Pyrazine Formation from Serine and Threonine

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The formation of pyrazines from L-serine and L-threonine has been studied. L-Serine and L-threonine, either alone or combined, were heated at 120 °C as low temperature for 4 h or at 300 °C as high temperature for 7 min. The pyrazines formed from each reaction were identified by GC/MS, and the yields (to the amino acid used, as parts per million) were determined by GC/FID. It was found that pyrazine, methylpyrazine, ethylpyrazine, 2-ethyl-6-methylpyrazine, and 2,6-diethylpyrazine were formed from serine, whereas 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, trimethylpyrazine, 2-ethyl-3,6-dimethylpyrazine, and 2-ethyl-3,5-dimethylpyrazine were formed from threonine. Mechanistically, it is proposed that the thermal degradation of serine or threonine is composed of various complex reactions. Among these reactions, decarbonylation followed by dehydration is the main pathway to generate the α-aminocarbonyl intermediates leading to the formation of the main product, such as pyrazine from serine or 2,5-dimethylpyrazine from threonine. Also, deamination after decarbonylation generates more reactive intermediates, α-hydroxycarbonyls. Furthermore, aldol condensation of these reactive intermediates provides α-dicarbonyls. Subsequently, these α-dicarbonyls react with the remaining serine or threonine by Strecker degradation to form additional α-aminocarbonyl intermediates, which then form additional pyrazines. In addition, decarboxylation and retroaldol reaction may also involve the generation of the intermediates.

Keywords: *Serine; threonine; pyrazine formation; decarbonylation; decarboxylation; deamination; α-aminocarbonyls; Strecker degradation; aldol condensation*

INTRODUCTION

Alkylpyrazines are generally considered as trace important flavor components in foods (Maga, 1992). The formation of alkylpyrazines has been widely investigated and reviewed (Vernin and Parkanyi, 1982; Heath and Reineccius, 1986; Ohloff et al., 1985; Vernin and Metzger, 1981). Categorically, there are two widely accepted mechanisms for the formation of pyrazines: the Strecker degradation and the ammonia/acyloin reaction. The Strecker degradation (Schonberg and Moubacher, 1952; Rizzi, 1972) is involved with α-amino acids and the reductones (α-dicarbonyls), which are derived either from the Maillard reaction or from caramelization of carbohydrates (Hodge, 1967). During the Strecker degradation, the α-dicarbonyls are converted into α-aminocarbonyls, which, in turn, condense to form alkylpyrazines. The ammonia/acyloin reaction is involved with ammonia and α-hydroxycarbonyls, which are also derived from caramelization of carbohydrates (Hodge, 1967). During this reaction, α-hydroxycarbonyls are converted into α-aminocarbonyls and then to alkylpyrazines. This reaction takes place very readily even at room temperature (Rizzi, 1988; Shu and Lawrence, 1995). Basically, these two mechanisms for alkylpyrazine formation require a carbohydrate source to provide the carbohydrate degradation products, either as α-dicarbonyls or as acyloins. However, without a carbohydrate source, alkylpyrazines were also found from the pyrolysis of hydroxyamino acids (Kato et al., 1970; Wang and Odell, 1973). These authors concluded that α-dicarbonyls are not required to form such alkylpyrazines, but they did not explain how such alkylpyrazines were formed.

The objective of the present study was to determine the major alkylpyrazines formed qualitatively and quantitatively from serine and threonine as well as from a combination of serine and threonine under different temperatures. On the basis of the results obtained, a mechanism for the formation of alkylpyrazines from serine and threonine is proposed.

EXPERIMENTAL PROCEDURES

Materials. L-Serine and L-threonine were purchased from Ajinomoto Co. (Tokyo, Japan), and *n*-hexadecane was purchased from Aldrich Chemical Co. (Milwaukee, WI).

