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Amino Acid Transformation and Decomposition in Saturated Subcritical Water Conditions

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The reactions of amino acids under subcritical water conditions in the temperature range 503–563 K using a pressure value corresponding to the saturated vapor pressure of water at the employed reaction temperature (hereafter called saturated subcritical water) were investigated. As reactants, solutions containing a single amino acid or a mixture of 17 different amino acids were used, and the obtained results in both cases were compared. Glycine, alanine, valine, and proline were produced as intermediate products during the thermal transformation of other amino acids. Generally, the results showed that the existence of amino acids together in a mixture decreased the overall stability since the activation energy values of decomposition of individual amino acids reduced significantly due to their presence in a mixture of amino acids. Most of the amino acids were found to be labile at acidic and near-natural pH values and more stable at a highly basic pH value.

Introduction

Amino acids are the building blocks of proteins. They join together to form peptides (short chains with 10–50 amino acids), polypeptides (much longer chains with >50 amino acids), or proteins (which are polypeptides of specific sequence, length, and folded conformation). Over 80 amino acids are known to occur naturally, with 20 found commonly in proteins. In industry, amino acids have many uses and applications. They can be used for the synthesis of new materials including medicines, taste enhancers, animal feeds, and even in electronic-related chemicals such as liquid crystals and exposure liquids for color copiers. Commercial interest in amino acids is an outgrowth of an understanding of the many functions that these substances perform in humans and animals. Four different methods are available for amino acid manufacturing. They are extraction, synthesis, fermentation, and enzymatic methods. The last two biotechnological processes, with their economic and ecological advantages, are responsible for this spectacular growth in interest.¹

Currently, amino acids (especially those used in products containing amino acids) are mainly manufactured by the fermentation method using natural materials. The enzymatic method suffers the disadvantage of being expensive, while the extraction method (in which natural proteins are degraded to various amino acids) is limited by many parameters, including the restriction of the production by the amount of each amino acid contained in the raw protein and the high production cost of the necessary enzymes. However, even when whole cell extract is used, the process is very difficult to control. Accordingly, other alternative and cost-effective production technologies are required to make large-scale production of these products more economical.

Recently, subcritical water technology was considered to be one of the efficient technologies for converting organic wastes to valuable materials through extraction and hydrolysis mechanisms. In general, the characteristics of water as a reactive

solvent can be evaluated with two parameters: its ion product and dielectric constant. Increasing the value of the ion product increases the concentration of both hydrogen and OH ions, which leads to a significant increase in the power of the hydrolysis reaction. Under high ion product values water possesses the effect of an acid catalyst. Conversely, a decrease in the dielectric constant decreases the water polarity and makes it possible to dissolve organic substances that do not dissolve in water under atmospheric conditions.² Therefore, the ion product becomes the measure of the interaction with hydrolysis reactions and the dielectric constant with solvation power. Both the ion product and dielectric constant of water can be controlled through regulating the temperature and pressure of water. On heating beyond the critical point of water (temperature >374 °C, pressure >22.1 MPa), water is referred to as supercritical water and it is extremely active and corrosive. On the other hand, when the temperature is controlled within the critical temperature under enough pressure to maintain a liquid state, water is called subcritical water and it can perform very selective extractions of polar (at lower temperatures), moderately polar, and nonpolar (at higher temperatures) compounds.^{3–5}

Different organic pollutants and wastes have been treated utilizing subcritical water, including hazardous organic wastes,⁶ municipal sewage sludge,⁷ biomass,⁸ degradation polymers,⁹ and marine wastes.⁵ Recently, the present authors reported on different applications of the subcritical water technology in waste reuse, recycling, and treatment.¹⁰

In our laboratory, subcritical water was found to be very efficient in extracting amino acids from proteinaceous waste compounds through a protein hydrolysis pathway.¹¹ The results showed that relatively large amounts of oil, organic acids, and amino acids could be extracted from fish, squid entrails, cow meat, and bone meal wastes using subcritical water technology.^{12–16} Using such wastes offers a priceless starting raw material which in turn seems to provide an economical route for the large-scale production of amino acids.

For a full understanding and better reactor design, both the reaction mechanism and rate equations governing the production of the amino acids from such organic wastes under subcritical water conditions should be clarified.

