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Degradation of the Amadori Compound *N*-(1-Deoxy-D-fructos-1-yl)glycine in Aqueous Model Systems

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The fate of the Amadori compound *N*-(1-deoxy-D-fructos-1-yl)glycine (DFG) was studied in aqueous model systems as a function of time and pH. The samples were reacted at 90 °C for up to 7 h while maintaining the pH constant at 5, 6, 7, or 8. Special attention was paid to the effect of phosphate on the formation of glycine and the parent sugars glucose and mannose, as well as formic and acetic acid. These compounds and DFG were quantified by high-performance anion-exchange chromatography. The rate of DFG degradation increased with pH. Addition of phosphate accelerated this reaction, particularly at pH 5−7. The rate of glycine formation increased with pH in both the absence and presence of phosphate. High glycine concentrations (60−70 mol %) were obtained, preferably at pH 6−8 with phosphate. However, the yield of glycine formed from DFG decreased at the advanced reaction stage for all pH values studied, both in water and in phosphate buffer. The rate of parent sugar formation increased from pH 5 to pH 7 in the absence of phosphate, leading to glucose and mannose in a constant ratio of 7:3. Addition of phosphate accelerated this reaction, yielding up to 18% parent sugars, most likely formed by reverse Amadori rearrangement. The formation rate of acetic and formic acid increased with increasing pH. The sum of both acids attained 76 mol %. However, the acetic acid concentrations were much higher than those of formic acid.

KEYWORDS: Maillard reaction; reverse Amadori rearrangement; Amadori compound; *N*-(1-deoxy-D-fructos-1-yl)glycine; anion-exchange chromatography

INTRODUCTION

Amadori compounds are N-substituted 1-amino-1-deoxyketoses, **V** (**Figure 1**), representing an important class of Maillard intermediates (*1*). They are formed in the initial phase of the Maillard reaction by Amadori rearrangement of the corresponding *N*-glycosylamines **II** (*2*), the latter obtained by condensation of amino acids and aldoses such as glucose (**I**) as shown in pathway A. The importance of Amadori compounds stems from the fact that their formation as well as decomposition can be initiated under physiological conditions and during food processing and storage. Thus, the formation of Amadori compounds represents a pathway requiring less energy for sugar degradation as compared to caramelization. The chemistry of Amadori compounds has recently been reviewed, including analysis, synthesis, and physical and spectrometric properties (*3*, *4*).

One of the important characteristics of Amadori compounds is their tendency to easily undergo enolization (**Figure 1**). Degradation of the Amadori compound **V** by 1,2-enolization (pathway B) and 2,3-enolization (pathway D) leads to the formation of 3-deoxy-2-hexosulose (**VII**) and 1-deoxy-2,3-hexodiulose (**XII**), respectively, as already suggested by Hodge (*5*). In parallel to these pathways, other α-dicarbonyls can be formed by enolization. For example, transition-metal-catalyzed oxidation of 1,2-enaminol **IV** can lead via pathway C to osones such as glucosone (**IX**) (*6*, *7*). Pathway E gives rise to the 1-amino-1,4-dideoxy-2,3-diulose (**XIII**) by elimination of the C4-OH group of the 2,3-enediol **VI** (*8*). If the iminoketone **VIII** formed by oxidation of 1,2-enaminol **IV** is not hydrolyzed, then Strecker aldehydes can be formed in the course of pathway C by direct oxidative degradation of the Amadori compound and decarboxylation of **X**, as recently proposed by Hofmann and Schieberle (*9*).

Other mechanisms of degradation of Amadori compounds involve retro-aldol cleavage of further aminated Amadori compounds yielding diimine derivatives of methylglyoxal (*10*) and dehydration of Amadori compounds in the cyclic form (*11*). Besides the above-mentioned reactions, Amadori compounds can also act as nucleophiles and react with another sugar molecule to form diketosyl derivatives (*1*).

Despite significant effort in the past decades, many questions still persist concerning the degradation of Amadori compounds. Among others, the reversibility of Amadori rearrangement and formation of carboxylic acids at early stages of the Maillard reaction need further clarification. The formation of parent sugars from the corresponding Amadori compound has been reported under physiological conditions (*12*, *13*) and more

