Influence of the Structural Characteristics of Amino Acids on Direct Methylation Behaviors by TMAH in Pyrolysis

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Direct methylation behaviors of 20 amino acids with tetramethylammonium hydroxide (TMAH) were studied under diluted conditions with silica. Amino acid concentration was controlled by dilution with silica (SiO_2) and the molar ratios of amino acid/silica were 0.20, 0.50, and 2.0. The molar ratios of amino acid/TMAH (0.51 - 4.64) also varied. It was found that arginine, asparagine, aspartic acid, cysteine, glutamic acid, and glutamine did not generate any directly methylated pyrolysis products, whereas alanine, glycine, isoleucine, leucine, methionine, phenylanaline, valine, and proline generated all the directly methylated pyrolysis products. Tri- and tetra methylated products of lysine consisted of two types. Histidine and threonine hardly generated the partly methylated products. Mono- and dimethylated products of serine, tryptophan, and tyrosine were not observed. Relative intensities of the methylated products varied with the amino acid concentration, TMAH concentration, and pyrolysis temperature. Direct methylation behaviors of amino acids were explained by the structural characteristics of amino acids.

Key Words: Amino acids, Direct methylation, TMAH, Py-GC/MS, Silica

Introduction

Analytical pyrolysis is widely utilized for the chemical characterization of complex organic materials of natural origin and pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) is one of the most widely used analytical tools to characterize recalcitrant macromolecules at the molecular level.[1-3] Amino acids are important constitutive units or starting components of various organic macromolecules such as proteins and humic substances. The pyrolytic behaviors of common amino acids have been investigated and the principal thermal degradation products have been identified through Py-GC/MS.[4-7] However, the usefulness of this technique is limited by unexpected secondary reactions of pyrolyzates during the pyrolysis process and difficulty with polar pyrolyzates analyzed by GC/MS. In order to overcome these limitations, pyrolysis in the presence of tetraalkylammonium hydroxide was developed. The most efficient reagent was proven to be tetramethylammonium hydroxide (TMAH) which is used as a methylating agent.[8-11] The application of TMAH thermochemolysis for the study of proteinaceous materials[12-15] is important because of the significant amounts of proteinaceous materials found in environmentally and geochemically important samples.

In previous studies,[8,16,17] TMAH was used excess for direct methylation of amino acids. These studies reported that partly methylated products were also generated along with fully methylated ones. The degree of direct methylation related to amino acids and the variation associated with methylated products have not been studied in detail. In this study, amino acids were pyrolyzed in the presence of silica to dilute amino acids. Pyrolysis behaviors based on amino acid concentration and pyrolysis temperature were investigated. Inorganic materials such as Al_2O_3, SiO_2, CaO, Na_2CO_3, and NaCl affected the formation of pyrolysis products.[18] The degree of direct methylation and variation of the relative abundances of the methylated products were also investigated. Differences in the direct methylation behaviors of amino acids were explained by the structural characteristics of the amino acids.

Experimental

Phenylalanine, tyrosine, tryptophan, serine, valine, and glycine were purchased from Daejung Chemicals & Metals Co. (Korea). Argine, aspartic acid, glutamic acid, and proline were purchased from Samchun Pure Chemical Co. (Korea). Alanine, histidine, leucine, glutamine, threonine, and asparagine were purchased from Junsei Chemaical Co. (Japan). Lysine and isoleucine were purchased from Acros Organics (USA), and Cysteine was purchased from Merck Co. (Germany). Methionine was purchased from Yakuri Pure Chemicals Co. (Japan). Fumed silica was purchased from Merck Co. (Germany). Tetramethylammonium hydroxide (TMAH, 25% in methanol) was purchased from Lancaster Synthesis Inc. (UK). We used silica after drying it in 150 °C oven to remove water. Each amino acid was mixed with silica (amino acid : SiO_2 = 1 : 5, 1 : 2, and 2 : 1 by the molar ratio) and pelletized. The 0.2 μL TMAH in methanol was added to the 0.3 mg mixture sample. After the methanol evaporated, the sample was placed into a quartz tube.

Pyrolysis-GC/MS was carried out by using a CDS Pyroprobe 1500 heated filament pyrolyzer (Chemical Data System, Oxford, USA) coupled to an Agilient 6890 gas chromatograph equipped with a 5973 mass spectrometer of Agilent Technology Inc. (USA). An HP-5MS (30 m × 0.25 mm i.d., 0.25 μm film thickness column, Agilent Technology Inc.) was used. The following analysis conditions were used: pyrolysis temperature and time, 700 °C and 3 sec; GC injector temperature, 250 °C; split ratio, 1 : 20; GC oven temperature program, 50 °C (held for 3 min) to 250 °C at 10 °C/min; carrier gas, helium (flow rate, 1.5 mL/min); the interface temperature of GC to MS, 250 °C. The electron impact ionization (electron energy 70 eV) was used to ionize the pyrolysis products. The

Table 1. Molar ratios of amino acids and TMAH (amino acid/ TMAH).