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In one of our previous works, fish-derived wastes were used to explore the kinetics of the protein hydrolysis and amino acid decomposition.¹⁷ However, the fish-derived wastes contained not only proteins, but also many other organic, inorganic, and metal-based compounds which were present in great amounts. The presence of such compounds might affect the mechanism and pathway of the hydrolysis reaction of the protein and amino acids as well. For this reason, it is necessary to use model compounds to investigate the general behavior of protein and amino acids in both hydrolysis and decomposition under subcritical water conditions. In very recent works we used bovine serum albumen (BSA) as a model protein to investigate protein hydrolysis under saturated subcritical water conditions. The products of the reaction were a water-insoluble solid phase and an aqueous phase. The results showed that BSA, under the tested conditions, passed through aggregation followed by gel formation processes which resulted in the formation of insoluble solid aggregates. Then, BSA unfolded, releasing polypeptides as an intermediate product and finally low-molecular-weight products such as amino and organic acids. The formed solids were found to be completely water insoluble with many plastic properties, and both aggregation and gelation processes were enhanced dramatically to the extent that we were able to synthesize a novel protein based plastic with a very strong polymer matrix using such formed solid aggregates.¹⁸ In subsequent research we studied the kinetics and mechanism of formation of such novel protein based plastics.^{19,20}

Sato and others reported the decomposition behavior of five selected amino acids (using solutions containing a single amino acid) under subcritical water conditions in the temperature range 200–340 °C and a fixed pressure of 20 MPa.²¹

Vallentyen also studied the decomposition of amino acids under subcritical water conditions. Despite the great efforts made in his research, he faced many technological problems due to the breaking of the glass reactors employed in his work.²²

Although useful data have been obtained from these studies, there is still a lack of systematic investigation on the hydrolysis and decomposition reactions that occur during the treatment of amino acids with subcritical water under a pressure value corresponding to the saturated vapor pressure of water at the employed reaction temperature (hereafter called the saturated subcritical water condition). For this reason, in the present work we investigated the kinetics and thermodynamic properties of different amino acid reactions at different pH values using a mixture of amino acids or solutions of a single amino acid under saturated subcritical water conditions. A series of experiments were made using solutions containing a mixture of 17 different amino acids and compared with the data obtained using solutions of a single amino acid. The logic behind these procedures was in part an attempt to develop a method whereby the decomposition rates of several amino acids could be determined simultaneously under saturated subcritical water conditions and in part an experimental test to see how the decomposition rates of individual amino acids might be affected by the presence of other amino acids, which represents the actual case of proteinaceous materials.

Materials and Methods

Materials. All the chemicals used in this study were produced by Wako Pure Chemical Industries, Ltd. (Osaka, Japan). A pure amino acid mixture in a liquid form containing glycine, alanine, valine, leucine, isoleucine, methionine, phenylalanine, tyrosine, serine, threonine, cysteine, proline, lysine, histidine, arginine, aspartic acid, and glutamic acid was used for the amino acid

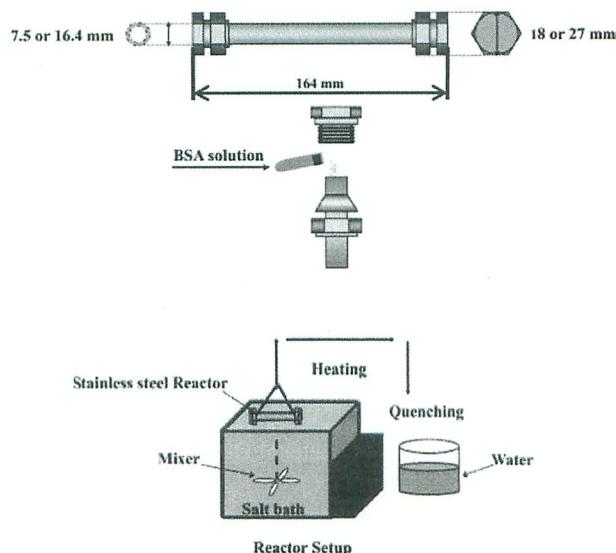


Figure 1. Batch reactor configuration and experimental setup.

decomposition experiments in the case of using a mixture of amino acids. The concentration of each individual amino acid in the mixture was 2.5 mM. Such a mixture was selected due to its availability in the market as a standard amino acid mixture. All amino acids were completely soluble in the mixture. The molecular weight is equivalent to the summation of the molecular weights of each amino acid included in the mixture.

Experimental Procedures. 1. Measuring the Change of Temperature vs Time inside the Reactor during the Heating and Cooling Periods. The temperature inside the reactor was measured instantaneously using a thermocouple installed inside the reactor and connected to a monitor showing the change in temperature. Using a digital camera, we recorded the change in temperature with snapshots of the recording monitor. Later, data were collected by viewing the slow-speed film. After reaching a steady-state temperature (equal to the bath temperature), the reactor was cooled by immersion in a water bath. The decrease in the temperature inside the reactor was recorded in the same way, by using a digital camera. All experiments were carried out in triplicate and presented together.

