Carbohydrate and Amino Acid Degradation Pathways in L-Methionine/D-[^{13}C] Glucose Model Systems

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Maillard model systems consisting of labeled D-[^{13}C]glucoses, L-[^{15}N]methionine, and L-[methyl-^{13}C]-methionine, have been utilized to identify the amino acid and carbohydrate fragmentation pathways occurring in the model system through Py-GC/MS analysis. The label incorporation analyses have indicated that the carbohydrate moiety produces 1-deoxy- and 3-deoxyglucosones and undergoes C_2/C_4 and C_3/C_3 cleavages to produce glycolaldehyde, tetrose, and C_3-reactive sugar derivatives such as acetol, glyceraldehyde, and pyruvaldehyde. Glycolaldehyde was found to incorporate C-1, C-2 (70%) and C-5, C-6 (30%) glucose carbon fragments, whereas the tetrose moiety incorporates only C-3, C-4, C-5, C-6 glucose carbon atoms. In addition, the major source of reactive C_3 fragments was found to contain C-4, C-5, C-6 sugar moiety. On the other hand, methionine alone also generated Strecker aldehyde as detected by its condensation product with 3-(methylthio)propylamine. Plausible mechanisms were proposed for the formation of the interaction products between sugar and amino acid degradation products on the basis of the label incorporation patterns.

Keywords: *Sugar and amino acid degradation mechanisms; Strecker aldehyde; L-methionine; Maillard reaction; ^{13}C-labeled glucoses; Py-GC/MS*

INTRODUCTION

Sulfur-containing amino acids such as L-methionine play an important role in the generation of characteristic aromas of coffee, fried potato, and processed meat. In addition, L-methionine is known to act as an antioxidant in both heated and unheated foods. This activity could be attributed partly to its ease of oxidation to form L-methionine sulfoxide and partly to the formation of 3-(methylthio)propylamine (decarboxylation product in heated systems) that has the same antioxidant activity as BHA (*1*). Fujimaki et al. (*2*) studied the volatile pyrolysis products of L-methionine alone at 300 °C and identified several amines, aldehydes (including the Strecker aldehyde), and methanethiol. The formation of Strecker aldehyde in the absence of sugars indicates the formation of oxidizing intermediates (such as dimethyl disulfide) from the decomposition of L-methionine, as amino acids are known to undergo oxidative decarboxylation with mild oxidizing agents (*3*) to form Strecker aldehydes. More detailed study of the pyrolysis of L-methionine alone was performed by Shirai and Hosogai (*4*). They also detected the formation of Strecker aldehyde when the pyrolysis was performed at 240 °C. In addition, when the pyrolysate was hydrolyzed with 6N HCl, glycine and alanine were detected in the mixture. The L-methionine/monosaccharide reaction was studied by Tressl et al. (*5*) and Rijke et al. (*6*). Both groups identified 3-(methylthio)propylamine, methional, methanethiol, and acrolein as principal reactive intermediates responsible for the formation of most of the products in the model system. Yu and Ho (*7*), on the other hand, quantified the amount of methional and dimethyl sulfides in L-methionine/D-glucose or L-methionine sulfoxide/D-glucose mixtures and tentatively identified various sulfur-containing heterocyclic compounds. Vernin et al. (*8*) studied the kinetics of formation and thermal degradation of L-methionine Amadori product and identified around 70 degradation products in the decomposition mixture. In the present study, using various ^{13}C-labeled D-glucoses and L-methionines, the mechanisms of carbohydrate and methionine degradation pathways were elucidated using Py-GC/MS as a microreactor. Recent findings (*9*) have indicated that the common products observed between pyrolytic and aqueous-phase reactions show similar label incorporation patterns, indicating the formation of similar sugar and amino acid reactive intermediates in both phases.

MATERIALS AND METHODS

All reagents, chemicals, and D-[1-^{13}C]glucose (99%), D-[2-^{13}C]glucose (99%), and D-[6-^{13}C]glucose were purchased from Aldrich Chemical Co. (Milwaukee, WI). D-[3-^{13}C]glucose (99%), D-[4-^{13}C]glucose 99%), D-[5-^{13}C]glucose (99%), L-[^{15}N]methionine, and L-[methyl-^{13}C]methionine were purchased from Cambridge Isotope Laboratories (Andover, MA). Methionine Amadori product was synthesized based on published procedures (*10*).