Amino acid/Silica	0.2	0.5	2.0
Alanine (Ala)	1.16	2.32	4.64
Arginine (Arg)	0.59	1.19	2.37
Asparagine (Asn)	0.78	1.57	3.13
Aspartic acid (Asp)	0.78	1.55	3.11
Cysteine (Cys)	0.85	1.71	3.42
Glutamic acid (Glu)	0.70	1.41	2.81
Glutamine (Gln)	0.71	1.42	2.83
Glycine (Gly)	1.38	2.75	5.51
Histidine (His)	0.64	1.28	2.57
Isoleucine (Ile)	0.79	1.58	3.15
Leucine (Leu)	0.79	1.58	3.15
Lysine (Lys)	0.71	1.42	2.83
Methionine (Met)	0.69	1.39	2.77
Phenylalanine (Phe)	0.63	1.25	2.50
Proline (Pro)	0.90	1.80	3.59
Serine (Ser)	0.98	1.97	3.94
Threonine (Thr)	0.87	1.74	3.47
Tryptophan (Trp)	0.51	1.01	2.03
Tyrosine (Tyr)	0.57	1.41	2.28
Valine (Val)	0.88	1.77	3.53

MS source temperature was 230 °C.

The quantitative analysis was performed using an Acme 6000 gas chromatograph of Younglin Co. (Korea) equipped with a flame ionization detector (FID). Nitrogen was used as carrier gas (flow rate, 2.0 mL/min). The FID and injector temperatures were set at 250 °C. An HP-5 (30 m × 0.25 mm i.d., 0.25 μm film thickness column, Agilent technology Inc.) was temperature programmed from 50 °C (held for 3 min) to 250 °C at a rate of 10 °C/min. The split ratio was 1 : 20. The sample was pyrolyzed at 400, 500, 700 and 900 °C.

Results and Discussion

The pyrolysis products of the amino acids were identified through GC/MS, the quantitative analysis was performed with GC-FID. The molar ratios of the amino acids, silica, and TMAH of the samples are summarized in Table 1. The molar ratios of the amino acid/silica were 0.2, 0.5, and 2.0, and those of the amino acid/TMAH were 0.51 to 5.51. The direct methylated pyrolysis products are summarized in Table 2. For arginine, asparagine, aspartic acid, cysteine, glutamic acid, and glutamine, no directly methylated pyrolysis products were observed. Gallois and coworkers[16] reported that arginine, asparagine, and aspartic acid did not generate direct methylated pyrolysis products but cysteine, glutamic acid, and glutamine produced

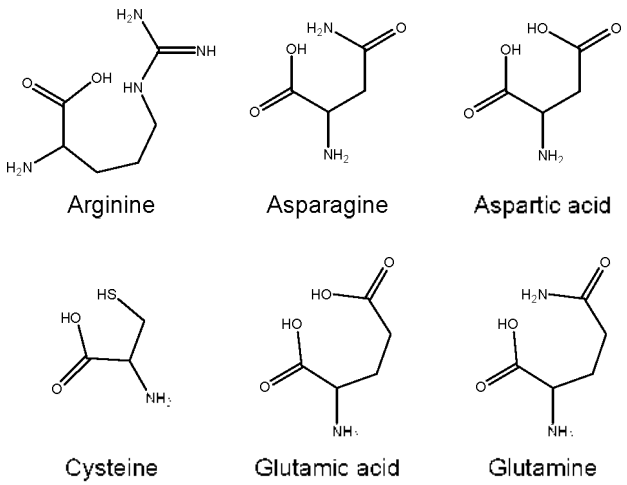
Table 2. Directly methylated pyrolysis products of amino acid monomers.

Amino acid	Degree of methylation	*m/z* (chemical formula)	Compound
Alanine	M1	103 ($C_4H_9NO_2$)	Alanine methyl ester
	M2	117 ($C_5H_{11}NO_2$)	*N*-Methylalanine methyl ester
	M3	131 ($C_6H_{13}NO_2$)	*N*,*N*-Dimethylalanine methyl ester
Arginine	None		
Asparagine	None		
Aspartic acid	None		
Cysteine	None		
Glutamic acid	None		
Glutamine	None		
Glycine	M1	89 ($C_3H_7NO_2$)	Glycine methyl ester
	M2	103 ($C_4H_9NO_2$)	*N*-Methylglycine methyl ester
	M3	117 ($C_5H_{11}NO_2$)	*N*,*N*-Dimethylglycine methyl ester
Histidine	M4	211 ($C_{10}H_{17}N_3O_2$)	*N*,*N*,*N*′-Trimethylhistidine methyl ester
Isoleucine	M1	145 ($C_7H_{15}NO_2$)	Isoleucine methyl ester
	M2	159 ($C_8H_{17}NO_2$)	*N*-Methylisoleucine methyl ester
	M3	173 ($C_9H_{19}NO_2$)	*N*,*N*-Dimethylisoleucine methyl ester
Leucine	M1	145 ($C_7H_{15}NO_2$)	Leucine methyl ester
	M2	159 ($C_8H_{17}NO_2$)	*N*-Methylleucine methyl ester
	M3	173 ($C_9H_{19}NO_2$)	*N*,*N*-Dimethylleucine methyl ester
Lysine	M3	188 ($C_9H_{20}N_2O_2$)	*N*′,*N*′-Dimethyllycine methyl ester
	M4	202 ($C_{10}H_{22}N_2O_2$)	*N*,*N*′,*N*′-Trimethyllycine methyl ester
	M3′	188 ($C_9H_{20}N_2O_2$)	*N*,*N*-Dimethyllycine methyl ester
	M4′	202 ($C_{10}H_{22}N_2O_2$)	*N*,*N*,*N*′-Trimethyllycine methyl ester
	M5	216 ($C_{11}H_{24}N_2O_2$)	*N*,*N*,*N*′,*N*′-Tetramethyllycine methyl ester

