of glycine present, but butylamine seemed to be slightly more reactive (**Figure 1**). Acrylamide content decreased linearly (r = −0.993, p = 0.00076) in the presence of glycine in the range of 0−15 μmol of the amino acid, and higher amounts of glycine did not produce further losses of the amide.

Acrylamide disappearance in the presence of glycine exhibited some changes as a function of pH (**Table 1**). Thus, the amount of disappeared acrylamide increased as a function of pH when using citrate buffer, and no clear effect was observed at pH 6−8.

Acrylamide also disappeared linearly as a function of time and temperature. **Figure 4A** shows the time course of acrylamide disappearance when 0.2 μmol of acrylamide reacted with 20 μmol of glycine at the different assayed temperatures (120−180 °C). When 50 μmol of glycine was employed, reaction rates were slightly higher (**Figure 4B**). However, the same activation energies

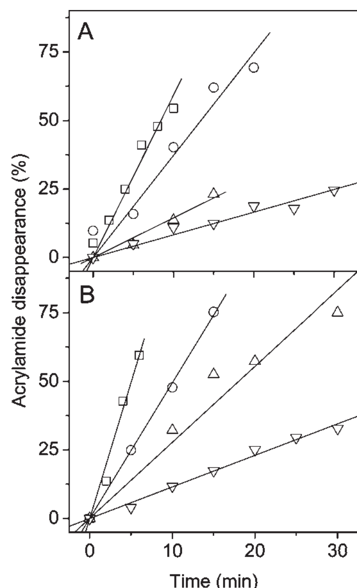

Figure 4. Effect of time and temperature on the acrylamide determined after heating (**A**) an acrylamide/glycine (0.2 μmol/20 μmol) model system or (**B**) an acrylamide/glycine (0.2 μmol/50 μmol) model system at 180 (□), 160 (○), 140 (△), and 120 (▽) °C and pH 7.

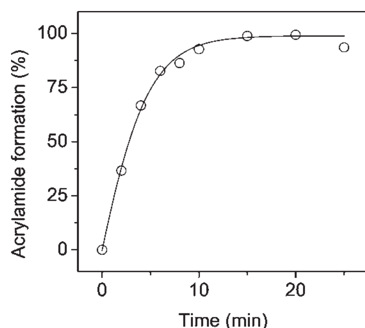

Figure 5. Time course of acrylamide formation in the thermal decomposition of 2-(bis(3-amino-2-oxopropyl)amino)acetic acid (**4**) at pH 7 and 180 °C.

for both reactions were determined by means of an Arrhenius plot (**Figure 3B**). As observed in this last figure, two lines were obtained, but the slopes were identical. The activation energy (E_a) found for the disappearance of acrylamide in the presence of glycine was 52 kJ/mol, which is slightly higher than that above-described for butylamine/acrylamide mixtures.

Acrylamide Formation by Thermal Decomposition of 2-(Bis(3-amino-2-oxopropyl)amino)acetic Acid (4). Analogously to the Michael adduct formed between butylamine and acrylamide, the Michael adduct produced between glycine and acrylamide should also suffer an elimination upon heating. Although this Michael adduct (formed by reaction of one molecule of glycine and one molecule of acrylamide) could not be prepared, the thermal heating of an analogous Michael adduct (formed by reaction of one molecule of glycine and two molecules of acrylamide) produced acrylamide very rapidly to a high extent (**Figure 5**).

Comparative Reactivity of Butylamine, Glycine, and Lysine for Acrylamide Removal. When 0.2 μmol of acrylamide was heated in the presence of 20 μmol of butylamine, glycine, or lysine, only slight differences in the reactivity of the different amino compounds were observed (**Figure 6**). Glycine exhibited the lowest reactivity, and lysine was the most reactive. The reactivity of butylamine was somewhat intermediate between them. At short heating times, butylamine reactivity was quite similar to that of lysine. However, at long heating times, it was close to that of glycine. These differences might be related to both a higher reactivity of butylamine and the presence of two amino groups in the molecule of lysine.