2. Decomposition of a Mixture of Amino Acids. The decomposition reactions were carried out using a batch reactor shown in Figure 1. A stainless steel tube (SUS16, i.d. 0.0075 m × 0.15 m, reactor volume 7.0 cm³) with Swagelok caps was used as a reactor. One milliliter of the amino acid mixture was completely mixed with 24 mL of Milli-Q water. From the previous mixture, a predetermined amount (estimated from the steam table according to the temperature used and the pressure used) was taken and charged into the reactor tube (the concentration of each individual amino acid inside the reactor was 100 μM). Then the dissolved oxygen in the sample was removed by purging it with argon gas. The reactor was then sealed and immersed in a preheated molten salt bath (Thomas Kagaku Co. Ltd.) containing a mixture of potassium nitrate and sodium nitrate. The decomposition reactions were carried out at 503–563 K. At a predetermined reaction time, the reactor was immediately cooled by soaking in a cold-water bath. The product of the reaction was recovered from the reactor for further analysis. It was observed that some gases were produced; however, in this study we ignored the gas products since very special reactor design and analysis tools are needed to collect and analyze the gas products.

3. Decomposition of Single Amino Acid Solutions. Solutions of individual amino acids were prepared using Milli-Q water and subjected to the subcritical water reaction at 503–563 K following the same procedures described above. The concentration of each individual amino acid inside the reactor was around 35 mM. It is important to report that the concentration of each amino acid in the case of using a mixture of amino acids was adopted to be within the solubility values of all amino acids in the mixture. However, in the case of a single amino acid a higher concentration was used because the concentration of the decomposition products was too low to be detected by even HPLC systems. Accordingly, we had to use a relatively higher concentration to be able to measure the quantity of the decomposition products.

Analytical Procedures. Measuring the Amino Acid Concentrations. The amino acid concentrations were measured using an HPLC system with a combination of an ion exclusion column (Shedex Rspak KC-811, JASCO Co., Osaka, Japan) and a post labeling method with a spectrofluorophotometer (Shedex Rspak KC-G, JASCO Co., Osaka, Japan). Seven different buffer systems with different concentrations, pH values, and flow rates were used under the manufacturer's standard method. Data acquisition and analysis software were provided by the instrument's manufacturer. The retention time of each organic acid was confirmed by injecting a sample in which a known amount of the authentic acid was added as an internal standard.

Theoretical Section

An amino acid (A) is expected to undergo several reactions under the saturated subcritical water condition including decarboxylation, deamination, transamination, and/or in some cases ring opening to produce direct decomposition intermediate (B), which undergoes further decomposition and produces a final decomposition product (C). Carbonic acid (which easily decomposes to carbon monoxide or dioxide), ammonia, and water are produced as the final decomposition products. The decomposition of amino acid can follow either a consecutive or a parallel reaction pathway with a first-order rate law. The kinetic analysis was done based on a general approach of the first-order reactions within the tested temperature and pressure. The data of the time course of concentration of amino acids under the subcritical water decomposition were fit to the first-order reaction approach, which is expressed as

$$C_A = \exp[-kt]C_{A_0} \quad (1)$$

where C_A is the amino acid concentration, k is the kinetic constant, t is the reaction time, and C_{A_0} is the initial amino acid concentration.

The decomposition reactions of amino acids were also analyzed thermodynamically. Both the Arrhenius and Eyring equations were used for describing the temperature dependence of the reaction rate. The Arrhenius equation is founded on empirical observation and common sense; that is, chemical perception suggests that the higher the temperature the faster a given chemical reaction will proceed (eq 2).

$$k = A \exp[-E/RT] \quad (2)$$

A is the preexponential factor (sometimes called frequency factor), E is the activation energy, and T is the absolute temperature.

On the other hand, the Eyring equation, which represents a theoretical construct, is based on transition-state theory. This theory provides a direct link between the preexponential factor

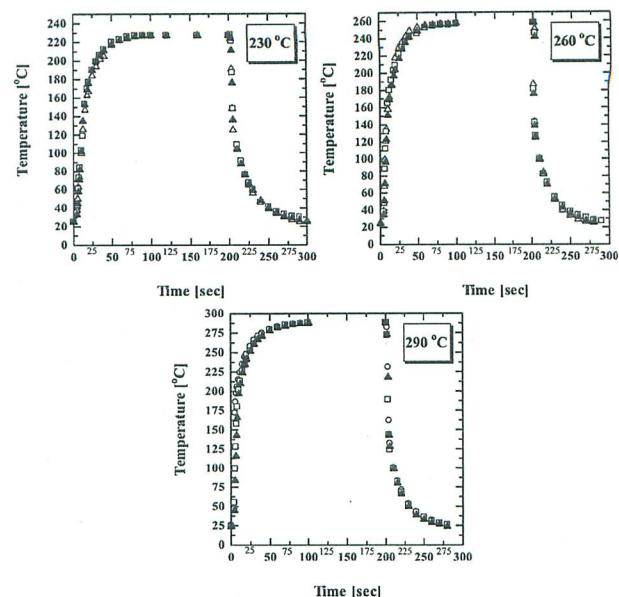


Figure 2. Profiles showing temperature vs time inside the batch reactor used in this study at different salt bath temperatures under the saturated subcritical water condition.