Table 2. Continued.

Amino acid	Degree of methylation	*m/z* (chemical formula)	Compound
Methionine	M1	163 ($C_6H_{13}NO_2S$)	Methionine methyl ester
	M2	177 ($C_7H_{15}NO_2S$)	*N*-Methylmethionine methyl ester
	M3	191 ($C_8H_{17}NO_2S$)	*N,N*-Dimethylmethionine methyl ester
Phenylalanine	M1	179 ($C_{10}H_{13}NO_2$)	Phenylalanine methyl ester
	M2	193 ($C_{11}H_{15}NO_2$)	*N*-Methylphenylalanine methyl ester
	M3	207 ($C_{12}H_{17}NO_2$)	*N,N*-Dimethylphenylalanine methyl ester
Proline	M1	129 ($C_6H_{11}NO_2$)	Proline methyl ester
	M2	143 ($C_7H_{13}NO_2$)	*N*-Methylproline methyl ester
Serine	M3	147 ($C_6H_{13}NO_3$)	*N*-Methyl-*O*-methylserine methyl ester
	M4	161 ($C_7H_{15}NO_3$)	*N,N*-Dimethyl-*O*-methylserine methyl ester
Threonine	M1	133 ($C_5H_{11}NO_3$)	Threonine methyl ester
	M4	175 ($C_8H_{17}NO_3$)	*N,N*-Dimethyl-*O*-methylthreonine methyl ester
Tryptophan	M3	246 ($C_{14}H_{18}N_2O_2$)	*N,N*-Dimethyltryptophan methyl ester
	M4	260 ($C_{15}H_{20}N_2O_2$)	*N,N,N'*-Trimethyltryptophan methyl ester
Tyrosine	M3	223 ($C_{12}H_{17}NO_3$)	*N*-Methyl-*O*-methyltyrosine methyl ester
	M4	237 ($C_{13}H_{19}NO_3$)	*N,N*-Dimethyl-*O*-methyltyrosine methyl ester
Valine	M1	131 ($C_6H_{13}NO_2$)	Valine methyl ester
	M2	145 ($C_7H_{15}NO_2$)	*N*-Methylvaline methyl ester
	M3	159 ($C_8H_{17}NO_2$)	*N,N*-Dimethylvaline methyl ester

direct methylated pyrolysis products. Possible direct methylating sites of an amino acid are α-carboxylic acid (-CO_2H) and α-amine (-NH_2) of the backbone and side polar groups such as amine, carboxylic acid, and hydroxyl groups. Of the direct methylating sites of the backbone, first the α-carboxylic acid group is methylated and then the α-amine group will be methylated. If the α-carboxylic acid group is blocked by intramolecular hydrogen bonding, the methylation reaction with the α-carboxylic acid group will be unlikely. Since the above six amino acids have polar side chains such as -CO_2H, -$CONH_2$, -NH_2, =NH, or -SH (Scheme 1), the intramolecular hydrogen bonding with the α-carboxylic acid group happens frequently. In addition, the amino acids can be fixed on the silica surface by hydrogen bonding with silanol groups. Since silica surface is acidic because of a number of hydroxyl groups (silanol groups) on the surface, which results in adsorption of polar materials by hydrogen bonds, especially it forms a strong hydrogen bond with basic materials.[19-22] In previous works,[8,16] the direct methylated products of cysteine, glutamic acid, and glutamine were reported, but these studies used TMAH excess and did not employ silica. Another reason for no direct methylation may have been more favorable other reactions such as cyclization and dissociation processes. Arginine, asparagine, glutamic acid, and glutamine predominantly generated cyclic pyrolysis products through intramolecular cylization reaction by loss of H_2O, NH_3, and other species. The major cyclic pyrolysis products formed from arginine, asparagine, glutamic acid, and glutamine were 1-methyl-3-(*N*-methylamino) piperidin-2-one, 1-methyl-pyrrole-2,5-dione, 1-methyl-3-hydro-pyrrole-2-one, and *N*-methyl-5-oxoproline methyl ester, respectively. Aspartic acid and cysteine generated dimerized pyrolysis products by loss of H_2O. The major dimerized pyrolysis products formed from aspartic acid and cysteine were 3,4-didehydropyrrolidine-2,5-dione and di(methylthio)methane, respectively.