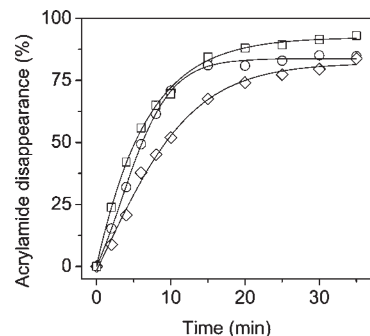

Figure 6. Time course of acrylamide disappearance in a model system containing 0.2 μmol of acrylamide and 20 μmol of butylamine (○), glycine (◇), or lysine (□) at pH 7 and 180 °C.

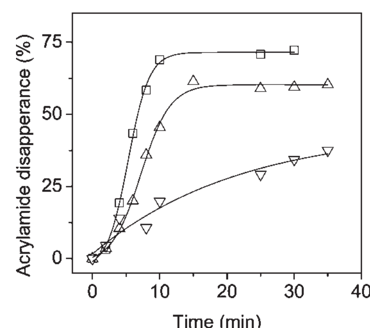

Figure 7. Time course of acrylamide disappearance in a model system containing 0.2 μmol of acrylamide and 2 mg of lysine (□), a polylysine of MW 4200 (△), or a polylysine of MW 68300 (▽) at pH 7 and 180 °C.

Table 2. Effect of Storage at 37 or 60 °C and Reheating at 180 °C on Acrylamide Disappearance[a]

	storage at 37 °C		storage at 60 °C	
time (days)	non-reheated	reheated	non-reheated	reheated
0	0.0 ± 2.8 b	77.8 ± 1.1 b	0.0 ± 2.8 b	77.8 ± 1.1 b
7	51.5 ± 3.2 c	nd	85.3 ± 1.6 c	nd
14	77.1 ± 4.5 d	nd	97.6 ± 0.1 d	nd
21	81.6 ± 3.4 d	nd	99.0 ± 0.1 d	nd
28	87.6 ± 3.8 d	90.8 ± 3.5 c	99.3 ± 0.1 d	89.4 ± 3.2 c

[a] Values are acrylamide disappearance (%) in a model system containing 0.2 μmol of acrylamide and 20 μmol of glycine. Values are mean ± SD for, at least, two independent experiments. Means with different letters in the same column are significantly different ($p < 0.05$). nd, not determined

Comparative Reactivity of Lysine and Polylysines for Acrylamide Removal. In addition to amines and amino acids, amino groups of proteins are also able to take part in acrylamide removal. However, to avoid the competition of amino groups with other functional groups, polylysines were employed in these studies. **Figure 7** shows the time course of the disappearance of 0.2 μmol of acrylamide in the presence of 2 mg of lysine or polylysines at 180 °C. As observed in the figure, reactivity was inversely proportional to the molecular weight. The most reactive was lysine, followed by the polylysine with the lower molecular weight, and the polylysine with the higher molecular weight was the least reactive. This is likely related to the number of

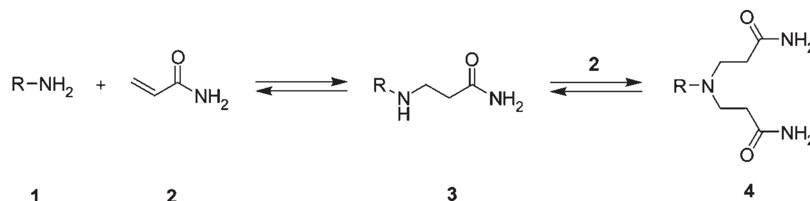

Figure 8. Chemical addition of amino compounds (**1**) to acrylamide (**2**) to produce the corresponding Michael adduct (**3**). A further addition of this Michael adduct to a new molecule of acrylamide would produce the adduct **4**. The elimination of adducts **3** and **4** produces acrylamide. The compounds prepared in this study were 3-(butylamino)propanamide (**3** with R = butyl) and 2-(bis(3-amino-2-oxopropyl)amino)acetic acid (**4** with R = CH_2COOH).

amino groups (the higher molecular weight, the smaller number of terminal amino groups) and also to the accessibility of amino groups (the higher molecular weight of the protein, the lesser accessibility of amino groups to acrylamide). In addition, there was a linear relationship ($r = 0.989$, $p = 0.095$) between the amount of acrylamide recovered at the end of the heating period and the natural logarithm of the molecular weight of the amino compound added.