Pyrolysis-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 (2.5 mg) of L-methionine, L-methionine Amadori product, L-methionine/D-glucose (labeled or unlabeled), L-methionine/glycolaldehyde (dimer), L-methionine/D-glyceraldehyde, L-methionine/acetol, or L-methionine/pyruvaldehyde mixtures (1:1) were introduced inside a quartz tube (0.3 mm thickness) which was plugged with quartz wool and inserted inside the coil probe. The Pyroprobe was set at 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

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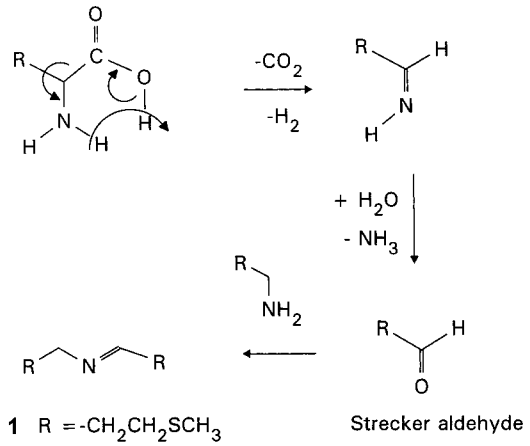

Figure 1. Oxidative decarboxylation of amino acids and formation of Strecker aldehyde in the absence of a carbohydrate source.

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 (60 m length x 0.25 mm i.d. x 25 um 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 270 °C at a rate of 8 °C/min and kept at 270 °C for 5 min. Chemical structures were identified through mass spectral library (NIST/Wiley) searches.

RESULTS AND DISCUSSION

In L-methionine model systems, the "amino acid fragmentation pool" (*11*) plays an important role in the generation of reactive intermediates that leads to the formation of sulfur-containing Maillard reaction products. This could be attributed to the relative stability of the products formed from the degradation of L-methionine. As in all Maillard systems "sugar fragmentation pool" provides the reactive dicarbonyls for the formation of various heterocyclic compounds. To investigate the origin of different Maillard reaction products formed in the methionine/glucose model system, the amino acid was reacted with ^{13}C-labled D-glucoses and D-glucose was reacted with ^{15}N- and [methyl-^{13}C]methinone.

Strecker Aldehyde Formation from *L*-Methionine Alone. The initial degradation of *L*-methionine alone proceeds through decarboxylation to produce 3-(methylthio)propylamine as the major product. However, in the presence of carbohydrates, 3-(methylthio)-propanal (methional) the Strecker aldehyde, becomes the major product. The Strecker aldehyde has been also detected in the decomposition mixtures of *L*-methionine alone (*2, 4, 7*). Although methional was not detected when *L*-methionine was pyrolyzed at 250 °C, however, its condensation product (**1**) with 3-(methylthio)propylamine was detected, indicating the formation of methional during pyrolysis as shown in Figure 1. Compound **1** contained, as expected, one nitrogen atom and two methyl groups of methionine but no sugar carbon atoms. A proposed general mechanism for the oxidative decarboxylation of amino acids in the absence of a carbohydrate source is shown in Figure 1. In the case of methionine, dimethyl disulfide could act as a possible oxidizing agent. The imine produced in the first step of this reaction can be easily hydrolyzed into the Strecker aldehyde and subsequently trapped as compound **1** after its reaction with 3-(methylthio)propylamine.