Table 1. Approximate Time Required To Attain a Steady-State Condition inside the Reactor at Different Temperatures

temp [K]	steady-state time [s]	
	heating	cooling
503	70	18
533	75	17
563	80	15

and the entropy of activation, which is the entropy change (ΔS) that appears in the thermodynamic form of the rate equation obtained from conventional transition-state theory. The pre-exponential factor is used to calculate the activation entropy from the following equation (eq 3):

$$A = \exp[\Delta S/R]k_B T e/h, \quad \text{where } \Delta S = R \ln[A/T(k_B e/h)] \quad (3)$$

k_B is the Boltzmann constant (1.38×10^{-23} J/K), h is Planck's constant (6.626×10^{-34} J s), e is the base of the natural logarithm (2.718), T is the average reaction temperature used, and A is the preexponential factor.

Results and Discussion

Kinetic Data. Prior to the kinetic study it was necessary to explore the profiles of temperature vs time during unsteady-state periods inside the batch reactor under the applied saturated subcritical water conditions. Figure 2 shows profiles of the temperature vs time in seconds at different salt bath temperatures. Both the heating and cooling times are listed in Table 1. It could be concluded that the ultimate heating time is about 80 s for reaction temperatures in the range 503–563 K while the cooling time was about 18 s to reach lower than 353 K. On the basis of these results, we designed the kinetic experiments to be in the range 2.5–40 min to avoid collecting any data during the thermal instability period inside the reactor and we ignored the cooling time (since it is very short compared to the reaction time).

For the kinetic analysis, the changes in the concentration of each amino acid during the reaction of the mixture of amino acids were measured as a function of the reaction time. The

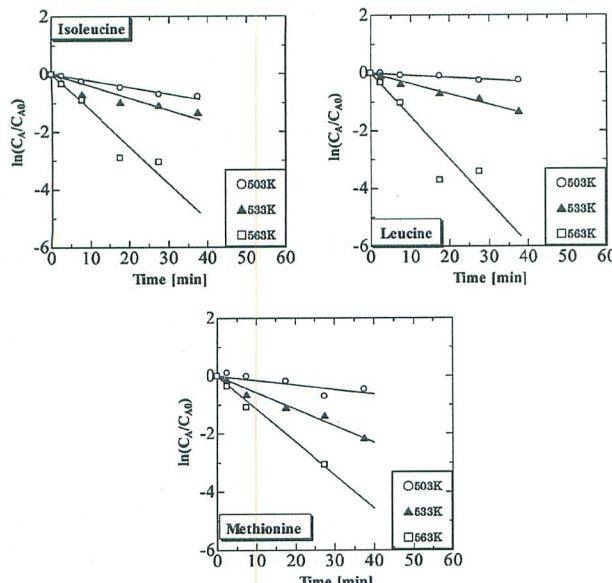


Figure 3. Effect of temperature on first-order decomposition rates of amino acids having a hydrophobic (nonpolar) aliphatic R group in a mixture of amino acids.

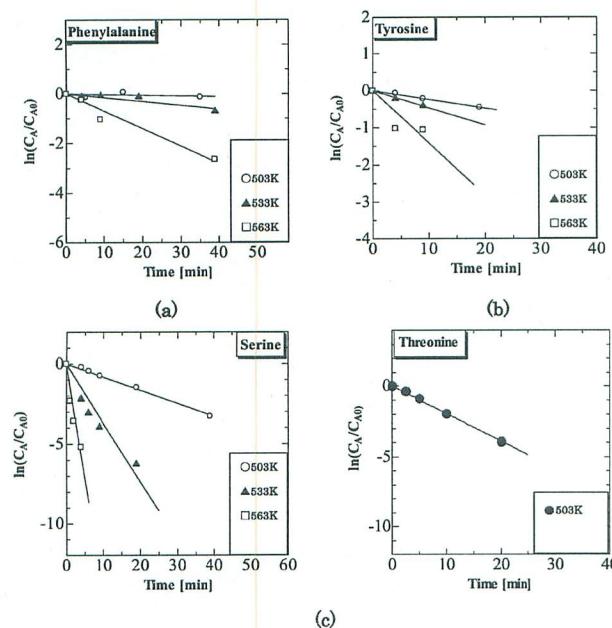


Figure 4. Effect of temperature on first-order decomposition rates of amino acids having a hydrophobic aromatic R group (a), a hydrophilic aromatic R group (b), and a hydrophobic polar aliphatic R group (c) in a mixture of amino acids at pH 6.2.

