Elucidation of the Mechanism of Pyrazinone Formation in Glycine Model Systems Using Labeled Sugars and Amino Acids

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Model studies using D-[^{13}C]glucoses and a series of C_2, C_3, and C_4 dicarbonyl compounds with labeled [^{15}N/^{13}C]glycines have indicated that methyl-substituted pyrazines and pyrazinones formed in these model systems have a common intermediate. The labeling studies have also helped to identify a new chemical transformation of α-dicarbonyls, affected by the amino acid, that lead to the addition of the C-2 atom of the amino acid to the α-dicarbonyl compounds, instead of the amino group as in the case of the Strecker-type interaction between the two reactants. Thus, glyoxal and pyruvaldehyde can be transformed into pyruvaldehyde and 2,3-butanedione, respectively, by the amino acid. Two pathways of formation of pyrazinones were distinguished on the basis of the labeling experiments, one involving the reaction of 3 mol of glycine and the other the interaction of the dipeptide glycylglycine with an α-dicarbonyl compound.

Keywords: *Amadori; decomposition mechanisms; Maillard reaction; ^{13}C-labeled glucose; [^{15}N]- and [^{13}C]-glycine; pyrazinones; pyrazines; Py/GC/MS*

INTRODUCTION

The role of glycine in the Maillard reaction as a flavor precursor has been investigated by many researchers. Rizzi (1972) showed the possible mechanism for the formation of pyrazines from glycine and alanine with diketones. Olsson et al. (1978) gave a detailed review and illustration for the possible formation pathways of various flavor compounds generated from the reaction between glycine and D-glucose in slightly acidic, aqueous solutions. The flavor compounds were comprised of furans, pyrroles, pyridines, phenols, carboxylic acids, and lactones. The formation of the majority of the nitrogen compounds was rationalized by a reaction scheme based on the dehydration of the D-glucose with or without Strecker degradation of the glycine. Chuyen et al. (1973) identified a series of 2-(3′-alkyl-2′-oxopyrazin-1′-yl)alkanoic acids in the equimolar reaction mixture of various dipeptides including glycine with glyoxal, heated at 100 °C. Oh et al. (1992) detected the decarboxylated analogs of the above alkanoic acids [alkyl-2(1*H*)-pyrazinones] in the equimolar reaction mixture of various glycine peptides and D-glucose heated at 180 °C and classified them as peptide-specific Maillard reaction products. The present paper explores the mechanism of formation of pyrazinones by pyrolysis coupled with gas chromatography/mass spectrometry (Py/GC/MS) from ^{13}C-enriched glucoses and ^{15}N/^{13}C-enriched glycines. Py/GC/MS has been demonstrated to be a fast and convenient technique for the analysis of Maillard reaction products (Huyghues-Despointes et al., 1994; Keyhani and Yaylayan, 1996), especially those arising from isotopically enriched compounds for mechanistic studies (Huyghues-Despointes and Yaylayan, 1996). Huyghues-Despointes et al. (1994) demonstrated that Py/GC/MS can be used to identify primary and secondary pyrolysis products of the proline Amadori compound with minimum sample preparation and analysis time and that the pyrolysis of the proline Amadori compound in the quartz tube for 20 s at 250 °C is comparable to autoclaving of proline/glucose mixture at 150 °C for 1.5 h in water. Quartz tube Py/GC/MS is specially suited to perform small scale reactions without the need to isolate or extract the reaction mixture, which may lead to the loss of valuable isotopically labeled products. The use of ^{13}C- and ^{15}N-enriched compounds for the elucidation of reaction mechanisms concerning the Maillard reaction has been well documented (Tressl et al., 1993; Heang et al., 1994; Amrani-Hemaimi, 1995).

MATERIALS AND METHODS

All reagents, chemicals, and D-[1-^{13}C]glucose, D-[2-^{13}C]-glucose, D-[6-^{13}C]glucose were purchased from Aldrich Chemical Co. (Milwaukee, WI). D-[3-^{13}C]glucose, D-[4-^{13}C]glucose, D-[5-^{13}C]glucose, [^{15}N]glycine, [1-^{13}C]glycine, [2-^{13}C]glycine, and [1,2-^{13}C]glycine were purchased from ICON Services Inc. (Summit, NJ). The synthesis of Amadori glycine was performed according to the method of Sosnovsky et al. (1993).