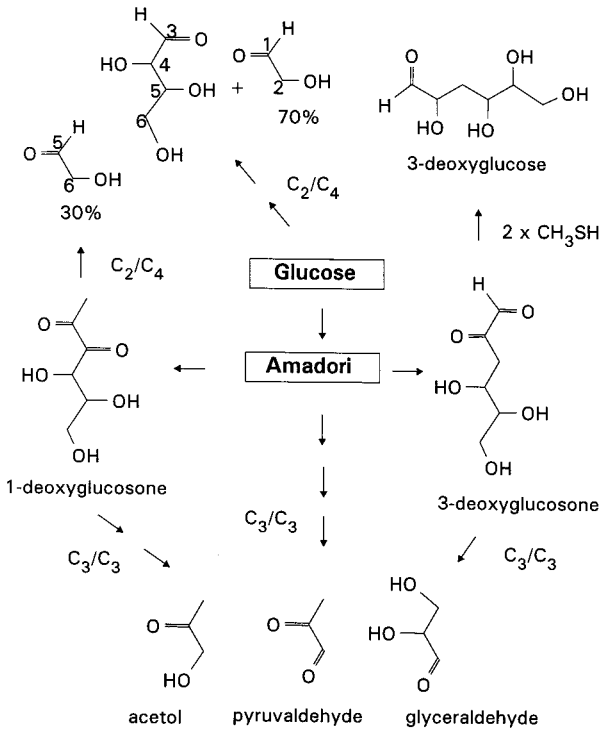

Figure 2. Carbohydrate degradation pathways and formation of C_2, C_3, C_4, and C_6 reactive intermediates in L-methionine/D-glucose model system based on labeling studies. Carbon numbers indicate original D-glucose carbon positions.

Sugar Fragmentation Pathways. The mechanism of sugar fragmentation in the L-methionine/D-glucose model system (see Figure 2) was inferred from the incorporation of glucose fragments in selected Maillard reaction products. Label incorporation studies have indicated that the sugar moiety undergoes C_2/C_4 and C_3/C_3 cleavages in addition to the formation of two C_6 reactive intermediates: 1-deoxy- and 3-deoxyglucosones. A similar carbohydrate fragmentation pattern was also identified in glucose/alanine system (*12*). Evidence for the cleavage of glucose into C_2 and C_4 fragments (glycolaldehyde and a tetrose) was found in the incorporation of intact C-1, C-2 (70%) and C-5, C-6 glucose atoms in 3-[(methylthio)methyl]furan (**2** in Figure 3), and C-3, C-4, C-5, C-6 glucose atoms in N-[(methylthio)-propyl]pyrrole (**3**). Evidence for the cleavage of the sugar moiety into two C_3 fragments was found in the incorporation of C-1, C-2, C-3 and C-4, C-5, C-6 intact glucose carbon atom sequences in 2,5-dimethylpyrazine and 2,5-dimethyl-3-[(methylthio)propyl]pyrazine (see Table 1 and Figure 4). On the other hand, 5-[(methylthio)-methyl]-2-methylfuran (**4** in Figure 5) and 5-[(methylthio)methyl]furfuryl alcohol (**5**) incorporated intact C_6 glucose carbon atoms, indicating the formation of 1-deoxy- and 3-deoxyglucosones (see below).

Mechanism of Formation of Heterocyclic Compounds Originating from C_6 and C_3 Sugar Fragments. Amadori compound, 1-deoxy- and 3-deoxyglucosones are known to undergo C_3/C_3 cleavages through carbonyl migrations and retro aldol reactions to produce intact three-carbon-containing moieties (C-1, C-2, C-3 and C-4, C-5, C-6) such as acetol, glyceraldehyde, and pyruvaldehyde (see Figure 2). All three intermediates are capable of forming α-amino carbonyl compounds

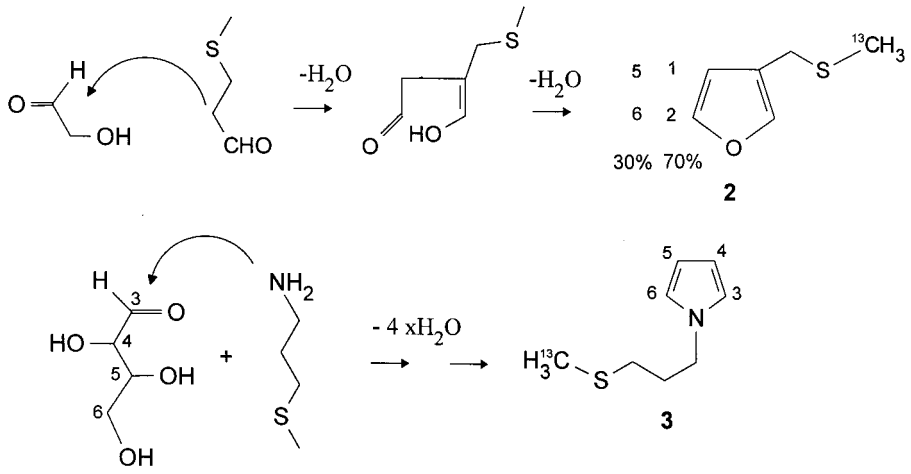

Figure 3. Amino acid and carbohydrate precursors of 3-[(methylthio)methyl]furan (**2**) and *N*-[(methylthio)propyl]pyrrole (**3**). Carbon numbers indicate original D-glucose carbon positions.