Preparation of the Reaction Mixtures. In an enclosed reaction vessel (Parr Instrument Co., Moline, IL), 300 mg of reactant and 36 μL of water (as 12% moisture level) were heated in an oven at 120 °C for 4 h or at 300 °C for 7 min. The reactants included L-serine, L-threonine, and a combination of 50% L-serine and 50% L-threonine (w/w). Each reaction mixture obtained was cooled to room temperature and extracted with methylene chloride (5 mL × 4). The methylene chloride extracts, which were combined from the reaction performed at 300 °C for 7 min, were directly analyzed by GC/MS, while the methylene chloride extracts, which were combined from the reaction performed at 120 °C for 4 h, were further concentrated under a stream of nitrogen to 0.5 mL prior to GC/MS analysis.

GC/MS Analysis. Each extract as prepared above was analyzed by GC/MS on a DB-Wax fused silica column (60 m × 0.32 mm, 0.15 μm film thickness) with a mass selective detector (EI; 70 eV). The oven temperature was programmed from 50 to 200 °C at 6 °C/min.

Quantitation of the Alkylpyrazines Formed in the Reaction Mixture. Each extract prepared above was also analyzed under the same chromatographic conditions as described above except that a flame ionization detector (FID) was used. *n*-Hexadecane was added to each extract as an internal standard for the quantitation of each alkylpyazine;

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Table 1. Pyrazines Identified and the Quantitative Data, at Parts per Million

	120 °C/4 h			300 °C/7 min		
pyrazine identified	from Ser	from Thr	from Ser/Thr	from Ser	from Thr	from Ser/Thr
pyrazine	97.6		32.3	1477		354
methyl-	6.1		32.0	245		880
2,5-dimethyl-		11.4	9.9		1100	898
2,6-dimethyl-			3.1		390	501
ethyl-	21.3		8.2	1025		348
2-ethyl-6-methyl-			12.6	131		1077
2-ethyl-5-methyl-						281
trimethyl-		10.0	7.4		271	705
2,6-diethyl-				391		247
2-ethyl-3,6-dimethyl-		0.4	11.4		632	2291
2-ethyl-3,5-dimethyl-					83	458
total	125.0	21.8	116.9	3269	2476	8040

it was assumed that the FID response factor of *n*-hexadecane was the same as those of the alkylpyrazines. The yield of each alkylpyrazine was reported as parts per million of serine or/and threonine used (ppm).

RESULTS AND DISCUSSION

Table 1 shows the pyrazines identified from all of the reactions along with the quantitative data. In general, heating at high temperature (300 °C/7 min) generated greater amounts of alkylpyrazines than heating at low temperature (120 °C/4 h). Also, under each condition, it is obvious that L-serine generates a higher yield of pyrazines than L-threonine, probably due to the melting point of L-serine (222 °C), which is lower than the melting point of L-threonine (256 °C). This melting point effect on the yield appears to be more significant for heating at low temperature than at high temperature.

When L-serine is heated to 120 °C, pyrazine, ethylpyrazine (major), and methylpyrazine (minor) were found, whereas when it is heated to 300 °C, pyrazine, ethylpyrazine, 2,6-diethylpyrazine (major), methylpyrazine, and 2-ethyl-6-methylpyrazine (minor) were found. Taking into account these results and relevant literature information, it is suggested that during thermal degradation of serine, alkylpyrazines are formed as shown in Figure 1.

Figure 1A shows the pathway to form the parent pyrazine and the reactive intermediates. Decarbonylation of serine followed by dehydration generates a precursor, α-aminoacetaldehyde (**1**), which is dimerized to form pyrazine. Meanwhile, decarbonylation followed by deamination forms α-hydroxyacetaldehyde (glycolaldehyde), which can also be converted to **1**. In addition, decarboxylation followed by deamination can lead to the formation of acetaldehyde. These reactive intermediates participate further in the reactions as described later. Decarbonylation of serine is essential to describe this mechanism. Basically, it is analogous to the acid-catalyzed decarbonylation of α-hydroxy acid, in which the primary products include CO and water. Carbon monoxide and other low molecular weight compounds were already found from the pyrolysis of amino acids (Lien and Nawar, 1974).