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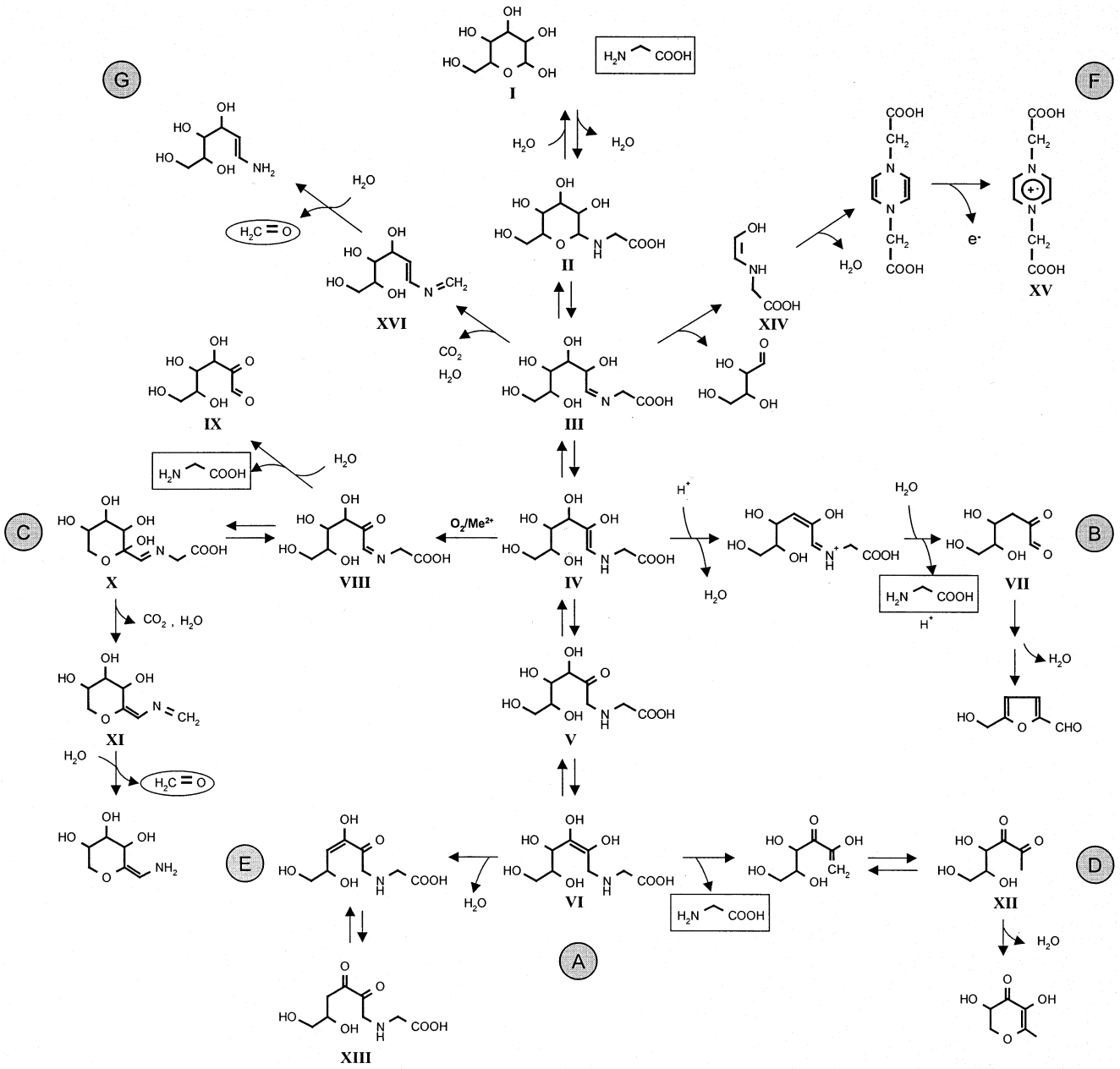

Figure 1. Reaction of glucose (**I**) and glycine leading to the Amadori compound *N*-(1-deoxy-D-fructos-1-yl)glycine (**V**, open-chain form) and related degradation reactions.

recently under pyrolysis conditions (*14*). However, no information is available about the yields of parent sugars formed under food processing conditions or about the effect of reaction conditions on the yields.

Generation of high levels of acetic and formic acid at early stages of the degradation of protein-bound Amadori compounds has recently been reported by Brands and van Boekel (*15*). C_1-C_2 cleavage of 3-deoxy-2-hexosulose (**VII**) and C_2-C_3 cleavage of 1-deoxy-2,3-hexodiulose (**XII**) were proposed as mechanisms leading to the formation of formic acid and acetic acid, respectively. However, as no labeling experiments were performed and neither 2-deoxypentose (remaining C5 fragment of **VII**) nor erythrose (remaining C4 fragment of **XII**) were detected, further experiments are necessary to confirm the proposed mechanisms.

The aim of our study was to gain more insight into the fate of the Amadori compound *N*-(1-deoxy-D-fructos-1-yl)glycine (DFG) in aqueous model systems. Special attention was paid to the effect of pH and phosphate on the formation of the parent sugars glucose and mannose as well as acetic and formic acid.

MATERIALS AND METHODS

Chemicals. The following compounds were obtained commercially: glycine, D-mannose, sodium acetate (Merck, Darmstadt, Germany); D-glucose, acetic acid, and formic acid (Sigma-Aldrich, Steinheim, Germany); maltitol (Fluka, Buchs, Switzerland); sodium hydroxide (NaOH), 46−48% solution (Fisher Scientific, Pittsburgh, PA); sodium hydrogen carbonate (Prolabo, Paris, France). DFG was prepared from D-glucose and glycine as reported by Staempfli et al. (*16*). The solutions and eluents were prepared using ultrapure deionized water (specific resistance ≥18.2 MΩ·cm) from a Milli-Q system (Millipore, Bedford, MA). NaOH solutions used as eluents were prepared by diluting a carbonate-free 46−48% (w/w) NaOH solution in water previously degassed with helium gas.

Model Reactions. Solutions of DFG (15 mmol) in water (150 mL) or in a phosphate buffer (150 mL, 100 mmol/L) were heated at 90 °C for 7 h. Experiments were performed at constant pH (5, 6, 7, or 8),

Table 1. Integrated Amperometry Waveform

time (s)	electrode potential (V)	comment
0	–0.1	
0.2	–0.1	start of signal integration
0.3	0.35	
0.4	0.35	
0.5	–0.1	
0.7	–0.1	stop of signal integration
0.71	0.9	
0.9	0.9	
0.91	–0.9	
1.0	–0.9	

measured at 90 °C, using a pH-stat device (Metrohm, Herisau, Switzerland) filled with a NaOH solution (3 mol/L) and equipped with an electrode permitting measurements at 90 °C. Aliquots of the reaction mixture were taken at regular time intervals and analyzed by high-performance anion-exchange chromatography (HPAEC) and UV/vis spectroscopy.

Quantification of DFG, D-Glucose, D-Mannose, and Glycine. An aliquot of the reaction mixture (1 mL) was diluted 1000 times with deionized water, filtered through a PVDF filter (polyvinylidene fluoride, 0.22 μm/25 mm), and analyzed by HPAEC. The analyses were performed using the DX-500 system from Dionex (Sunnyvale, CA) composed of an autosampler (model AS-50 with a 50 μL sample loop), a gradient pump (model EG-40) with on-line degas, and an electrochemical detector (model ED-40). The separation was accomplished on a 250 × 4 mm i.d. CarboPac PA1 anion-exchange column (Dionex) and a 50 × 4 mm i.d. CarboPac PA1 guard column (Dionex) using the following gradient: starting with a mixture (0/96/4, v/v/v) of sodium acetate (300 mmol/L), water, and NaOH (300 mmol/L), the sodium acetate was first increased to 3% (3/93/4, v/v/v) in 1 min and kept isocratic for 28 min and then increased to 80% (80/16/4, v/v/v) in 5 min and kept isocratic for 5 min. Each analytical cycle was followed by cleaning and regeneration of the column with NaOH (300 mmol/L) for 15 min and equilibration of the column with the initial gradient condition for 10 min. The flow rate was kept constant at 1 mL/min throughout the program. D-Glucose (RT 18.07 min), D-mannose (RT 19.47 min), glycine (RT 27.35 min), DFG (RT 36.53 min), and maltitol (internal standard, RT 11.48 min) were quantified with an electrochemical detector working in the integrated amperometry mode and equipped with a gold working electrode. The waveform used is shown in **Table 1**. All compounds were quantified on the basis of calibration curves by comparing the peak areas with those of standard solutions containing known amounts of pure compounds. All samples were analyzed in duplicate.