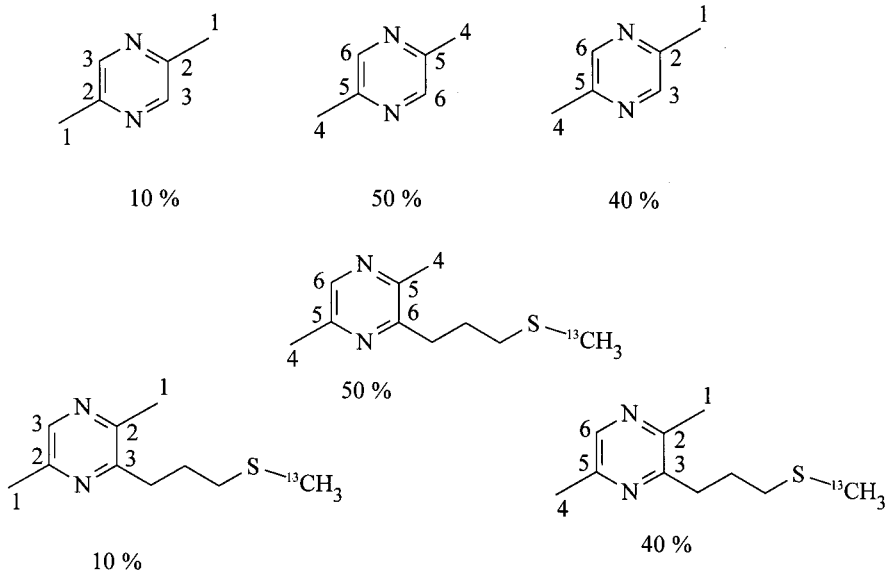

Figure 4. Percent distribution of glucose carbon atoms in 2,5-dimethyl- and 2,5-dimethyl-3-[(methylthio)propyl]pyrazines. Carbon numbers indicate original D-glucose carbon positions. $^{13}CH_3$ indicates presence of terminal methyl group of L-methionine.

Table 1. Percent Label Distribution in Acetol, 2,5-Dimethylpyrazine (DMP), and 2,5-Dimethyl-3-[(methylthio)propyl]pyrazine (DMTP) Formed from Labeled D-glucoses (Glu) and L-methionines (Met)

	acetol		DMP			DMTP		
model	M	M+1	M	M+1	M+2	M	M+1	M+2
met/glu	100	0	100	0	0	100	0	0
met/[1-^{13}C]glu	30	70	50	40	10	50	40	10
met/[2-^{13}C]glu	30	70	50	40	10	50	40	10
met/[3-^{13}C]glu	30	70	50	40	10	50	40	10
met/[4-^{13}C]glu	70	30	10	40	50	10	40	50
met/[5-^{13}C]glu	70	30	10	40	50	10	40	50
met/[6-^{13}C]glu	70	30	10	40	50	10	40	50
[^{15}N]met/glu	0	0	0	0	100	0	0	100
[methyl-^{13}C]met/glu	0	0	0	100	0	0	100	0

through Strecker reaction to eventually form pyrazine derivatives. The involvement of these three sugar fragments in the formation of 2,5-dimethylpyrazine and 2,5-dimethyl-3-[(methylthio)propyl]pyrazines was confirmed by observing an increase in the intensity of the peaks associated with the pyrazines when L-methionine was reacted separately with acetol, glyceraldehyde, and pyruvaldehyde. Furthermore, these intermediates can also react with amino acids to produce α-dicarbonyl compounds containing the amino acid side chain (see Figure 6). Label incorporation studies have also indicated that 2,5-dimethylpyrazine and 2,5-dimethyl-3-[(methylthio)propyl]pyrazine are formed through reaction of the same C_3 reactive intermediates (see Table 1 and Figure 4) and that the C_3 fragment containing a C-4, C-5, C-6 sequence of glucose carbon atoms is the major fragment in the C_3 reactive pool. On the other hand, the detection of 5-[(methylthio)methyl]-2-methylfuran (**4**) and 5-[(methylthio)methyl]furfuryl alcohol (**5**) incorporating an intact sequence of all six carbon atoms of glucose could be justified by the formation of 1-deoxy- and 3-deoxyglucosones and their subsequent interaction with methanethiol as shown in Figures 2 and 5.