Scheme 1. Structures of amino acids which do not generate any directly methylated products.

Lysine has five direct methylating sites and tri-, tetra-, and pentamethylated species were observed. The tri- and tetramethylated products were found to be two types each as shown in Figure 1. The peaks at 12.70 and 12.96 min are trimethylated products assigned to *N',N'*-dimethyllycine methyl ester and *N,N*-dimethyllycine methyl ester, respectively, while the peaks at 12.89 and 13.38 min are tetramethylated products assigned to *N,N',N'*-trimethyllycine methyl ester and *N,N,N'*-trimethyllycine methyl ester, respectively. Figures 2 and 3 show the mass spectra of the tri- and tetramethylated products. Another

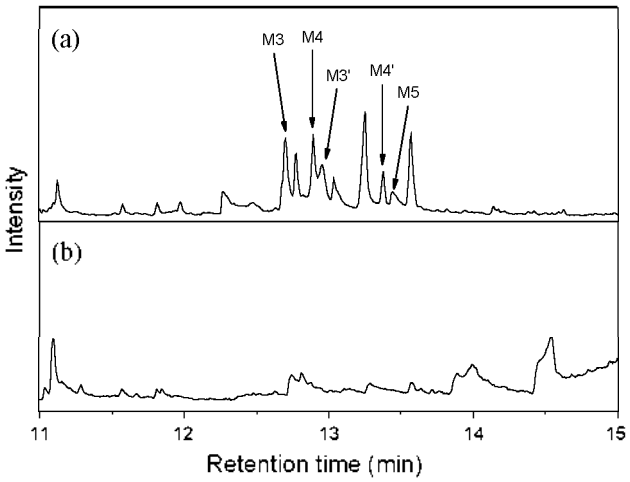


Figure 1. Pyrolysis-GC/MS chromatograms of the lysine/silica ratios of 0.2 (a) and 2.0 (b) at 700 °C.

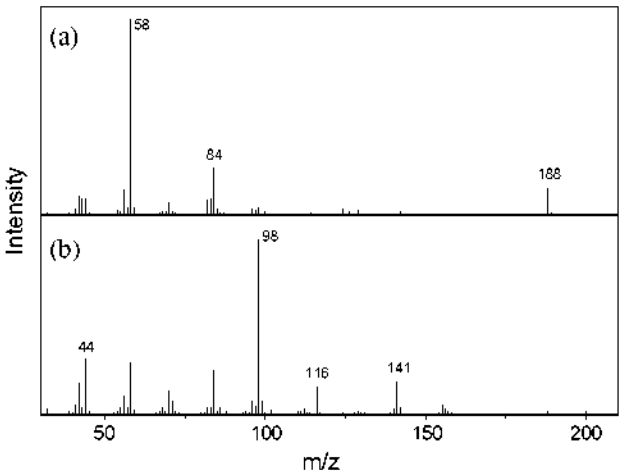


Figure 2. Mass spectra of the trimethylated pyrolysis products M3 (a) and M3' (b) of lysine. The M3 (12.71 min) and M3' (12.96 min) are *N',N'*-dimethyllycine methyl ester and *N,N*-dimethyllycine methyl ester, respectively.

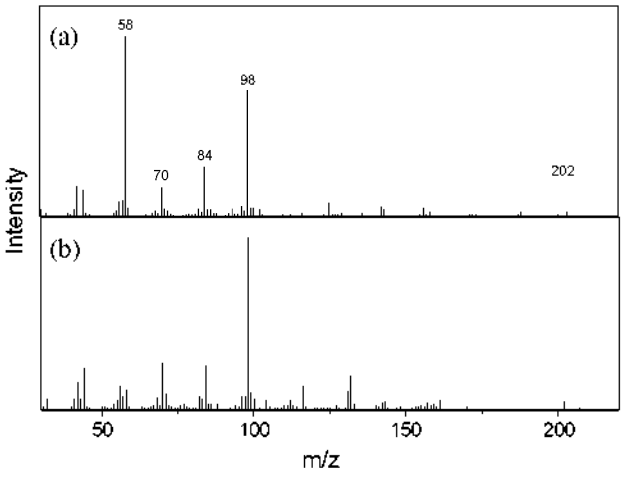
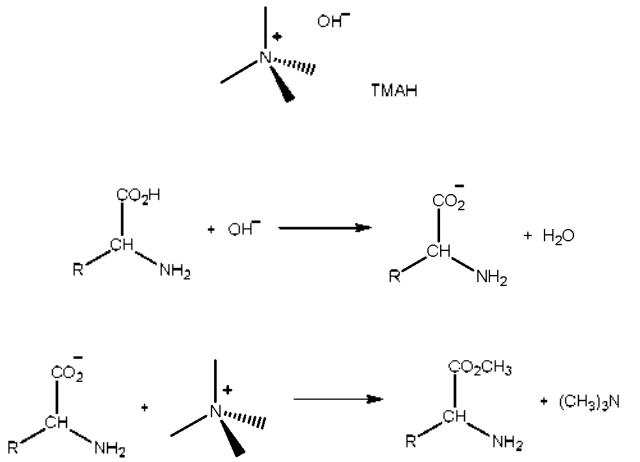
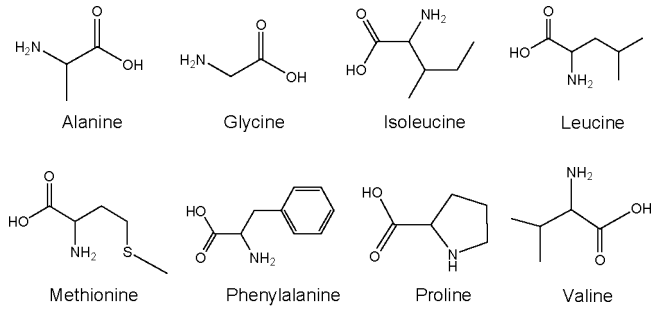