Quantification of Acetic and Formic Acid. An aliquot of the reaction mixture (1 mL) was diluted 50 times with deionized water and analyzed by HPAEC. The analyses were performed using a DX-120 Dionex system (Dionex Corp., Sunnyvale, CA) equipped with an autosampler (model AS-40), a 25 μL sample loop, and a conductivity detector. The anion self-regenerating suppressor (ASRS-Ultra, 4 mm) was working in the autosuppression recycle mode. The isocratic separation was accomplished on a 250 × 4 mm i.d. IonPac AS-14 anion-exchange column (Dionex) and a 50 × 4 mm i.d. IonPac AG-14 guard column (Dionex) using sodium hydrogen carbonate (2.5 mmol/L) as the mobile phase at a flow rate of 1.2 mL/min. Acetic acid (RT 11.07 min) and formic acid (RT 11.81 min) were quantified on the basis of calibration curves by comparing the peak areas with those of standard solutions containing known amounts of pure compounds. All samples were analyzed in duplicate.

Browning of Reaction Mixtures. UV/vis spectrometry measurements were performed on a Uvikon UV-942 (Kontron Instruments, Milan, Italy). The absorbance of the 50 times diluted reaction mixtures was measured at $\lambda = 420$ nm in a 10 mm cell.

RESULTS AND DISCUSSION

The effect of pH and phosphate on the decomposition of *N*-(1-deoxy-D-fructos-1-yl)glycine (DFG) was studied at 90 °C using the same initial concentration of DFG (0.1 mol/L). All experiments were carried out at constant pH (5, 6, 7, or 8) to obtain comparable data between experiments performed in water and in phosphate buffer. Quantification of DFG was achieved by HPEAC coupled with an electrochemical detector working in integrated amperometry mode. This method, developed in our laboratories, will be published elsewhere in detail. It permits quantification of not only DFG but also its parent compounds (glucose, mannose, and glycine) in the same analytical run.

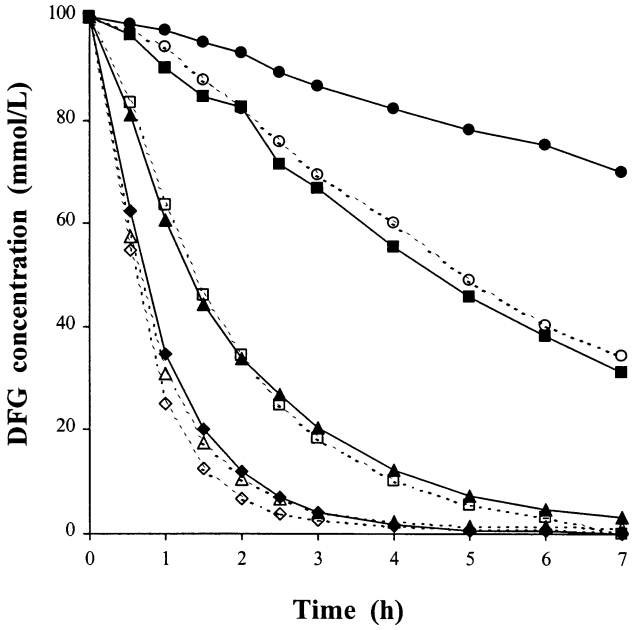

Figure 2. Thermal degradation of *N*-(1-deoxy-D-fructos-1-yl)glycine (DFG) in water (—) and in phosphate buffer (•••) at pH 5 (●), pH 6 (■), pH 7 (▲), and pH 8 (◆). The coefficient of variation was lower than 10% for concentrations above 1.5 mmol/L.

Degradation of *N*-(1-Deoxy-D-fructos-1-yl)glycine (DFG). The rate of DFG degradation in water was relatively slow at pH 5 (**Figure 2**). After 7 h of heating at 90 °C, about 70% of DFG remained unreacted. Increasing the pH considerably favored the degradation rate of DFG. While 6 h was required to degrade 25% of DFG at pH 5, only 135, 40, and 20 min were needed to reach the same degree of degradation at pH 6, 7, and 8, respectively.

A similar trend was observed for the effect of pH on the degradation of DFG in the presence of phosphate buffer. However, the rate of DFG degradation increased with pH, particularly in the pH range 5−7, and there was only a small increase between pH 7 and pH 8. Interestingly, the addition of an equimolar amount of phosphate showed a similar effect on the decomposition of DFG as increasing the pH of the unbuffered solution by one unit. These findings are in agreement with data reported in the literature (*17*). The effect of phosphate on the reaction of glucose with glycine was described as a general base catalysis with an optimum in the pH range between 5 and 7. Dihydrogen phosphate ions, which act as a base abstracting a proton during the Amadori rearrangement, were found to be the principal catalytic species leading to enhanced degradation of Amadori compounds.