decomposition rates of different amino acids were determined simultaneously in the mixture by means of a general kinetic approach for the first-order reaction within the temperature range 503–563 K and reaction intervals of 2.5–40 min. The amount of water used in the reactions was adjusted to attain a pressure corresponding to the saturated vapor pressure of water at the tested temperature. The water vapor pressure was estimated from a steam table to be 2.8–7.4 MPa within the tested temperature range. Figures 3–5 show the first-order plots of decomposition rates of different amino acids using solutions containing a mixture of amino acids under a constant condition of pH (pH 6.2). As the experimental data was correlated in straight lines,

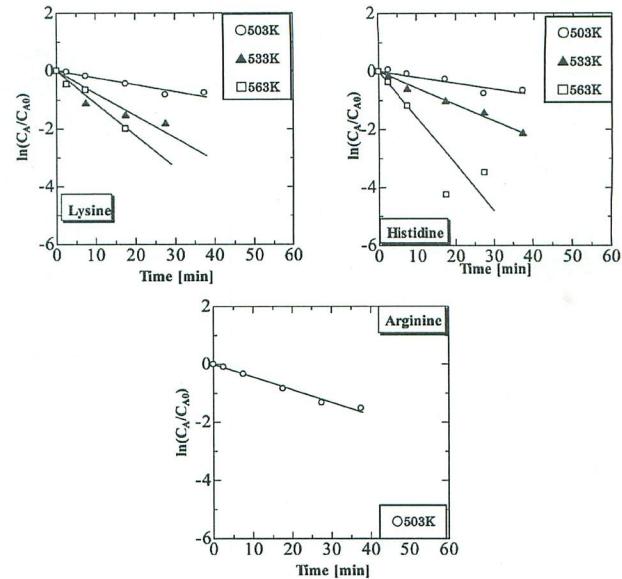


Figure 5. Effect of temperature on first-order decomposition rates of amino acids having a positively charged R group in a mixture of amino acids at pH 6.2.

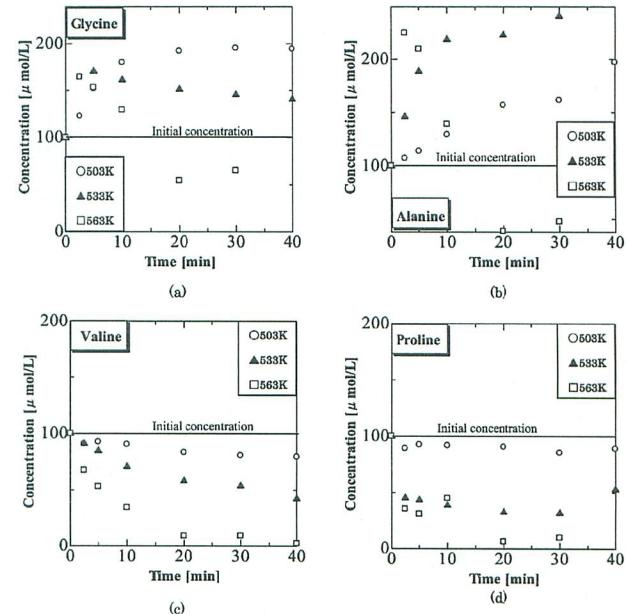


Figure 6. Effect of temperature on the decomposition of glycine (a), alanine (b), valine (c), and proline (d) in a mixture of amino acids at pH 6.2.

it could be concluded that the decompositions of the tested amino acids are considered to be first-order reactions. Such a result agrees with what has been reported elsewhere.^{18,22–25} However, it was noticed that for some amino acids the experimental data slightly deviated from the theoretical line at 563 K, in the case of using a mixture of amino acids. These amino acids are isoleucine, leucine (Figure 3), tyrosine (Figure 4), and histidine (Figure 5). We were uncertain about the exact order of their decomposition reaction rates due to such deviations. However, performing the decomposition reactions using solutions containing only one individual amino acid did not show the same deviation and the results could be correlated well with the first-order approach (as will be explained in Figures 7–9). Accordingly, such deviations may be explained as being due to some side decomposition reactions with other