Figure 3. Mass spectra of the tetramethylated pyrolysis products M4 (a) and M4' (b) of lysine. The M4 (12.89 min) and M4' (13.38 min) are *N,N',N'*-trimethyllycine methyl ester and *N,N,N'*-trimethyllycine methyl ester, respectively.



Scheme 2. Direct methylation reaction mechanism of α-carboxylic acid of amino acid with TMAH.



Scheme 3. Structures of amino acids which generate all the directly methylated products.

possible trimethylated product of lysine is *N,N'*-dimethyllycine methyl ester, but this was not observed in this study. All the product ions were identified based on interpretation of the fragment ions and the literature data.[8,16]

Histidine generates only fully methylated product, *N,N,N'*-trimethylhistidine methyl ester. This implies that the methylation reaction of histidine with TMAH occurs very fast and the equilibrium constant is also very large. The acid dissociation constant (K_a) of histidine is relatively higher than other amino acids. The pK_a values of histidine are 1.60, 9.28, and 5.97 for α-carboxylic acid, α-amine, and side group amine, respectively.[23] The direct methylation reaction requires abstracting a proton from an amino acid by a hydroxide ion of TMAH and then methyl is added to the amino acid as shown in Scheme 2. Thus, amino acids with higher acid dissociation constants may be more favorable for methylation. For threonine, the fully methylated product was observed but the partly ones were not generated except for the monomethylated product (M1) which was observed by trace amount.

Peak intensity ratios of the partly methylated products were plotted as a function of the amino acid concentration (amino acid/silica) to investigate the silica effect and the fully methylated product was employed as a reference. Alanine, glycine, isoleucine, leucine, methionine, phenylalanine, valine, and proline generated all the methylated pyrolysis products. Their

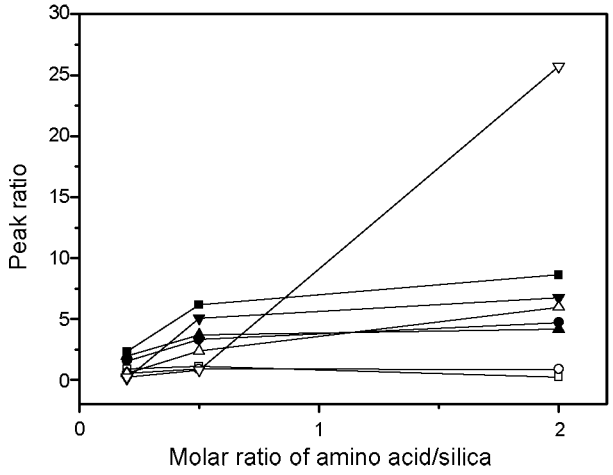


Figure 4. Variation of the methylated product ratios of alanine with the amino acid concentration (amino acid/silica (SiO_2) by molar ratio). Squares, circles, up-triangles, down-triangles indicate the pyrolysis temperatures of 400, 500, 700, and 900 °C, respectively. Solid and open symbols stand for the M1/M3 and M2/M3 ratios, respectively.