Py/GC/MS Analysis. A Hewlett-Packard GC/mass selective detector (5890 GC/5971B MSD) interfaced to a CDS pyroprobe 2000 unit was used for the Py/GC/MS analysis. Solid samples (1−4 mg) of glycine/glucose in different ratios were introduced inside a quartz tube (0.3 mm thickness) and plugged with quartz wool and inserted inside the coil probe. The pyroprobe was set at the desired temperature (250 °C) at a heating rate of 50 °C/ms and with a total heating time (THT) of 20 s. The pyroprobe interface temperature was set at 250 °C. The GC column flow rate was 0.8 mL/min for a split ratio of 92:1 and a septum purge of 3 mL/min. Capillary direct MS interface temperature was 180 °C; ion source temperature was 280 °C. The ionization voltage was 70 eV, and the electron multiplier was 1682 V. The mass range analyzed was 30−300 amu. The column was a fused silica DB-5 column (30 m length × 0.25 mm i.d. × 25 μm film thickness; Supelco, Inc.). Unless otherwise specified, the column initial temperature was −5 °C for 3 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. Products that were not found in the mass spectral libraries were identified by comparison with literature mass spectral data or by generating the products from their proposed

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Table 1. Mass Spectrometric Data

pyrazinone	*m/z* (relative intensity)
1,5-dimethyl-2(1*H*)-pyrazinone[a]	124 (80), 95 (100), 81 (22), 68 (34), 54 (19), 42 (47), 41 (46), 39 (42)
1,5-dimethyl-2(1*H*)-pyrazinone	124 (80), 95 (100), 81 (22), 68 (39), 54 (22), 42 (45), 41 (12), 39 (18)
1,6-dimethyl-2(1*H*-)pyrazinone[a]	124 (95), 95 (100), 81 (14), 68 (27), 56 (48), 42 (31), 39 (31)
1,6-dimethyl-2(1*H*)-pyrazinone	124 (98), 95 (100), 81 (15), 68 (30), 56 (56), 42 (29), 39 (25)
1,5,6-trimethyl-2(1*H*)-pyrazinone[a]	138 (75), 109 (100), 95 (33), 82 (8), 68 (20), 56 (42), 42 (30)
1,5,6-trimethyl-2(1*H*)-pyrazinone	138 (80), 109 (100), 95 (42), 82 (8), 68 (22), 56 (43), 42 (28)

[a] Oh et al. (1992).

precursors and comparing mass spectra and chromatographic retention times.

RESULTS AND DISCUSSION

The reactions of various dipeptides, including glycine, with glyoxal at 100 °C have yielded a series of 2-(3′-alkyl-2′-oxopyrazin-1′-yl)alkanoic acids (Chuyen et al., 1973) that have been characterized by different spectroscopic techniques. Oh et al. (1992) obtained alkyl-2(1*H*)pyrazinones, the decarboxylated analogs of the alkanoic acids, when glycine dipeptide was reacted with pyruvaldehyde or D-glucose at a higher temperature (180 °C). The authors referred to alkyl-2(1*H*)pyrazinones as peptide-specific Maillard reaction products, since in an equimolar mixture of D-glucose and glycine, pyrazinones were not detected. In the course of our study of different Amadori products and mixtures of D-glucose and amino acids by Py/GC/MS, it was found that trace amounts of dimethyl-2(1*H*)-pyrazinones and 1,5,6-trimethyl-2(1*H*)-pyrazinone were formed from the glycine Amadori product and from glycine/D-glucose equimolar mixtures when pyrolyzed at 250 °C for 20 s. The efficiency of formation of these compounds increased dramatically when an equimolar amount of glycine was added to the glycine Amadori compound or when a 3- or 2-fold excess of glycine was reacted with D-glucose. Table 1 shows the mass spectrometric data of pyrazinones. To confirm the origin of the carbon atoms incorporated into the pyrazinone structures and to elucidate the role of excess glycine in the formation of pyrazinones, D-[^{13}C]glucoses labeled at 1-^{13}C, 2-^{13}C, 3-^{13}C, 4-^{13}C, 5-^{13}C, and 6-^{13}C positions were systematically reacted with glycine. In addition, [1-^{13}C]-, [2-^{13}C]-, [1,2-^{13}C]-, and [^{15}N]glycines were also reacted with D-glucose and the glycine Amadori compound to confirm the incorporation of carbon and nitrogen atoms originating from glycine in the pyrazinone structure. The distribution of the isotope labeling was calculated from the intensities of the parent ions. The results were corrected to account for the natural ^{13}C content of the corresponding unlabeled reference compounds. Tables 2−5 summarize the results obtained. Table 2 indicates that dimethylpyrazinones (1:4 mixture of 1,5- to 1,6-dimethylpyrazinones) mostly incorporate two carbon units originating from glucose: C1−C2 (∼30%), C2−C3 (∼10%), and C4−C5 (∼60%). The reactive two-carbon sugar fragment could be either glyoxal (Chuyen et al., 1973) or glycoladehyde. Inspection of Table 3, on the other hand, reveals that trimethylpyrazinone is predominantly formed by the incorporation of three carbon units from glucose: C1−C2−C3 (∼25%) and C4−C5−C6 (∼75%). It has already been demonstrated that pyruvaldehyde reacts with glycylglycine to produce both di- and trimethylpyrazinones (Oh et al., 1992). The authors speculated that pyruvaldehyde is converted into 2,3-butanedione through a free radical mechanism; however, as we demonstrate below, this conversion is effected by glycine through aldol-type condensation.