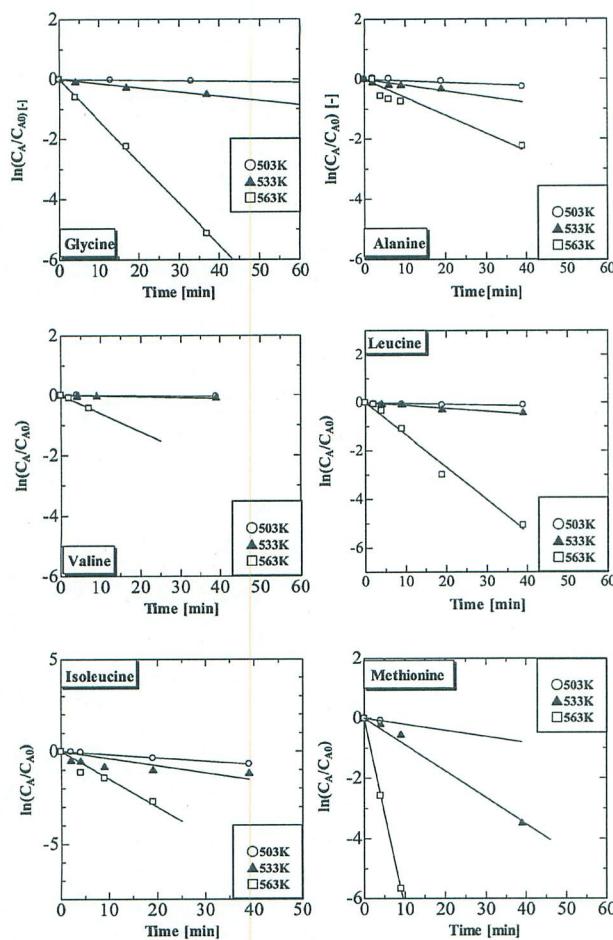


Figure 7. Effect of temperature on first-order decomposition rates of amino acids having a hydrophobic (nonpolar), aliphatic R group in the solution of an authentic amino acid.

amino acids existing in the mixture rather than as deviations from the first-order approach.

In general, amino acids differ only in the nature of the substituent R group. Accordingly, we classified them on the basis of the attached R groups. Therefore, they were classified into amino acids with hydrophobic aliphatic, hydrophobic aromatic, hydrophilic aromatic, or hydrophilic aliphatic R groups and amino acids carrying negatively or positively charged R groups. As a general observation, the results showed that the decomposition rates of amino acids in the mixture under the tested conditions corresponded to the reaction temperature. In the cases of glycine, alanine, valine, and proline, the decomposition rate constants could not be calculated in these cases because these amino acids are produced as intermediate products from the decomposition of other amino acids in the amino acid mixture. Figure 6 shows the time course for the decomposition of these amino acids at different temperatures. The results clearly show that the concentration of glycine and alanine exceeded their initial concentrations ($100 \mu\text{M}$) at all tested temperatures. Although this phenomenon was not clearly observed in the case of valine and proline, the production of these amino acids was confirmed during the study of the decomposition products of each individual amino acid (which will be presented in more detail in a future work) as an intermediate product of the decomposition of lysine and arginine, respectively.

The first-order decomposition rate constants of all tested amino acids in the mixture were evaluated again using solutions

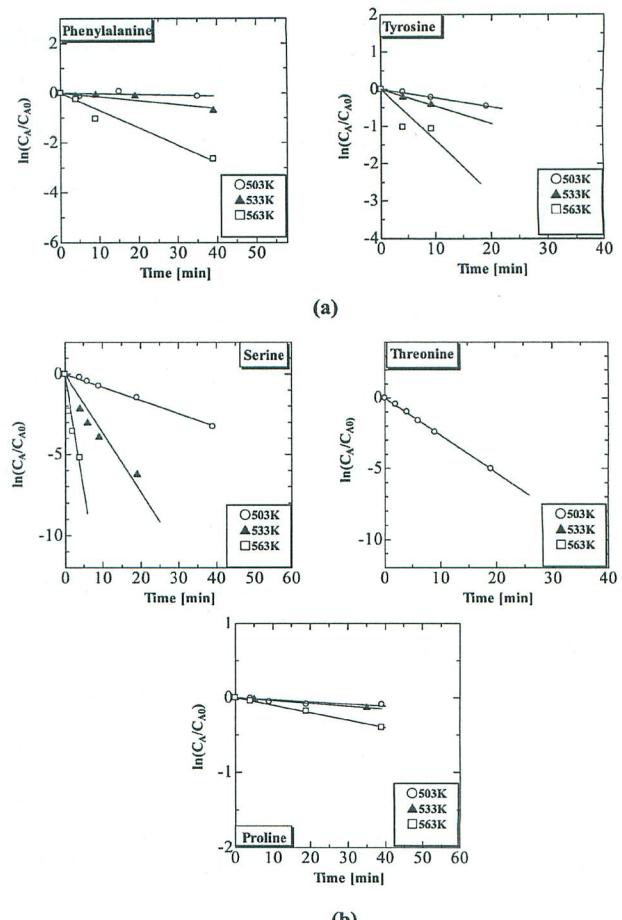


Figure 8. Effect of temperature on first-order decomposition rates of amino acids having a hydrophilic (polar), aliphatic R group (a) and amino acids having a hydrophilic (polar), aliphatic R group (b) in the solution of an authentic amino acid.

containing only one amino acid (Figures 7–9). The reaction rate constants were calculated from the regression lines and compared with those obtained using a mixture of amino acids and are listed in Table 2.