Formation of Glycine. The rate of glycine formation increased with pH in both the absence and presence of phosphate. However, there was almost no difference between the rate of formation of glycine at pH 7 and 8 in the presence of phosphate (**Figure 3**). This is in line with the data shown in **Figure 2** as glycine is liberated during the decomposition of

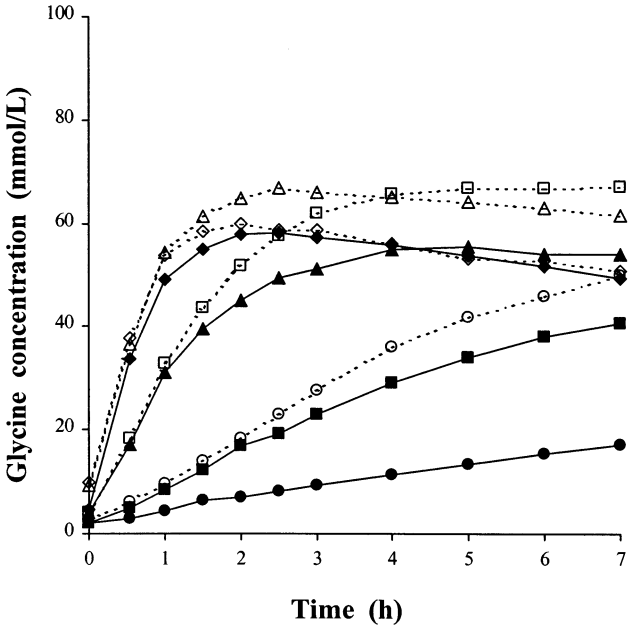

Figure 3. Formation of glycine during thermal degradation of *N*-(1-deoxy-D-fructos-1-yl)glycine in water (—) and in phosphate buffer (···) at pH 5 (●), pH 6 (■), pH 7 (▲), and pH 8 (◆). The coefficient of variation was lower than 10% for concentrations above 2.5 mmol/L.

DFG. Formation of glycine from DFG mainly occurs by 1,2-enolization (pathway B) and 2,3-enolization (pathway C), yielding 3-deoxy-2-hexosulose (**VII**) and 1-deoxy-2,3-hexodiulose (**XII**), respectively (**Figure 1**). In theory, 1 mol of DFG should yield 1 mol of glycine. However, the yield of glycine was generally lower, especially for the advanced reaction stages. This indicates that part of the liberated glycine reacted further with other compounds present in the reaction mixture (e.g., α-dicarbonyls and α-hydroxycarbonyls). These reactions may include Strecker degradation and formation of imidazolium compounds (*18*) and 1,4-dialkylpyrazinium radicals (*7*) as well as incorporation of glycine into melanoidins.

However, glycine can also be degraded by mechanisms that do not include glycine liberation from DFG. For example, the mechanism reported by Namiki and co-workers (*19*−*21*) involves cleavage of the sugar moiety of Schiff base **III** (**Figure 1**, pathway F), leading to a C2 fragment (**XIV**, glycolaldehyde imine in the enol form), which upon dimerization and oxidation forms a 1,4-dialkylpyrazinium radical (**XV**). Alternatively, the Schiff base **III** can also be decarboxylated, resulting in **XVI**, which hydrolyzes to form a Strecker aldehyde as shown in pathway G (*22*). Another mechanism (*9*) involves direct degradation of the Amadori compound in the presence of oxygen via oxidation of enaminol **IV** to iminoketone **VIII**, which is stabilized by cyclization (**X**). Decarboxylation and dehydration lead to **XI**, resulting in the Strecker aldehyde upon hydrolysis (**Figure 1**, pathway C).

To obtain more insight into the importance of glycine degradation under different reaction conditions, the amount of glycine generated per 1 mol of DFG decomposed was calculated for five reaction stages (**Table 2**), i.e., at the beginning of the reaction when only 5% of DFG was decomposed and at the time when 25%, 50%, 75%, and 95% of DFG was decomposed. The data set is incomplete for some reaction stages, as the reaction at pH 5 or 6 did not proceed far enough under the conditions chosen (90 °C, 7 h). The yield of glycine formed from DFG decreased at the advanced reaction stage for all pH values studied both in water and in phosphate buffer. This is not surprising as new compounds, such as α-dicarbonyls and α-hydroxycarbonyls, are formed with progressing reaction time and glycine is more importantly involved in reactions such as Strecker degradation.

Table 2. Amount of Glycine Generated from *N*-(1-Deoxy-D-fructos-1-yl)glycine (DFG) at Different Reaction Stages

amount of DFG (%) decomposed	amount of glycine (mol/mol of DFG) generated in water				amount of glycine (mol/mol of DFG) generated in phosphate buffer			
	pH 5	pH 6	pH 7	pH 8	pH 5	pH 6	pH 7	pH 8
5	0.74	0.74	0.80	0.84	0.88	0.88	0.80	0.78
25	0.54	0.62	0.77	0.84	0.86	0.85	0.81	0.79
50		0.46	0.69	0.77	0.79	0.78	0.79	0.77
75			0.64	0.68		0.74	0.74	0.69
95			0.55	0.59		0.62	0.68	0.60

Similarly, the yield of glycine decreased as the pH value of the phosphate buffer increased. These data are coherent with the fact that increasing pH favors retro-aldol reactions, which leads to the formation of short-chain α-dicarbonyls or α-hydroxycarbonyls such as glyoxal, methylglyoxal, and glycol aldehyde (*7*, *19*). These compounds are known to be highly reactive in Strecker degradation of amino acids (*23*, *24*) and also in other reactions such as the formation of 1,4-dialkylpyrazinium radicals (*7*). Similarly to the observation that the formation of carboxymethyllysine increased with phosphate concentration and pH (*13*), the formation of carboxymethylglycine (CMG) is another reaction that could cause decrease in yield of glycine with increasing pH. However, this reaction does not seem to be significant under our reaction conditions as enhanced formation of CMG should lead to lower yields of glycine in phosphate buffer as compared to water. However, opposite results were found as shown in **Table 2**.

Contrary to the data obtained in the presence of phosphate buffer, the yield of glycine in water increased with increasing pH (**Table 2**). For example, when 25% of DFG was reacted, the yield of glycine reached 84% at pH 8 but only 54% at pH 5. These results are surprising, as the opposite trend would be expected as explained above. Comparison of the yields obtained in the presence and in the absence of phosphate buffer indicates that phosphate slowed the degradation of glycine at lower pH values (mainly pH 5 and 6). These results could indicate that reactions such as decarboxylation of the Schiff base **III** and subsequent hydrolysis gain in importance in the absence of phosphate (**Figure 1**, pathway G). The relative importance of this reaction should decrease at higher pH values as the Schiff base formation is less favored. If phosphate decreases the relative importance of pathway G, then the increase of the yield of glycine in the presence of phosphate should be more visible at lower pH values, which was observed in this study. However, more experiments are necessary to validate these hypotheses.