Mechanism of Formation of Heterocyclic Compounds Originating from C_2 and C_4 Sugar Fragments. Retro aldol cleavages initiated by the C-3 hydroxyl group of the free glucose and the C-5 hydroxyl group of 1-deoxyglucosone can result in the formation of two isotopomeric glycolaldehydes (see Figure 2). The former can also generate a tetrose moiety incorporating the last four carbon atoms of glucose with potential carbonyl groups at both terminal carbon atoms. The formation of two isotopomeric glycolaldehydes was

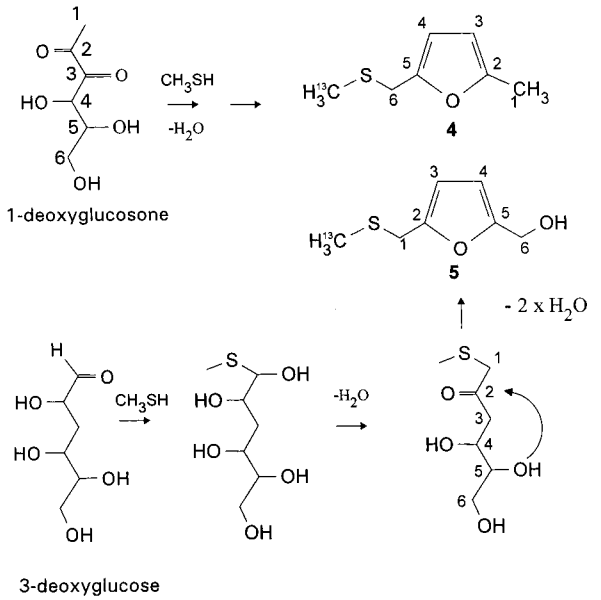

Figure 5. Amino acid and carbohydrate precursors of 5-[(methylthio)methyl]-2-methylfuran (**4**) and 5-[(methylthio)methyl]-furfuryl alcohol (**5**). Carbon numbers indicate original D-glucose carbon positions. $^{13}CH_3$ indicates presence of terminal methyl group of L-methionine.

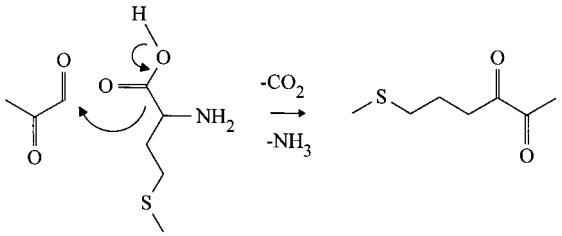

Figure 6. Proposed mechanisms of amino-acid-assisted chain elongation reaction of pyruvaldehyde.

inferred from the analysis of label incorporation in 3-[(methylthio)methyl]furan (**2**) which indicated the formation of two isotopomers incorporating C-1, C-2 (70%) and C-5, C-6 (30%) intact glucose carbon chains. Aldol condensation between methional and glycolaldehyde followed by dehydrations and cyclization can result in the formation of **2** as shown in Figure 3. When L-methionine was reacted in the presence of glycolaldehyde, the intensity of the chromatographic peak associated with compound **2** increased significantly implicating glycolaldehyde as the immediate precursor of **2**. On the other hand, the remaining C_4 glucose fragment (C-3, C-4, C-5, C-6) was incorporated as part of the pyrrole ring in *N*-[(methylthio)propyl]pyrrole (**3**) through its reaction with 3-(methylthio)propylamine. Both **2** and **3** also incorporated, as expected, the terminal methyl group of L-methionine, and compound **3** showed the incorporation of a single nitrogen atom. The fact that compound **3** incorporated only the tetrose fragment C-3, C-4, C-5, C-6 is consistent with the proposed mechanism because the formation of pyrrole moiety requires the presence of two terminal carbonyl groups.

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