First, let us discuss about the effect of the attached R group on the decomposition behavior of amino acids.

For the first group (that having a hydrophobic aliphatic R group) we found that the rate of the decomposition has no general tendency. For example, methionine and isoleucine respectively were found to be the most labile amino acids in this group at 503 K (regardless of presence in the mixture of amino acids or alone) while having decomposition rates of (0.02 and 0.018) and (0.016 and 0.023) min^{-1} in the cases of being alone and being in the mixture, respectively. However, valine was the most stable (0.0011 min^{-1}). On the other hand, at the higher temperature of 563 K, methionine recorded the highest rate of decomposition while alanine and valine were the lowest. The same phenomenon was also observed for the amino acids having positively charged R groups, lysine and histidine. It could be concluded from these results that the attached R group has no direct effect on the amino acid stability under the saturated subcritical water condition.

Among all tested amino acids, cysteine and glutamic acid were the most labile amino acids while glycine and valine were the most stable. Concerning glutamic acid, it was reported that it readily lactamizes when it is heated to form pyroglutamic

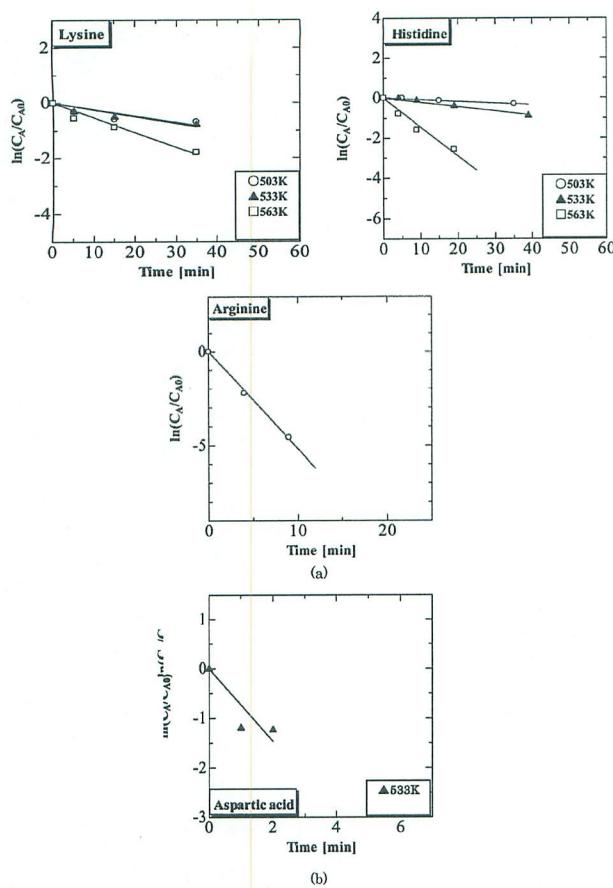


Figure 9. Effect of temperature on first-order decomposition rates of amino acids having a positively (a) or negatively (b) charged R group in the solution of an authentic amino acid.

acid. Such a reaction is somewhat reversible, though equilibrium is achieved much more rapidly from left to right (direction of decomposition) than the reverse and in a near-natural pH condition (as in our case, pH 6.2) the position of equilibrium lies far to the right (in the direction of decomposition). Glutamic

acid can be quantitatively regenerated from its pyro form by a 3 h period of hydrolysis with 1 N HCl.²² Aspartic acid, threonine, and arginine, respectively, came second in order after cysteine and glutamate for their lability. All these labile amino acids vanished at temperatures higher than 503 K or less as in the cases of cysteine and glutamate. Therefore, their decomposition rates were too fast to be calculated. Accordingly, it was not possible to calculate the thermodynamic parameters for these amino acids.

It was good to find that the rate of decomposition data for the amino acids glycine, alanine, leucine, aspartic acid, and serine obtained in our work agree with the data obtained elsewhere under quite different conditions. Miller and Bada²⁴ investigated the decomposition rate of these amino acids in an aqueous solution. In their experiment, a mixture containing 10^{-3} M alanine, leucine, aspartic acid, and serine was heated to 250 °C at a pressure of 26.8 MPa in 0.01 M HEPES buffer (*N*-2-hydroxyethylpiperazine). The ionic strength was initially adjusted to 0.7 M with NaCl and pH was adjusted to 7.0. Samples were collected over a period of 6 h and were analyzed for amino acids. Aspartic acid, followed by serine, displayed the most rapid decrease in concentration with time. Glycine appeared in the analysis after 1 h and increased initially rapidly and continued to increase but at a diminishing rate with time. Likewise, the concentration changes of alanine and leucine were rapid initially but the changes in concentration decreased with time.