Formation of Glucose and Mannose. The formation of glucose and mannose was studied in parallel to the degradation of DFG. The ratio of glucose to mannose was 7:3 and was stable under different reaction conditions (**Table 3**). Therefore, only the sums of glucose and mannose concentrations are presented. In the absence of phosphate, the formation rate of these sugars increased with pH in the pH range 5−7 (**Figure 4**). Further increase to pH 8 led to lower concentrations of glucose and mannose as compared to pH 7. This could be explained by the lower stability of the sugars formed. However, a decrease in the formation of glucose and mannose from DFG at pH 8 cannot be excluded. The data also illustrate that addition of phosphate

Table 3. Ratio of Glucose to Mannose Generated from *N*-(1-Deoxy-D-fructos-1-yl)glycine (DFG) under Different Reaction Conditions

	glucose:mannose ratio	
pH value	in water	in phosphate buffer
5	67:33	69:31
6	67:33	68:32
7	69:31	69:31
8	69:31	71:29

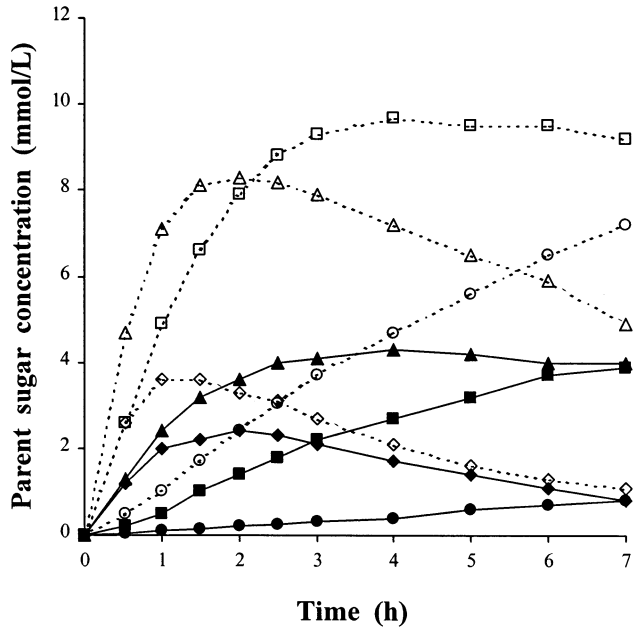

Figure 4. Formation of glucose and mannose during the thermal degradation of *N*-(1-deoxy-D-fructos-1-yl)glycine in water (—) and in phosphate buffer (•••) at pH 5 (●), pH 6 (■), pH 7 (▲), and pH 8 (◆). The curves represent the sum of glucose and mannose concentrations. The coefficient of variation was lower than 10% for concentrations above 1.5 mmol/L.

increases the formation rate of glucose and mannose at all pH values studied.

To gain more insight into the importance of glucose and mannose formation under different reaction conditions, the yields of these sugars per mole of DFG were calculated as shown in **Table 4**. In water, the highest yields of glucose and mannose were observed at pH 6 and 7, reaching up to 7%. However, much higher yields were obtained in phosphate buffer, indicating that phosphate favors the formation of glucose and mannose from DFG, particularly at pH 5. At this pH, the yields were 13−18%, about four to six times higher than in water. The data also indicate that high yields were obtained already at very early stages of the reaction.

Theoretically, the formation of glucose and mannose from DFG can be explained by several pathways: (i) reverse Amadori rearrangement (**Figure 1**, pathway A), (ii) aldol-type condensations of sugar fragments, and (iii) reduction of glucosone (**IX**) by reducing substances such as reductones and aminoreductones. The formation of parent carbonyls from Amadori compounds under physiological conditions has been attributed to reverse Amadori rearrangement (*12*, *13*). However, the other mechanisms cannot be excluded as no labeling experiments were performed. Thermal decomposition of the Amadori compound *N*-(deoxy-D-fructos-1-yl)proline in *N*,*N*-dimethylformamide (130 °C, 20 min) resulted in the parent sugars glucose and mannose,

Table 4. Amount of Glucose and Mannose Generated from *N*-(1-Deoxy-D-fructos-1-yl)glycine (DFG) at Different Reaction Stages

amount of DFG (%) decomposed	glucose and mannose (mol/mol of DFG) generated in water				glucose and mannose (mol/mol of DFG) generated in phosphate buffer			
	pH 5	pH 6	pH 7	pH 8	pH 5	pH 6	pH 7	pH 8
5	0.03	0.06	0.07	0.03	0.18	0.15	0.09	0.05
25	0.03	0.06	0.06	0.03	0.13	0.12	0.11	0.06
50		0.07	0.06	0.03	0.11	0.13	0.11	0.06
75			0.05	0.03		0.12	0.1	0.05
95			0.04	0.02		0.1	0.08	0.03

amount of DFG (%) decomposed	acetic acid (mol/mol of DFG) generated in water				acetic acid (mol/mol of DFG) generated in phosphate buffer			
	pH 5	pH 6	pH 7	pH 8	pH 5	pH 6	pH 7	pH 8
5	0	0.13	0.36	0.60	0	0.24	0.40	0.55
25	0.05	0.24	0.39	0.60	0.06	0.27	0.43	0.54
50		0.42	0.42	0.60	0.10	0.32	0.46	0.54
75			0.48	0.58		0.37	0.53	0.59
95			0.51	0.64		0.46	0.59	0.63

amount of DFG (%) decomposed	formic acid (mol/mol of DFG) generated in water				formic acid (mol/mol of DFG) generated in phosphate buffer			
	pH 5	pH 6	pH 7	pH 8	pH 5	pH 6	pH 7	pH 8
5	0	0.05	0.03	0.06	0	0	0.02	0.08
25	0.03	0.07	0.04	0.06	0	0.01	0.03	0.09
50		0.09	0.05	0.07	0.01	0.04	0.05	0.09
75			0.07	0.08		0.06	0.10	0.10
95				0.11		0.1	0.14	0.13

which has also been explained by reverse Amadori rearrangement (*14*). The formation of parent sugars by aldol-type condensations was excluded by labeling experiments. However, reduction of glucosone has not been considered as a mechanism leading to the parent sugars, and the design of labeling experiments does not exclude this possibility.