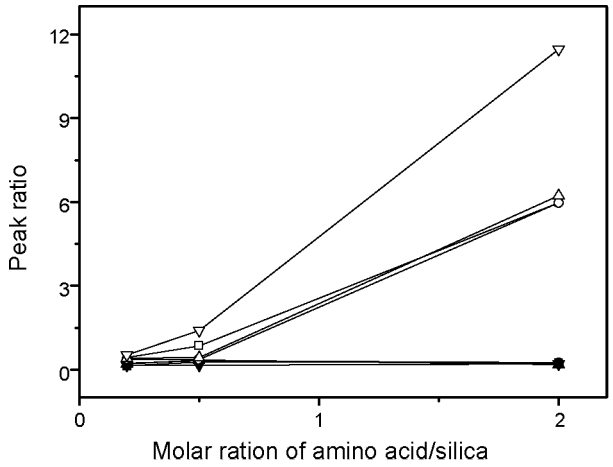


Figure 5. Variation of the methylated product ratios of glycine with the amino acid concentration (amino acid/silica (SiO_2) by molar ratio). Squares, circles, up-triangles, down-triangles indicate the pyrolysis temperatures of 400, 500, 700, and 900 °C, respectively. Solid and open symbols stand for the M1/M3 and M2/M3 ratios, respectively.

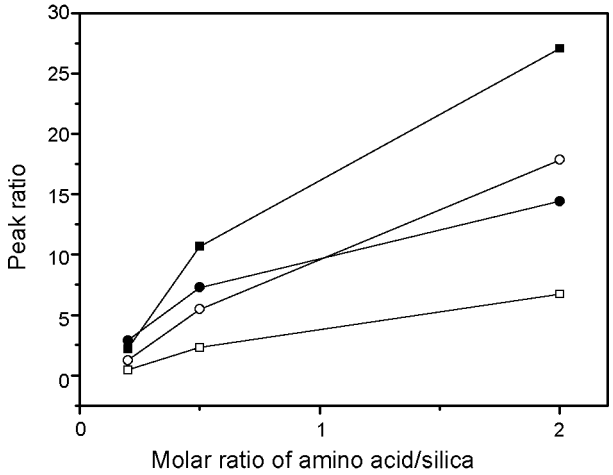


Figure 6. Variation of the methylated product ratios of isoleucine and leucine with the amino acid concentration (amino acid/silica (SiO_2) by molar ratio). The pyrolysis temperature was 500 °C. Squares and circles indicate the pyrolysis products of isoleucine and leucine, respectively. Solid and open symbols stand for the M1/M3 and M2/M3 ratios, respectively.

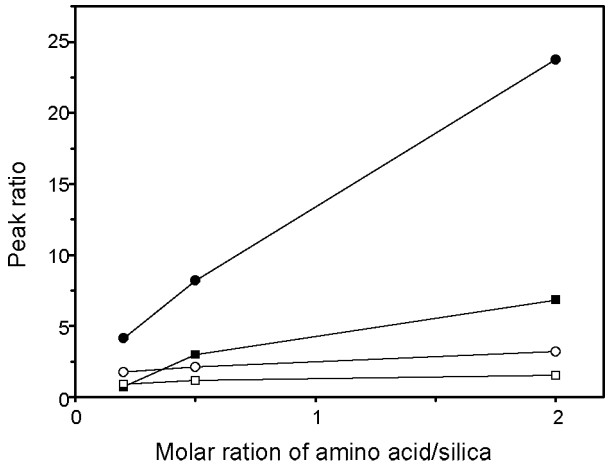


Figure 7. Variation of the methylated product ratios of methionine and phenylalanine with the amino acid concentration (amino acid/silica (SiO_2) by molar ratio). The pyrolysis temperature was 500 °C. Squares and circles indicate the pyrolysis products of methionine and phenylalanine, respectively. Solid and open symbols stand for the M1/M3 and M2/M3 ratios, respectively.

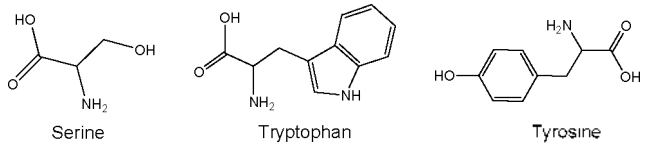
common characteristic is that they do not have any side polar groups as shown in Scheme 3. Figures 4 ~ 7 show their peak intensity ratios of the partly methylated products compared to the fully methylated one. For alanine, the monomethylated product (M1) is more abundant than the fully methylated product (M3) and the difference increases with increasing the amino acid concentration (amino acid/silica) as shown in Figure 4. The dimethylated product (M2) is less abundant than the M3 except under the high pyrolysis temperature and high amino acid concentration. The M1/M3 and M2/M3 ratios tend to increase with increasing the amino acid concentration except for the M2 at 400 and 500 °C. The pyrolysis temperature effect on the relative abundances did not show a specific trend, but only the M2/M3 at 2.0 of the amino acid concentration increases with increasing the pyrolysis temperature. For glycine, only the dimethylated product (M2) at high amino acid concentration of 2.0 is more abundant than the fully methylated product (M3). The M2/M3 ratios increases with increasing the amino acid concentration and the ratio at the amino acid concentration of 2.0 also increases with increasing the pyrolysis temperature as shown in Figure 5.