Figure 1B shows how the other major alkylpyrazines, ethylpyrazine and 2,6-diethylpyrazine, may be formed. Aldol condensation of the reactive intermediates, glycolaldehyde and acetaldehyde, followed by dehydration, generates 1,2-butanedione, which can participate in the Strecker degradation with the remaining serine to form

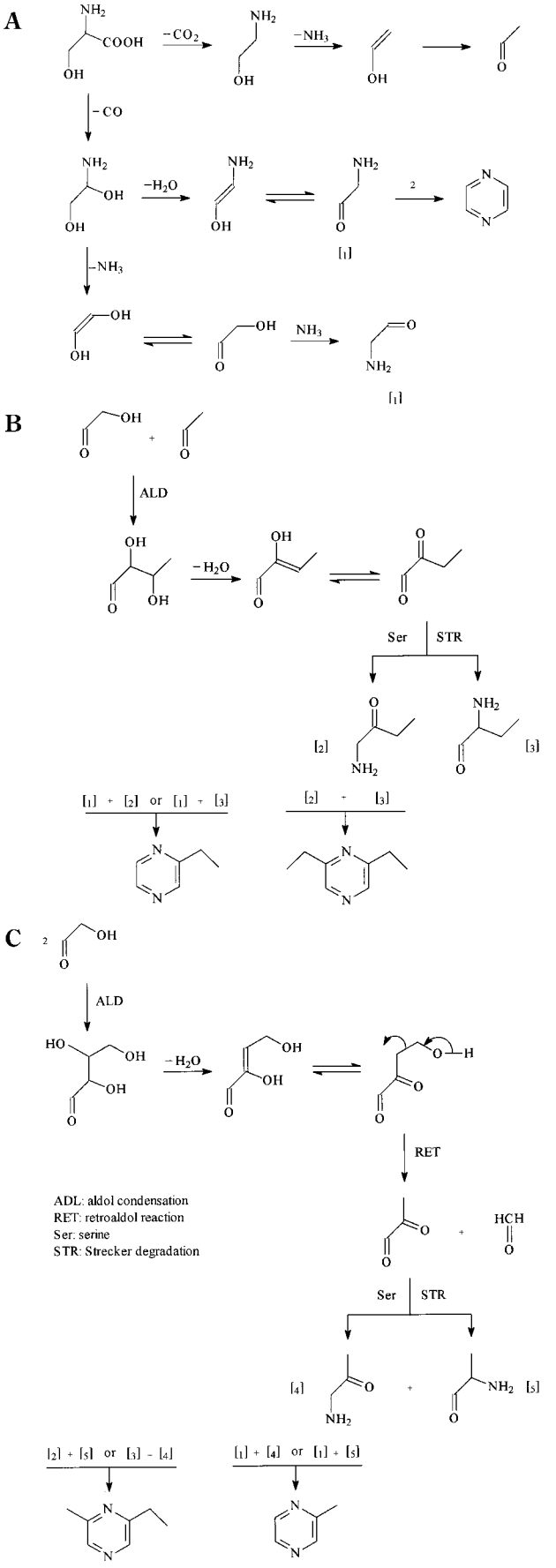

Figure 1. Mechanism proposed for the formation of pyrazines from serine: (A) formation of parent pyrazine; (B) formation of ethylpyrazine and 2,6-diethylpyrazine; (C) formation of 2-ethyl-6-methylpyrazine and methylpyrazine.