Table 2. Percent Label Distribution in 1,6- and 1,5-Dimethylpyrazinone Mixture (4:1) Formed from Labeled D-Glucoses and Glycines[a]

model	M	M + 1	M + 2	M + 3	M + 4
glycine/glucose	100	0	0	0	0
glycine/[1-^{13}C]glucose	68	30	2	0	0
glycine/[2-^{13}C]glucose	68	31	1	0	0
glycine/[3-^{13}C]glucose	91	9	0	0	0
glycine/[4-^{13}C]glucose	88	12	0	0	0
glycine/[5-^{13}C]glucose	42	58	0	0	0
glycine/[6-^{13}C]glucose	38	57	4	0	0
[^{15}N]glycine/glucose	0	0	100	0	0
[1-^{13}C]glycine/glucose	0	98	2	0	0
[2-^{13}C]glycine/glucose[b]	0	0	30	70	0
[2-^{13}C]glycine/glucose	0	0	40	60	0
[2-^{13}C]glycine/glucose[c]	15	14	35	36	0
[1,2-^{13}C]glycine/glucose	0	0	0	30	70

[a] Ratio of amino acid/sugar (3:1) unless otherwise specified. [b] Ratio (2:1). [c] Includes 10% (w/w) of the unlabeled dipeptide glycylglycine.

Table 3. Percent Label Distribution in 1,5,6-Trimethylpyrazinone Formed from Labeled D-Glucoses and Glycines

model	M	M + 1	M + 2	M + 3	M + 4
glycine/glucose	100	0	0	0	0
glycine/[1-^{13}C]glucose	77	23	0	0	0
glycine/[2-^{13}C]glucose	77	23	0	0	0
glycine/[3-^{13}C]glucose	70	30	0	0	0
glycine/[4-^{13}C]glucose	27	73	0	0	0
glycine/[5-^{13}C]glucose	27	73	0	0	0
glycine/[6-^{13}C]glucose	26	74	0	0	0
[^{15}N]glycine/glucose	0	0	100	0	0
[1-^{13}C]glycine/glucose	0	98	2	0	0
[2-^{13}C]glycine/glucose[b]	0	0	20	80	0
[2-^{13}C]glycine/glucose	0	0	30	70	0
[2-^{13}C]glycine/glucose[c]	2	10	38	50	0
[1,2-^{13}C]glycine/glucose	0	0	0	20	80

[a] Ratio of amino acid to sugar (3:1) unless otherwise specified. [b] Ratio 2:1. [c] Includes 10% (w/w) of the unlabeled dipeptide glycylglycine.