Direct methylation patterns of isoleucine and leucine show similar trends as shown in Figure 6. For isoleucine, the monomethylated product (M1) is more abundant than the dimethylated product (M2) and the partly methylated products are more abundant than the fully methylated product (M3) when the amino acid concentration is high or the pyrolysis temperature is low. The partly methylated products increase with increa-

sing the amino acid concentration. For leucine, the partly methylated products (M1 and M2) also tend to increase with increasing the amino acid concentration. The partly methylated products are also greater than the fully methylated product (M3) when the amino acid concentration is high.

For methionine, the monomethylated product (M1) is greater than the dimethylated product (M2) except for the low amino acid concentration of 0.2 and the partly methylated products are more abundant than the fully methylated product (M3) when the amino acid concentration is high (Figure 7). The M1/M3 ratios increase with increasing the amino acid concentration. For phenylalanine, the monomethylated product (M1) is also more abundant than the dimethylated product (M2) except for the low amino acid concentration of 0.2 and the partly methylated products are more abundant than the fully methylated product (M3) (Figure 7). The M1/M3 ratios increase with increasing the amino acid concentration.

Proline generated generated partly methylated product (M1) and fully methylated product (M2). The M1 is less abundant than the M2 except for the high amino acid concentration of 2.0 at low pyrolysis temperatures. For valine, the partly methylated products (M1 and M2) are more abundant than the fully methylated product (M3) when the amino acid concentration is high. Of the eight amino acids, except glycine, the monomethylated products of the seven amino acids increase with increasing the amino acid concentration. This is because the TMAH concentration is relatively low at the high amino acid concentration. Only for glycine, the monomethylated products are less abundant than the dimethylated ones. This implies that the methylation reaction of the amine group of glycine is very fast after methylation of the carboxylic acid group.



Scheme 4. Structures of amino acids which generate the trimethylated and fully methylated products.

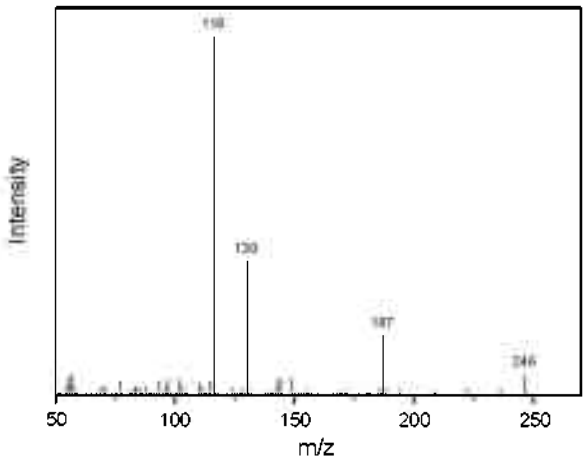


Figure 8. Mass spectrum of the trimethylated pyrolysis product of tryptophan. This is assigned to *N,N*-dimethyl tryptophan methyl ester.

Though serine, tryptophan, and tyrosine have four methylation sites, they generate only the trimethylated product and the fully methylated product (M4). They have three methylation groups including α-carboxyic acid, α-amine, and the side polar group as shown Scheme 4. Serine and tyrosine have a hydroxyl side group and tryptophan has an amine side group. The trimethylated products of serine and tyrosine are *N*-methyl-*O*-methylserine methyl ester and *N*-methyl-*O*-methyltyrosine methyl ester, respectively. Thus, the order of the methylation is α-carboxylic acid → hydroxyl → α-amine. The methylation order is closely related with the acidities of the func-

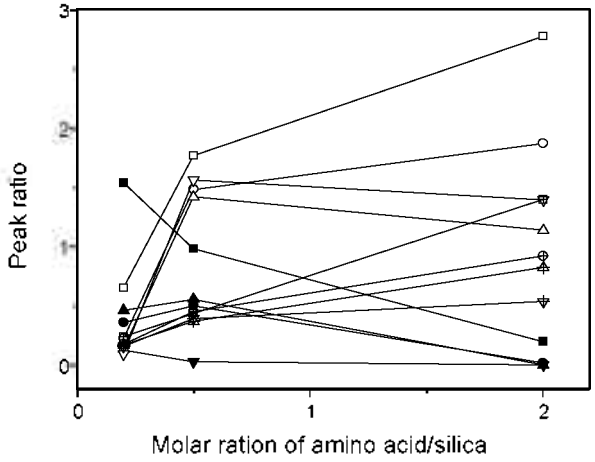


Figure 9. Variation of the methylated product ratios (M3/M4) of serine, tryptophan, and tyrosine with the amino acid concentration (amino acid/silica (SiO_2) by molar ratio). Squares, circles, up-triangles, down-triangles indicate the pyrolysis temperatures of 400, 500, 700, and 900 °C, respectively. Solid, open, and crossed symbols stand for serine, tryptophan, and tyrosine, respectively.