The results shown in this study represent for the first time quantitative data concerning the formation of parent sugars from an Amadori compound under conditions relevant to food processing (aqueous system, cooking temperature). Similarly, the effect of pH and phosphate on the yield of glucose and mannose has not been reported previously. Although the high yields of parent sugars found already at the early stage of DGF degradation support the hypothesis of reverse Amadori rearrangement under our experimental conditions, additional experiments are currently being performed to substantiate this hypothesis. Irrespective of the formation mechanism, our data clearly demonstrate that rather significant amounts of the parent sugars can be regenerated at the early stages of the Maillard reaction under conditions relevant to food processing.

Formation of Acids. The concentrations of acetic and formic acid were monitored by HPAEC to verify their formation from DFG. The rate of formation of both acids increased with increasing pH over the whole pH range studied (**Figure 5**). However, in the presence of phosphate there was only a small difference between the formation rate at pH 7 and 8. Thus, the effect of pH was similar on both the formation of these acids and on the decomposition of DFG. Phosphate accelerated the formation of acetic acid in the pH range studied, as it promoted the degradation of DFG. However, this effect was most pronounced at pH 5−7. Phosphate also accelerated the formation

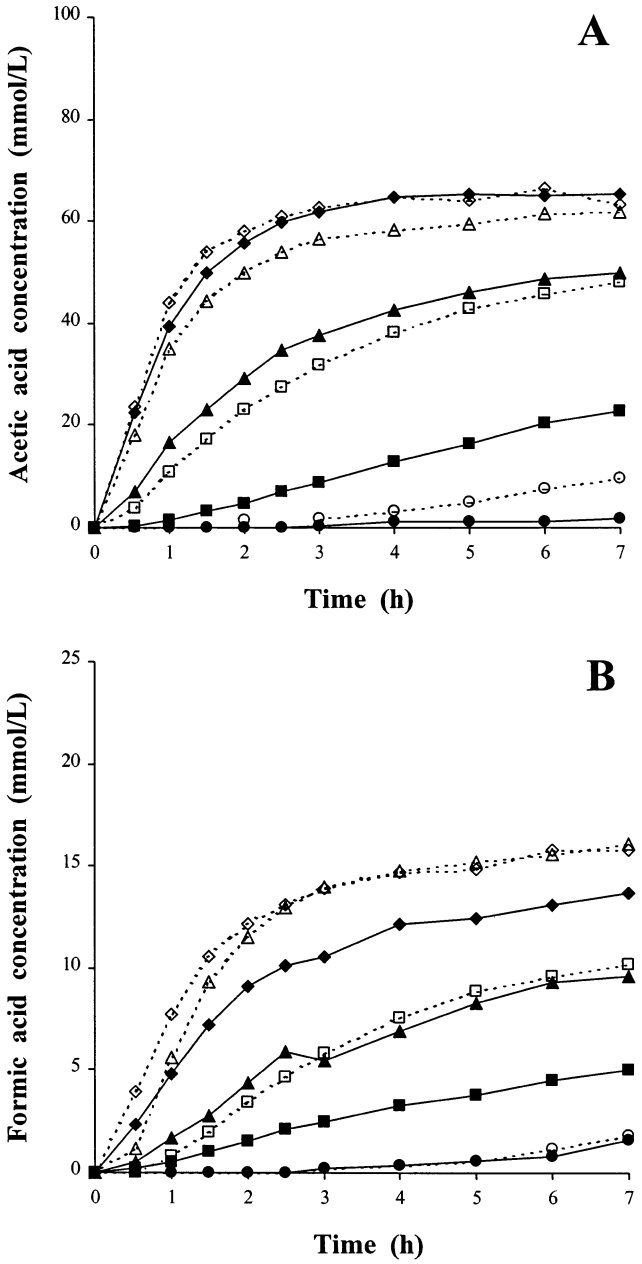

Figure 5. Formation of (A) acetic acid and (B) formic acid during the thermal degradation *N*-(1-deoxy-D-fructos-1-yl)glycine in water (—) and in phosphate buffer (···) at pH 5 (●), pH 6 (■), pH 7 (▲), and pH 8 (◆). The coefficient of variation was lower than 10% for concentrations above 3 mmol/L.

of formic acid, but only in the pH range 6−8. With the exception of pH 8, the total amounts of acetic and formic acid were generally lower than the amounts of NaOH added to keep pH constant (**Table 5**). Hence other acids had to be formed. On the other hand, the amounts of acetic and formic acid at pH 8 were higher than the amounts of NaOH added. This means that part of the acids was neutralized by compounds created during the reaction.

The yields of both acids were calculated at different reaction stages to gain more insight into the formation of acetic and formic acid under different reaction conditions. The yields of acetic acid increased with increasing pH and with increasing degree of DFG degradation in both water and phosphate buffer (**Table 4**). The same trend was observed for the yield of formic acid in the presence of phosphate buffer (**Table 4**). Interestingly,

Table 5. Comparison of Amounts of Acetic and Formic Acid Generated with Amounts of NaOH Added To Keep the pH Value Constant

reaction time (h)	sum of acetic and formic acid (mmol) reaction in water				NaOH added (mmol) reaction in water			
	pH 5	pH 6	pH 7	pH 8	pH 5	pH 6	pH 7	pH 8
1	0.0	0.3	2.7	6.6	0.1	1.2	4.3	5.0
2	0.0	0.9	5.0	9.7	0.2	2.5	6.8	7.8
3	0.1	1.7	6.5	10.9	0.4	3.2	8.5	10.1
4	0.2	2.4	7.4	11.5	0.5	4.1	9.8	11.1
5	0.3	3.0	8.1	11.7	0.7	5.0	10.8	11.9
6	0.3	3.7	8.7	11.7	0.9	5.8	11.5	12.5
7	0.5	4.1	8.9	11.8	1.1	6.5	12.0	13.1

reaction time (h)	sum of acetic and formic acid (mmol) reaction in phosphate buffer				NaOH added (mmol) reaction in phosphate buffer			
	pH 5	pH 6	pH 7	pH 8	pH 5	pH 6	pH 7[a]	pH 8
1	0.0	1.7	6.1	7.8	0.6	4.1		5.6
2	0.2	4.0	9.2	10.5	1.2	6.5		8.6
3	0.3	5.7	10.6	11.5	2.0	8.4		9.9
4	0.5	6.9	10.9	11.9	2.6	9.7		10.6
5	0.9	7.8	11.2	11.8	3.5	11.2		10.6
6	1.3	8.3	11.5	12.4	4.2	11.6		10.6
7	1.7	8.7	11.7	11.9	4.2	12.1		10.6