Previous studies (Huyghues-Despointes and Yaylayan, 1996) have indicated that pyruvaldehyde can be produced by the interaction of glyceraldehyde with amino acids and that glyceraldehyde itself can be generated by retro-aldol reaction of Amadori compounds. To verify whether glyoxal, glycoladehyde, and glyceraldehyde produce pyrazinones when they interact with glycine, model systems containing unlabeled and [2-^{13}C]glycines were analyzed by Py/GC/MS, and the results are given in Table 4. The data in this table indicate that indeed pyrazinones can be produced by the two- and three-carbon sugar fragments in the presence of free glycine. In addition, the model studies have indicated that both glyoxal and glycoladehyde can be converted into a three-carbon reactive unit (pyruvaldehyde) by the incorporation of C-2 of glycine through an aldol condensation reaction as described below and outlined in Scheme 1.

Pyrazines and Pyrazinones from Glyoxal Model Systems. To investigate the mechanistic relationship

Table 4. Percent Distribution of Unlabeled and Labeled Pyrazinones Formed from Glycine and Glycoladehyde, Glyoxal, or Glyceraldehyde Mixtures[a]

model	M	M + 1	M + 2	M + 3	M + 4	M + 5
[^{13}C-2]glycine + glycoladehyde						
dimethylpyrazinone	0	2	3	95	0	0
trimethylpyrazinone	0	2	29	48	17	4
[^{13}C-2]glycine + glyoxal						
dimethylpyrazinone	0	1	10	89	0	0
trimethylpyrazinone	0	0	3	21	73	2
[^{13}C-2]glycine + glyceraldehyde						
dimethylpyrazinone	0	0	0	0	0	0
trimethylpyrazinone	0	0	14	82	4	0

[a] Ratio of amino acid to dicarbonyl compounds (3:1).

Table 5. Percent Label Distribution in Dimethylpyrazinones[a] and Selected[b] Pyrazines Formed from D-Glucose and [2-^{13}C]Glycine

compound	M	M + 1	M + 2	M + 3
1,5-dimethylpyrazinone	0	0	94	6
1,6-dimethylpyrazinone	0	0	40	60
methylpyrazine	90	10		
2,5-dimethylpyrazine	25	70	5	

[a] The isomers were separated using the following temperature programing of the GC column: 40−260 °C at 2 °C/min and held at 260 °C for 40 min. [b] Methyl and 2,5-dimethylpyrazines are formed by condensation of 1-amino-2-propanone, fragment b in Scheme 1 (Strecker degradation product of pyruvaldehyde).