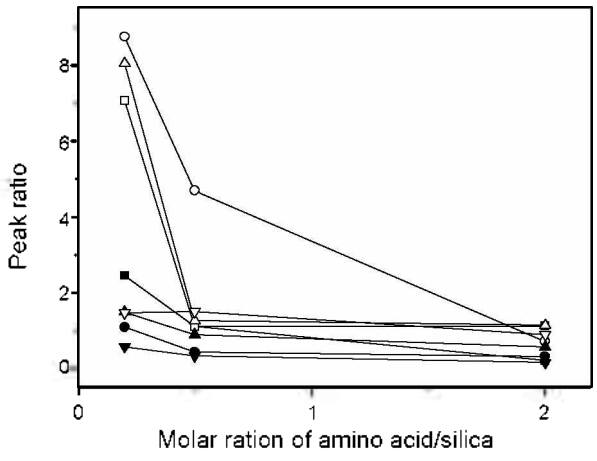


Figure 10. Variation of the methylated product ratios (M3/M5, M3'/M5, M4/M5, and M4'/M5) of lysine with the amino acid concentration (amino acid/silica (SiO_2) by molar ratio). Solid and open symbols stand for the pyrolysis temperatures of 500 and 900 °C, respectively. Squares, circles, up-triangles, down-triangles indicate the pyrolysis product ratios of M3/M5, M3'/M5, M4/M5, and M4'/M5, respectively. The M3, M3', M4, and M4' are *N',N'*-dimethyllycine methyl ester, *N,N*-dimethyllycine methyl ester, *N,N',N'*-trimethyllycine methyl ester, and *N,N,N'*-trimethyllycine methyl ester, respectively.

tional groups. The trimethylated product of tryptophan is *N,N*-dimethyltryptophan methyl ester, but Gallois and coworkers[16] reported that it was *N'*-methyl-*N*-methyltryptophan methyl ester. Principal ions (and the relative ion intensities) in the mass spectrum of the trimethylated product of tryptophan are *m/z* 116 (100%), 130 (37%), 187 (17%), and 246 (6%) as shown in Figure 8. The *m/z* 246 is the molecular ion and the *m/z* 116, 130, 187 are the fragment ions assigned to $CH_3O_2CCH=N(CH_3)_2^+$, $[M - CH_3O_2CCH\text{-}N(CH_3)_2]^+$, and $[M - CO_2CH_3]^+$, respectively. Thus, the trimethylated product of tryptophan is *N,N*-dimethyltryptophan methyl ester not *N'*-methyl-*N*-methyltryptophan methyl ester. Figure 9 shows the peak intensity ratios of the M3/M4 of serine, tryptophan, and tyrosine with the amino acid concentration. Only for tryptophan, the trimethylated product is more abundant than the fully methylated product (M4) when the amino acid concentrations are high of 0.5 and 2.0. The M2/M3 ratios of serine decrease with increasing the amino acid concentration whereas those of tyrosine increase. Variations of the M2/M3 ratios with the pyrolysis temperature do not show clear trends, but the ratios tend to increase with increasing the pyrolysis temperature.

Tri- and tetramethylated products of lysine consisted of two types as discussed previously. Figure 10 shows the peak intensity ratios of the M3 (or M3')/M5 and M4 (or M4')/M5 as a function of the amino acid concentration. The M3 (or M3')/M5 and M4 (or M4')/M5 ratios tend to decrease with increasing the amino acid concentration. This implies that formation of the tri- and tetramethylated products of lysine requires excess TMAH. At the low amino acid concentration of 0.2, the partly methylated products tend to increase with increasing the pyrolysis temperature. This implies that formation of the partly methylated products requires high energy. Thus, formation of the partly methylated products of lysine is dependent on high TMAH concentration and high energy. Consistent trends for the intensity ratios between the isomers of M3/M3' and M4/M4' were not found. For high yield conditions at high pyrolysis temperature and low amino acid concentration, the M3' (*N,N*-dimethyllycine methyl ester) is more abundant than the M3 (*N',N'*-dimethyllycine methyl ester) whereas the M4' (*N,N,N'*-trimethyllycine methyl ester) is less abundant than the M4 (*N,N',N'*-trimethyllycine methyl ester).

Conclusions

Arginine, asparagine, aspartic acid, cysteine, glutamic acid, and glutamine did not generate any directly methylated products, whereas alanine, glycine, isoleucine, leucine, methionine, phenylanaline, valine, and proline produced all the directly methylated products. The non-producing amino acids have structures composed of intramolecular hydrogen bonding while the others do not have any side functional groups such as amine, alcohol, or carboxylic acid. Lysine generated two trimethylated products (*N',N'*-dimethyllycine methyl ester and *N,N*-dimethyllycine methyl ester) and two tetramethylated products (*N,N',N'*-trimethyllycine methyl ester and *N,N,N'*-trimethyllycine methyl ester). High TMAH concentration and high energy were required to form partly methylated products of lysine. Histidine and threonine mainly generated only the fully methylated products. Even though serine, tryptophan, and tyrosine have four methylation sites, they generated only the trimethylated product and the fully methylated product (M4).

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