[a] Data not available.

the yield of formic acid in water increased only with increasing DFG degradation, but pH had almost no effect.

The yield of acetic acid was much higher than that of formic acid. The sum of the yields of both acids reached up to 0.76 mol/mol of DFG, which indicates that fragmentation of DFG was considerable. These high yields are in line with literature data (*15*, *25*). The degradation of protein-bound Amadori compound (0.43 mmol/L, 120 °C, pH 6.7) yielded more than 0.22 mmol/L acetic acid and 0.15 mmol/L formic acid. The authors explained the formation of formic acid by a C_1-C_2 cleavage of 3-deoxy-2-hexosulose. A C_2-C_3 cleavage of 1-deoxy-2,3-hexodiulose and a C_1-C_2 cleavage of methylglyoxal were proposed to explain the formation of acetic acid. Our data on acetic acid fit well the proposed mechanism as the yield of acetic acid increased with increasing pH. This is coherent with the fact that sugar fragmentation as well as 2,3-enolization is favored at higher pH values. Moreover, the formation of acetic acid was very rapid at the beginning of the reaction. However, it almost stopped when no more DFG was present in the reaction mixture. This implies direct involvement of DFG or its early degradation product in the formation of acetic acid.

In contrast, the formation of formic acid by the proposed mechanism is more difficult to explain. The yield of formic acid either was not affected by pH (reaction in water) or was even enhanced at higher pH values (reaction in phosphate buffer). However, the formation of 3-deoxy-2-hexosulose, the proposed precursor of formic acid (*15*), via 1,2-enolization should be favored at lower pH values. This discrepancy could be explained by the favored fragmentation at higher pH values. Thus, although less 3-deoxy-2-hexosulose is formed at higher pH values, it is more efficiently fragmented, resulting finally in the same or even higher amounts of formic acid as compared to lower pH values.

However, the proposed mechanism does not explain why the ratio of acetic acid to formic acid decreased as the reaction progressed (**Table 4**). This indicates that, apart from the possible formation by a C_1-C_2 cleavage of 3-deoxy-2-hexosulose, formic

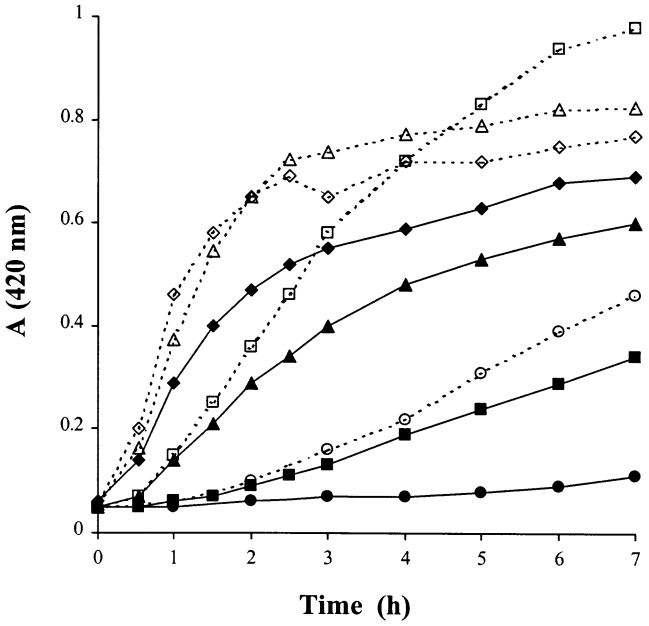

Figure 6. Color development during the thermal degradation of *N*-(1-deoxy-D-fructos-1-yl)glycine in water (—) and in phosphate buffer (•••) at pH 5 (●), pH 6 (■), pH 7 (▲), and pH 8 (◆).

acid is formed by additional pathways. These pathways, active in advanced reaction stages, could include further fragmentation of intermediates arising from the sugar molecule but also Cannizzaro reaction of formaldehyde, the Strecker aldehyde of glycine, or formation of formic acid by Strecker-type reaction (*24*). To clarify the formation mechanism of formic acid, further experiments using labeled compounds are necessary.

Development of Browning. The rate of browning of unbuffered DFG solutions increased with pH over the whole pH range studied (**Figure 6**). In the presence of phosphate, the browning rate also increased with pH, but only between pH 5 and pH 7. These data are well in line with the data obtained by Westphal et al. (*26*). These authors reported acceleration of the browning of fructosylalanine (100 °C) in phosphate buffer at pH 7 as compared to pH 5, but no further acceleration was observed at pH 8. The presence of phosphate accelerated the browning of DFG over the whole pH range studied. However, at pH 8 the effect of phosphate was lower than at pH 5−7.

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LITERATURE CITED

(1) Ledl, F.; Schleicher, E. New aspects of the Maillard reaction in foods and in the human body. *Angew. Chem.* **1990**, *29*, 565−594.

(2) Hodge, J. E. The Amadori rearrangement. *Adv. Carbohydr. Res.* **1955**, *10*, 169−205.

(3) Yaylayan, V. A.; Huyghues-Despointes A. Chemistry of Amadori rearrangement products: analysis, synthesis, kinetics, reactions and spectroscopic properties. *Crit. Rev. Food Sci. Nutr.* **1994**, *34*, 321−369.