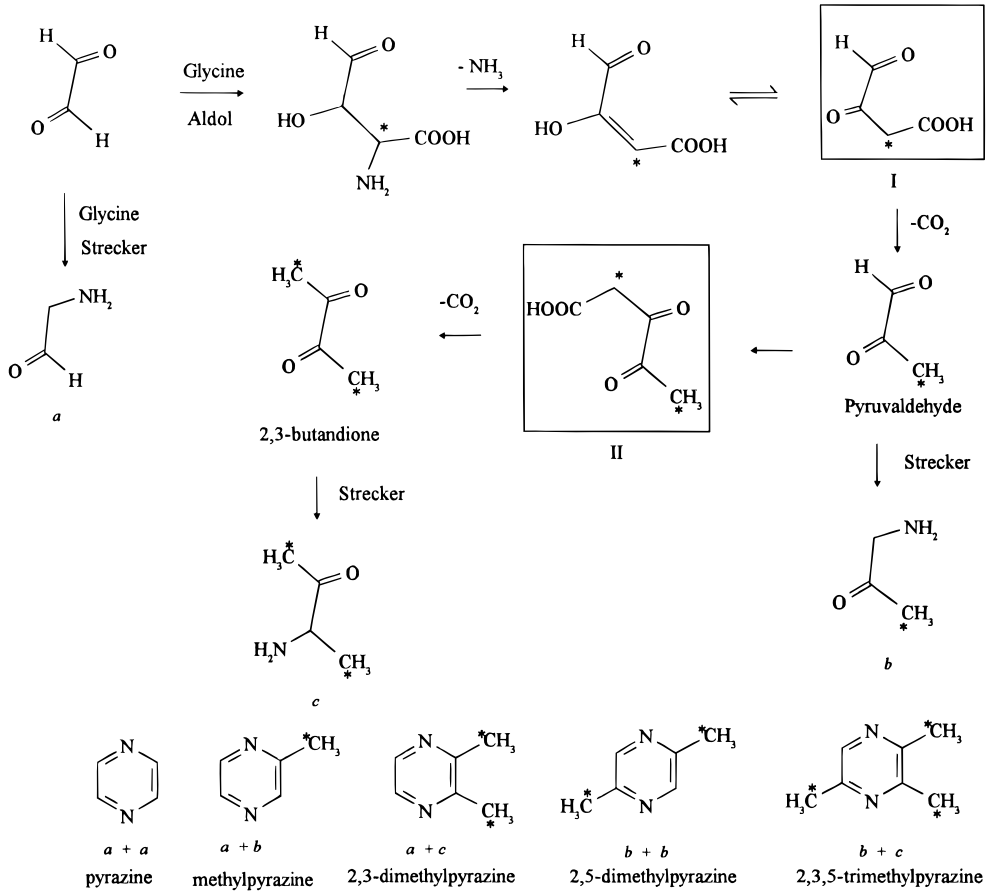
between the structurally related pyrazines and pyrazinones, the model system containing glyoxal/glycine in 1:3 molar ratio was also analyzed for the formation of pyrazines. The system produced the parent pyrazine and pyrrolo[1,2-*a*]pyrazine in addition to five methylated pyrazines: methyl-, 2,3-dimethyl-, 2,5-dimethyl-, and 2,3,5-trimethylpyrazines and a trace amount of tetramethylpyrazine. Analysis of the isotopic distribution of the resulting methylated pyrazines from glyoxal/[2-^{13}C]glycine model system indicated that all of the methyl groups incorporated onto the pyrazine rings arose from the C-2 of glycine (100% incorporation in all methylpyrazine derivatives). Scheme 1 illustrates a plausible mechanism of formation of pyrazines from the glyoxal/glycine model system. According to Scheme 1, glyoxal can undergo two types of interaction with glycine: one is the commonly accepted Strecker degradation that generates α-aminoacetaldehyde (*a* in Scheme 1), and the other is the aldol condensation followed by deamination and formation of intermediate I which decarboxylates to form pyruvaldehyde. Pyruvaldehyde, in turn, can undergo a similar reaction to form 2,3-butanedione, through the intermediate II. Both pyruvaldehyde and 2,3-butanedione were detected in the glyoxal/[2-^{13}C]glycine model systems with the expected label incorporation. The net result of this newly observed transformation is the chain elongation of reactive C_2 and C_3 α-dicarbonyl fragments by one carbon unit originating from C-2 atom of glycine. If the universality of this transformation can be verified with other amino acids, they could be viewed also as alkyl donors during the Maillard reaction. Glyoxal, pyruvalaldehyde, and 2,3-butanedione subsequently can react with glycine through Strecker degradation and produce α-aminocarbonyls (*a*, *b*, and *c* in Scheme 1) which eventually can interact and generate all of the observed alkylpyrazines by condensation as illustrated in Scheme 1. Pyrrolo-[1,2-*a*]pyrazine, on the other hand, incorporated only one ^{13}C-2 atom of glycine, and a plausible mechanism for its formation, starting with intermediate I, is shown in Scheme 2.

Mechanism of Alkyl(1*H*)pyrazinone Formation. From the model and labeling studies it was evident that the main sugar fragments incorporated into the dimethyl- and trimethylpyrazinones were glyoxal and pyruvaldehyde, respectively. Scheme 3 illustrates the mechanism of formation of pyruvaldehyde from the glycine Amadori compound (**1**). According to this scheme, the Amadori compound undergoes a retro-aldol cleavage at C3−C4 to generate compound **2** (from the first three carbon atoms of glucose) and glyceraldehyde (from the last three carbon atoms of glucose), which can react with free glycine by Amadori rearrangement and produce more of compound **2** that subsequently undergoes a *β*-elimination to form pyruvaldehyde and releases free glycine. In effect, this process can convert glycerladehyde into pyruvaldehyde (see Scheme 4) through the catalytic action of amino acid, which was also observed with proline Amadori compound (Huyghues-Despointes and Yaylayan, 1996). Glyoxal, on the other hand, can be formed by similar retro-aldol cleavages of glucosone and 1-deoxyglucosone as illustrated in Scheme 5.