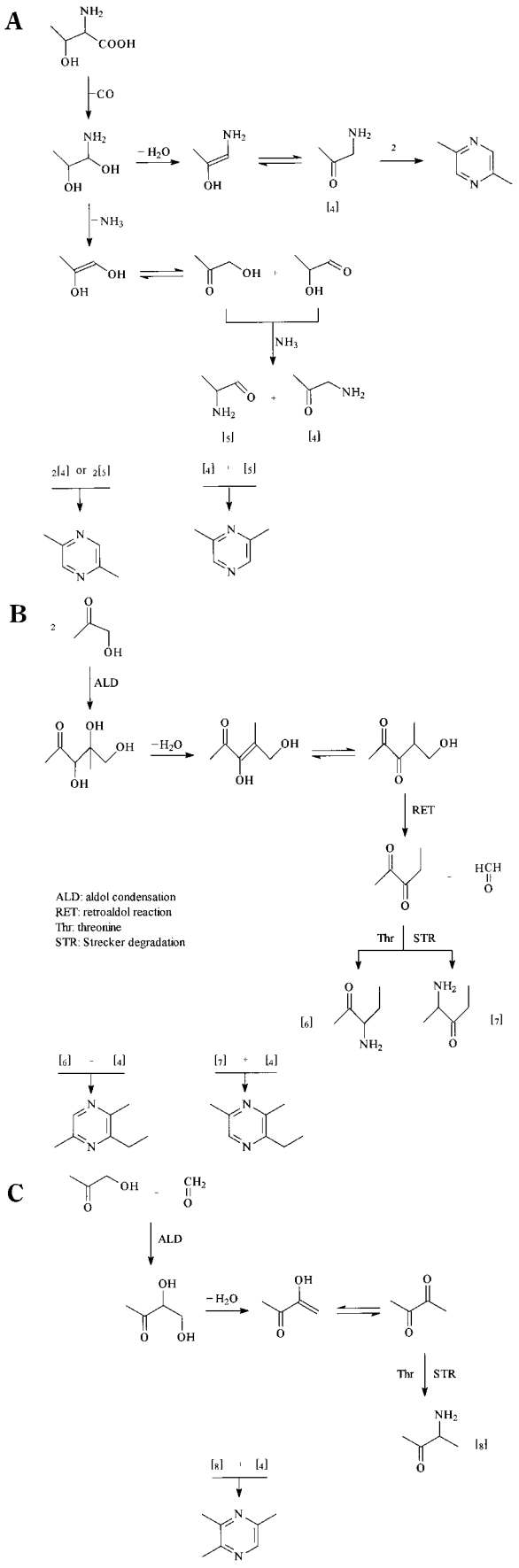

Figure 2. Mechanism proposed for the formation of pyrazines from threonine: (A) formation of 2,5-dimethylpyrazine and 2,6-dimethylpyrazine; (B) formation of 2-ethyl-3,6-dimethylpyrazine and 2-ethyl-3,5dimethylpyrazine; (C) formation of trimethylpyrazine.

precursors 1-amino-2-butanone (**2**) and 2-aminobutanal (**3**). As a result, the combination of **1** and **2** or **1** and **3** can form ethylpyrazine, whereas the combination of **2** and **3** can form 2,6-diethylpyrazine. Because the amount of parent pyrazine found is greater than that of other pyrazines (Table 1), it appears that decarbonylation followed by dehydration is the predominant pathway, compared with all other pathways. Similarly, Figure 1C shows the possible formation of precursors 1-amino-2-propanone (**4**) and 2-aminopropanal (**5**) from glycoaldehyde via aldol condensation and retroaldol reaction. Therefore, the combination of **2** and **5** or **3** and **4** can generate 2-ethyl-6-methylpyrazine, whereas the combination of **1** and **4** or **1** and **5** can generate methylpyrazine.

When threonine is heated to 120 °C, 2,5-dimethylpyrazine, trimethylpyrazine (major), and 2-ethyl-3,6-dimethylpyrazine (minor) were found, whereas when it is heated to 300 °C, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, trimethylpyrazine, 2-ethyl-3,6-dimethylpyrazine (major), and 2-ethyl-3,5-dimethylpyrazine (minor) were found. On the basis of these results and the literature information, a formation mechanism of the alkylpyrazines is proposed in Figure 2. In general, these formation pathways from threonine are similar to those described earlier for serine.