(4) Wrodnigg, T. M.; Eder, B. The Amadori and Heyns rearrangements: Landmarks in the history of carbohydrate chemistry or unrecognized synthetic opportunities? *Top. Curr. Chem.* **2001**, *215*, 115−152.

(5) Hodge, J. E. Dehydrated foods: Chemistry of browning reactions in model systems. *J. Agric. Food Chem.* **1953**, *1*, 928−943.

(6) Kawakishi, S.; Ushida, K. Autoxidation of Amadori compounds in the presence of copper ion and its effect on the oxidative damage of protein. In *Maillard reaction in food processing, human nutrition and physiology*; Finot, P. A., Aeschbacher, H. U., Hurrel, H. U., Liardon, R., Eds.; Birkhäuser: Basel, 1990; pp 475−480.

(7) Hofmann, T.; Bors, W.; Stettmaier, K. Studies on radical intermediates in the early stage of the nonenzymatic browning reaction of carbohydrates and amino acids. *J. Agric. Food Chem.* **1999**, *47*, 379−390.

(8) Hubert, B.; Ledl, F. Formation of 1-amino-1,4-dideoxy-2,3-hexodiuloses and 2-aminoacetylfurans in the Maillard reaction. *Carbohydr. Res.* **1990**, *204*, 215−220.

(9) Hofmann, T.; Schieberle, P. Formation of aroma active Strecker-aldehydes by a direct oxidative degradation of Amadori compounds. *J. Agric. Food Chem.* **2000**, *48*, 4301−4305.

(10) Hayashi, T.; Namiki, M. Role of sugar fragmentation in the Maillard reaction. In *Amino carbonyl reactions in foods and biological systems*; Fujimaki, M., Namiki, M., Kato, H., Eds.; Elsevier: Amsterdam, 1986; pp 29−38.

(11) Yaylayan, V. A.; Sporn, P. Novel mechanism for the decomposition of 1-(amino acid)-1-deoxy-D-fructoses (Amadori compounds): A mass spectrometric approach. *Food Chem.* **1987**, *26*, 283−305.

(12) Acharya, A. S.; Sussman, L. G. The reversibility of ketosamine linkages of aldose with proteins. *J. Biol. Chem.* **1984**, *259*, 4372−4378.

(13) Ahmed, M. U.; Thrope, S. R.; Bayens, J. W. Identification of N^{ϵ}-carboxymethyllysine as degradation product of fructoselysine in glycated protein. *J. Biol. Chem.* **1986**, *261*, 4889−4894.

(14) Yaylayan, V. A.; Huyghues-Despointes, A. Identification of per-*O*-(trimethylsilyl) derivatives of aldoses generated from thermal decomposition of *N*-(1-deoxy-D-fructopyranos-1-yl)proline: Reversibility of the Amadori rearrangement. *Carbohydr. Res.* **1996**, *286*, 179−187.

(15) Brands, C. M. J.; van Boekel, M. A. J. S. Reaction of monosaccharides during heating of sugar-casein system: Building of reaction network model. *J. Agric. Food Chem.* **2001**, *49*, 4667−4675.

(16) Staempfli, A. A.; Blank, I.; Fumeaux, R.; Fay, L. B. Study on the decomposition of the Amadori compound *N*-(1-deoxy-D-fructos-1-yl)-glycine in model systems: Quantification by fast atom bombardment tandem mass spectrometry. *Biol. Mass Spectrom.* **1994**, *23*, 642−646.

(17) Potman, R. P.; van Wijk T. A. Mechanistic studies of the Maillard reaction with emphasis on phosphate-mediated catalysis. In *Thermal Generation of Aromas*; Parliment, T. H., McGorrin, R. J., Ho, C.-T., Eds.; ACS Symposium Series 409; American Chemical Society: Washington, DC, 1989; pp 182−195.

(18) Velisek, J.; Davidek, T.; Davidek, J.; Trska, P.; Kvasnicka, F.; Velcova, K. New imidazoles in nonenzymatic browning reactions. *J. Food Sci.* **1989**, *54*, 1544−1646.

(19) Hayashi, T.; Namiki, M. Formation of two-carbon sugar fragment at an early stage of the browning of sugar with amine. *Agric. Biol. Chem.* **1980**, *44*, 2575−2580.

(20) Namiki, M.; Hayashi, T. Formation of novel free radical products in early stage of Maillard reaction. *Prog. Food Nutr. Sci.* **1981**, *5*, 81−91.

(21) Namiki, M.; Hayashi, T. A new mechanism of the Maillard reaction involving sugar fragmentation and free radical formation. In *The Maillard reaction in foods and nutrition*; Waller, G. R., Feather, M. S., Eds.; ACS Symposium Series 215; American Chemical Society: Washington, DC, 1983; pp 21−46.

(22) Cremer, D. R.; Vollenbroeker, M.; Eichner, K. Investigation of the formation of Strecker aldehydes from the reaction of Amadori rearrangement products with α-amino acids in low moisture model system. *Eur. Food Res. Technol.* **2000**, *211*, 400−403.

(23) Cremer, D. R.; Eichner, E. The influence of pH value on the formation of Strecker aldehydes in low moisture model systems and plant powders. *Eur. Food Res. Technol.* **2000**, *211*, 247−251.

(24) Hofmann, T.; Münch, P.; Schieberle, P. Quantitative model studies on the formation of Aroma active aldehydes and acids by Strecker-type reactions. *J. Agric. Food Chem.* **2000**, *48*, 434−440.

(25) van Boekel, M. A. J. S.; Brands, C. M. J. Heating of sugar casein solutions: Isomerisation and Maillard reaction. In *The Maillard reaction in food and medicine*; O'Brien, J., Nursten, H. E., Crabbe, M. J. C., Ames, J. M., Eds.; The Royal Society of Chemistry: Cambridge, U.K., 1998; pp 154−159.

(26) Westphal, G.; Kroh, L.; Föllmer, U. Studies of the Maillard reaction. Part 16. Reactivity of Amadori compounds dependent on the reaction medium. *Food (Nahrung)* **1988**, *32*, 117−120 (in German; *Chem. Abstr*. **1988**, *109*, 127413m).

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