Tables 2 and 3 also indicate that pyrazinones incorporate two nitrogen atoms, one C-1, and three or two C-2 atoms from glycine. These data clearly show that there is more than one pathway of formation of alkylpyrazinones, utilizing either two or three glycine molecules per pyrazinone. The major pathway of formation of pyrazinones incorporates three C-2 atoms of glycine (70% in the case of dimethylpyrazinone mixture and 80% in the case of trimethylpyrazinone) and the minor pathway incorporates two C-2 atoms of glycine (94% in the case of 1,5-dimethylpyrazinone, 40% in the case of 1,6-dimethylpyrazinone, and 20% in the case of trimethylpyrazinone). Schemes 6 illustrates the formation of pyrazinones through both pathways A and B. Pathway B requires pyruvaldehyde to form dimethylpyrazinones and 2,3-butanedione to form trimethylpyrazinone. On the other hand, pathway A requires glyoxal and pyruvaldehyde to generate dimethyl- and trimethylpyrazinones, respectively, and consequently it incorporates three glycine molecules in the pyrazinone structures, whereas in pathway B the number of glycine molecules incorporated depends on whether pyruvaldehyde and 2,3-butanedione originate from Amadori compound or are formed through transformation of glyoxal and pyruvaldehyde by glycine as shown in Scheme 1.

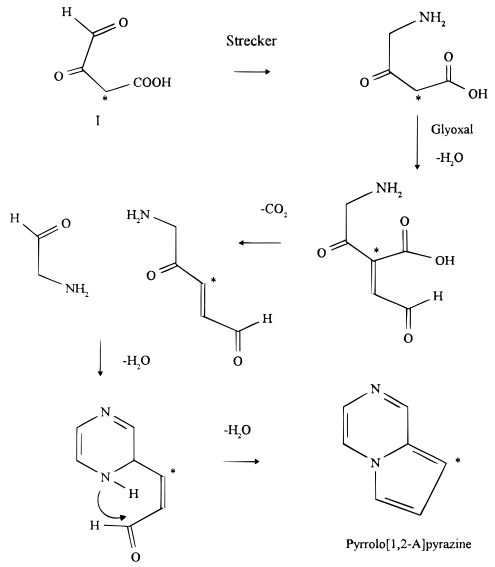
Pyrazinone Formation through Incorporation of Three Glycine Molecules—Pathway A. Intermediates I and II shown in Scheme 1 can be considered as the common mechanistic link between pyrazines and pyrazinones. These intermediates that are formed by aldol condensation of glycine with glyoxal and pyruvaldehyde, respectively, can lead to 1,6-dimethyl- and 1,5,6-trimethylpyrazinones but not to the 1,5-dimethyl isomer, as described in Scheme 6. Intermediates I and II

Scheme 1. Proposed Mechansim of Formation of Methylpyrazines in Glyoxal/Glycine Model System[a]



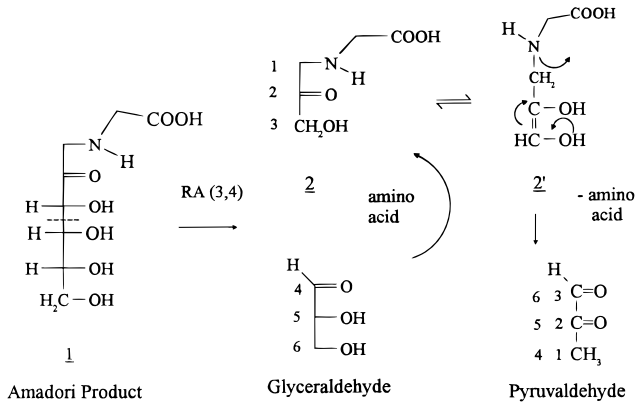
[a] Asterisk indicates C-2 of glycine.

Scheme 2. Proposed Mechansim of Formation of Pyrrolo[1,2-*a*]pyrazine in Glyoxal/Glycine Model System[a]



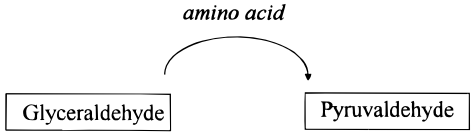
[a] Asterisk indicates C-2 of glycine.