Effect of Storage at Low/Moderate Temperatures on the Acrylamide Content of Acrylamide/Glycine Reaction Mixtures. The disappearance of acrylamide in acrylamide/glycine reaction mixtures was observed not only at high temperatures but also at low or moderate temperatures such as 37 or 60 °C. **Table 2** shows the acrylamide disappearance at 37 °C. Acrylamide disappeared as a function of the heating time. After 28 days, 88% of the initial acrylamide had disappeared. However, when the unheated samples and the samples heated for 28 days at 37 °C were heated for 20 min at 180 °C, the acrylamide contents from both samples were 22 and 9% of the initial acrylamide content, respectively.

When the same acrylamide/glycine mixtures were heated at 60 °C the results were slightly different (**Table 2**). After 14 days, samples contained negligible amounts of acrylamide. However, when the unheated samples and the samples heated for 28 days at 60 °C were heated for 20 min at 180 °C, the acrylamide recovered from both samples was 22 and 11% of the initial acrylamide content, respectively.

DISCUSSION

The above results confirm that acrylamide reacts with nucleophilic amino groups on amino acid side chains. However, this reaction is not as simple as generally accepted. **Figure 8** shows the different reactions involved. Thus, acrylamide (**2**) reacted very rapidly and easily with amino compounds (**1**) to produce the corresponding Michael adduct. In this study, the Michael adduct between acrylamide and butylamine was prepared and identified in these reactions (compound **3**). Because this Michael adduct still has a nucleophilic group (NH), it might be added to another molecule of acrylamide to produce the new adduct **4**. However, this last addition did not take place under the conditions employed in this study, in which acrylamide content was much lower than amino compound content. In addition, it is also not expected to be produced in foods because acrylamide will usually be produced to a much lower extent than the content of amino compounds. Although the Michael adduct **3** is produced very easily, it is not stable, and its thermal decomposition via an elimination reaction is also produced very easily (the reaction mechanism of the elimination of aminopropionamide and alkylaminopropionamides was previously described by Zamora et al., see ref *20*). This reaction, which also takes place from the adduct involving two molecules of the amino compound, as observed in the thermal decomposition of compound **4**, produces acrylamide.

Therefore, the data obtained in this study suggest that the reaction between acrylamide and amino compounds is reversible. However, activation energies (E_a) of one reaction and its reverse are not the same. In fact, acrylamide seems to be converted into its Michael adduct with a lower activation energy than the elimination reaction of the Michael adduct. Thus, when butylamine was employed, the activation energy of the first reaction was 44 kJ/mol and the activation energy of its reverse was 52 kJ/mol. For this reason, when acrylamide was stored in the presence of glycine at 60 °C (**Table 2**), acrylamide disappeared after 14 days. However, when samples heated for 28 days at 60 °C were heated again for 20 min at 180 °C, the equilibrium was reestablished and a significant amount of acrylamide was detected in samples in which acrylamide was previously absent. In fact, when both unheated samples and samples heated for 28 days at 37 or 60 °C were heated for 20 min at 180 °C, all assayed samples showed very similar amounts of acrylamide contents, therefore suggesting that these last conditions are sufficient to reestablish the equilibrium. In addition, these results also point out that although acrylamide cannot be determined in a food, it might be still present as a Michael adduct, which would be able to produce acrylamide by a simple procedure, such as a reheating.

Although samples previously stored, or not, at low temperatures and then heated at high temperature showed very similar amounts of acrylamide contents, this content was not exactly the same. In fact, the content of previously stored samples was lower. This difference in acrylamide content might be a consequence of the destruction of acrylamide during storage, which should take place by other reaction mechanisms, such as oxidation or polymerization. Different from the formation of the Michael adduct, these acrylamide losses are real because acrylamide cannot be produced again from these reaction products. Nevertheless, under the reaction conditions employed in this study, these other reactions contributed to only ~10% of acrylamide losses observed in samples stored for 1 month at either 37 or 60 °C.

All of these results suggest that amino compounds may play a significant role in the changes observed in acrylamide content in foods upon storage. In addition, they also point to *N*-alkylaminopropionamides as possible compounds in which acrylamide might be potentially hidden.

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