Figure 2A shows the main pathway through decarbonylation and dehydration to form precursor **4**, which is dimerized to yield 2,5-dimethylpyrazine. Also, decarbonylation followed by deamination generates 1-hydroxy-2-propanone and 2-hydroxyypropanal, the reactive intermediates, which may react back with ammonia to form precursors **5** and **4**. Furthermore, self-combination of **4** or **5** can form 2,5-dimethylpyrazine, whereas cross-combination of **4** and **5** can form 2,6-dimethylpyrazine. Because decarbonylation/dehydration is the main pathway, 2,5-dimethylpyrazine is preferentially formed over 2,6-dimethylpyrazine.

Figure 2B shows that the aldol condensation of 1-hydroxy-2-propanone followed by retroaldol reaction and Strecker degradation generates precursors 3-amino-2-pentanone (**6**) and 2-amino-3-pentanone (**7**). As a result, the combination of **6** and **4** can form 2-ethyl-3,6-dimethylpyrazine, whereas the combination of **7** and **4** can form 2-ethyl-3,5-dimethylpyrazine. Similarly, Figure 2C shows the pathway of the reaction of 1-hydroxy-2-propanone and formaldehyde to generate the precursor 3-amino-2-butanone (**8**). The combination of **8** and **4** can form trimethylpyrazine.

It should be noted that in addition to the pyrazines listed in Table 1, there are several other pyrazines present in trace amounts. For instance, trace amounts of 2,5-dimethylpyrazine and 2,6-dimethylpyrazine were also found from the thermal degradation of serine, probably due to reaction of **4** and **5** (Figure 1C). Self-combination of **4** or **5** forms 2,5-dimethylpyrazine, whereas cross-combination of **4** and **5** forms 2,6-dimethylpyrazine as in the case of threonine (Figure 2A). However, during this study, the detailed information of the formation of trace level pyrazines was not considered.

It is interesting to examine the pyrazines formed from the reactions of a mixture of 50% serine and 50% threonine (Table 1). It has been found that all of the pyrazines formed from the reaction of serine or threonine are formed from the reaction of serine/threonine, but the major pyrazines formed from the reaction of

serine/threonine are different from those formed by the reaction of serine or threonine alone. For example, heating at low temperature, methylpyrazine is one of the two most abundant pyrazines formed from the reaction of serine/threonine. In contrast, methylpyrazine is not the major pyrazine formed from the reaction of either serine or threonine. The difference may involve the precursors of methylpyrazine, α-aminoacetaldehyde (**1**) and 1-amino-2-propanone (**4**), which are derived from serine and threonine, respectively It is also observed that with heating at high temperature, 2-ethyl-3,6-dimethylpyrazine and 2-ethyl-6-methylpyrazine are the most abundant pyrazines formed from the reaction of serine/threonine, but they neither is the most abundant pyrazine from the reaction of serine or threonine. This may also involve their precursors, α-aminocarbonyls.

SUMMARY OF PROPOSED MECHANISM

The results obtained from this study indicate that the thermal degradation of serine or threonine, either at low temperature (120 °C) or at higher temperature (300°), is composed of various complex reactions including decarbonylation, dehydration, deamination, amination, aldol condensation, retroaldol reaction, decarboxylation, and Strecker degradation. Of these reactions, decarbonylation followed by dehydration is the main pathway to generate the α-aminocarbonyl intermediates leading to the formation of the main product, such as pyrazine from serine or 2,5-dimethylpyrazine from threonine. In addition, decarbonylation and decarboxylation followed by deamination generate reactive intermediates such as α-hydroxycarbonyls and aldehydes. Furthermore, aldol condensation and retroaldol reaction of these reactive intermediates lead to the formation of α-dicarbonyls. Subsequently, these α-dicarbonyls react with the remaining serine or threonine by Strecker degradation to form additional α-aminocarbonyl intermediates and additional pyrazines. When serine and threonine are combined in a mixture for the thermal reaction, the pyrazine profile obtained quantitatively depends on the α-aminocarbonyl intermediates formed.

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