Scheme 3. Proposed Mechanism of Formation of D-Glycerladehyde from Amadori Product and Its Subsequent Transformation into Pyruvaldehyde[a]



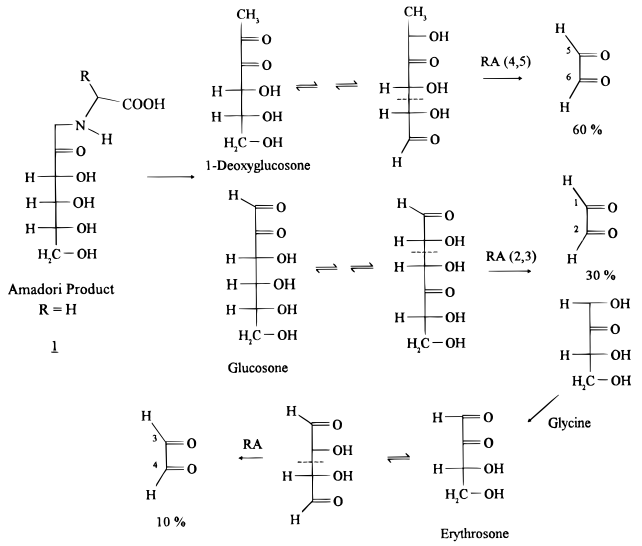
[a] Carbon numbers indicate original D-glucose carbon locations. RA[*x*,*y*] = retro-aldol cleavage at C*x*−C*y*.

Scheme 4. Conversion of D-Glyceraldehyde into Pyruvaldehyde by the Action of Amino Acids



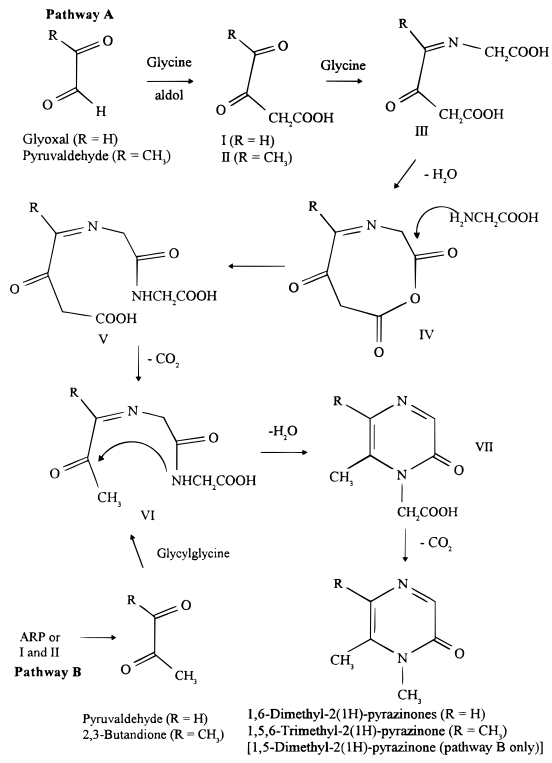
can react with a second mole of glycine to produce the diacid III, which upon dehydration can be converted into the acid anhydride IV containing an activated carbonyl group which reacts with a third mole of glycine to produce V. After decarboxylation and cyclization steps, intermediate V produces 2-(2′-oxopyrazin-1′-yl)alkanoic acids (VII), which have been isolated and identified previously from model systems containing glyoxal and equimolar amounts of glycylglycine (Chuyen et al., 1973). Upon decarboxylation intermediate V produces 1,6-dimethyl- (R = H, glyoxal) and 1,5,6-trimethyl-2(1*H*)-pyrazinone (R = CH_3; pyruvaldehyde). This pathway cannot produce the 1,5-dimethyl pyrazinone isomer.

Scheme 5. Proposed Mechanism of Formation of Glyoxal from Amadori Product[a]



[a] RA[*x*,*y*] = retro-aldol cleavage at C*x*−C*y*.

Scheme 6. Proposed Mechanisms of Formation of Pyrazinones[a]



[a] ARP, Amadori rearrangement product.

Pyrazinone Formation through Incorporation of Glycylglycine—Pathway B. Evidence from labeling experiments indicates that in the mixture of excess glycine with D-glucose, glycine can form the dipeptide glycylglycine and that added glycylglycine (unlabeled) in the reaction mixture of [^{13}C-2]glycine/glucose can hydrolyze to form free glycine as evidenced by observing M and M + 1 pyrazinone species (Tables 2 and 3). The formed dipeptide can eventually react with pyruvaldehyde and 2,3-butanedione to produce pyrazinones according to a previously published mechanism (Chuyen et al., 1973) through the common intermediates VI and VII as shown in Scheme 6. A model system containing 2,3-butadione and glycine produced 1,5,6-trimethyl-2(1*H*)-pyrazinone. In the case of pyruvaldehyde, the amino terminal of the dipeptide can react either with the aldehydo carbonyl or the keto carbonyl of the pyruvaldehyde to produce 1,6- and 1,5-dimethylpyrazinones, respectively. This pathway requires two glycine molecules as a dipeptide to produce pyrazinones, if the reacting dicarbonyls (pyruvaldehyde and 2,3-butanedione) are formed directly from the Amadori product and not through the intermediates I and II. If they are produced through the intermediates I and II (as outlined in Scheme 1) that already incorporate one C-2 of glycine, then the total number of C-2 incorporated into the pyrazinone structures will be three, similar to pathway A. To assess the extent of pyruvaldehyde formation through intermediate I, label incorporation from [2-^{13}C]-glycine into the methylpyrazine and 2,5-dimethylpyrazine identified in [2-^{13}C]glycine/glucose model system was calculated (Table 5) and was found to be 10 and 5%, respectively, which indicates that for every mole of pyruvaldehyde formed through intermediate I, approximately 9 mol is produced through retro-aldol reaction of the Amadori intermediate (Scheme 3).

Table 6. Percent Distribution of Unlabeled and Labeled Pyrazinones Formed from Unlabeled Glycine Amadori Compound and Variously Labeled Free Glycines

model	M	M + 1	M + 2	M + 3
[^{15}N]glycine + ARP (2:1)				
dimethylpyrazinone	18	40	42	
trimethylpyrazinone	10	30	60	
[^{13}C-1]glycine + ARP (1:1)				
dimethylpyrazinone	46	54		
trimethylpyrazinone	23	77		
[^{13}C-2]glycine + ARP (1:1)				
dimethylpyrazinone	30	24	26	20
trimethylpyrazinone	9	10	31	50

Evidence for the Release of Intact Amino Acid from the Amadori Products. 2,3-Enolization of Amadori products followed by β-elimination is known to produce 1-deoxyglucosones and free amino acid. Analysis of model systems containing unlabeled Amadori product and labeled free glycine (see Table 6) can confirm the release of free amino acid from Amadori products, if they can be trapped in a volatile product that can be detected. As was demonstrated above, pyrazinones are formed by incorporation of at least two glycine molecules of which one remains intact as part of the pyrazinone structure, and as such could be used as indicator compound for the presence of free glycine (released from Amadori product) in a model system containing unlabeled Amadori product and labeled free glycine. Inspection of Table 6 shows that mixtures of labeled free glycine and unlabeled Amadori products produce significant amounts of unlabeled pyrazinones, indicating the presence of released amino acid from the Amadori product. In addition, the percent of unlabeled dimethylpyrazinones was relatively higher than that of unlabeled trimethylpyrazinone, which indicates that dimethylpyrazinones are formed at a later stage, on the decomposition time scale of Amadori products, than the trimethylpyrazinones, since higher concentrations of unlabeled glycine released from Amadori product can compete more effectively with the already present labeled free glycine to produce unlabeled pyrazinones.

Conclusion. Carbon chain elongation in Maillard reactions so far has been thought to occur by aldol-type condensations of smaller sugar fragments. This study provides evidence that amino acids could be also involved in such processes by interaction with the aldehydo end of an α-ketoaldehyde and subsequent trans-

formation into an α-diketone. This process is in direct competition with Strecker-type interaction that generates Strecker aldehydes and α-aminocarbonyl compounds.

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