Second, in this study we have been concerned with the effect of other amino acids on the decomposition rate of each individual acid. For the sake of investigating this point, let us compare the data obtained for the first-order decomposition rate constants of amino acids determined by using solutions containing only one of each of the amino acids and measuring that using a solution containing a mixture of all the amino acids. A comparison of the two experimental sets is presented in Table 2. The results show that the use of a mixture of amino acids had some effect on the relative stability order of the amino acids which was determined using single solutions of amino acids. In the cases of leucine, isoleucine, phenylalanine, and histidine, the stability is roughly reduced almost 2-fold or less due to the presence of the mixture of amino acids. Such behavior indicates

Table 2. Effect of the Presence of Amino Acids in a Mixture of Amino Acids at pH 6.2 or Solutions of Authentic Amino Acids on Decomposition Rate Constants at Different Temperatures

R group type	amino acid	reaction rate const, k [min ⁻¹], at different temps					
		503 K authentic	503 K mixture	533 K authentic	533 K mixture	563 K authentic	563 K mixture
hydrophobic nonpolar aliphatic	glycine ^a	0.0018	—	0.014	—	0.14	—
	alanine ^a	0.0057	—	0.020	—	0.061	—
	valine ^a	0.0011	—	0.0027	—	0.061	—
	leucine	0.0038	0.0076	0.012	0.036	0.13	0.15
	isoleucine	0.018	0.023	0.039	0.042	0.15	0.13
	methionine	0.020	0.016	0.088	0.058	0.63	0.11
hydrophobic aromatic	phenylalanine	0.0029	0.0079	0.016	0.036	0.070	0.11
hydrophilic aromatic	tyrosine	0.024	0.0079	0.047	0.044	0.14	0.13
hydrophilic polar aliphatic	serine ^b	0.082	0.021	0.37	0.17	1.44	—
	threonine ^b	0.26	0.20	—	—	—	—
	cysteine ^b	—	—	—	—	—	—
	proline ^a	0.0028	—	0.0037	—	0.010	—
positively charged	lysine	0.023	0.024	0.025	0.077	0.053	0.087
	histidine	0.0086	0.020	0.022	0.056	0.14	0.16
	arginine ^b	0.52	0.044	—	—	—	—
negatively charged	aspartic acid	0.73	—	—	—	—	—
	glutamic acid ^b	—	—	—	—	—	—

^a The rate constant k could not be calculated for these amino acids because they were produced by other amino acids. ^b These amino acids decomposed very quickly, and the rate constant k could not be calculated.

Table 3. Effect of pH on the First-Order Decomposition Rate Constants in a Mixture of Amino Acids at 503 K

R group type	amino acid	reaction rate const, k [min $^{-1}$], at different pHs		
		pH 2.5	pH 6.2	pH 10.5
hydrophobic nonpolar aliphatic	leucine	0.0072	0.0076	0.0011
	isoleucine	0.026	0.023	0.0073
	methionine	0.0041	0.016	0.011
hydrophobic aromatic	phenylalanine	0.0067	0.0078	0.00012
hydrophilic aromatic	tyrosine	0.0023	0.0078	0.0042
hydrophilic polar aliphatic	serine	0.082	0.021	0.013
	threonine	0.19	0.20	0.055
positively charged	lysine	0.046	0.024	0.034
	histidine	0.038	0.017	0.011
	arginine	0.012	0.044	0.75
negatively charged	aspartic acid	0.32		0.48

that some interactions among these amino acids and other amino acids present in the mixture occurred and led to an accelerated rate of decomposition. By contrast, tyrosine, serine, and arginine behaved oppositely, since the stability was enhanced roughly 2-fold. This may signify that these amino acids are protected by other amino acids present in the solution. Methionine and lysine showed no significant differences in both cases. Other amino acids could not be compared because these amino acids are produced as intermediate products from the decomposition of other amino acids, as mentioned before.

Third, let us explore the effect of changing the pH of the amino acid solution on the decomposition rate of each tested amino acid under the saturated subcritical water condition. Because both α -carboxylic and α -amino groups in amino acids are capable of ionizing, it is expected that the pH will have a significant effect on the decomposition rate by altering the equilibrium condition of the amino acids. At a neutral pH solution of amino acid, the zwitterion is the dominant species; however, this species can undergo acid–base reactions by adding either a strong acid or a strong base to the solution. In an acidic pH, the α -carboxylic end of the amino acid picks up an H^+ ion to form a molecule with a net positive charge, while in an alkaline pH the α -amino end of the amino acid loses an H^+ ion to form a molecule with a net negative charge. Table 3 shows the first-order decomposition rate constants of different amino acids (classified in the same way as in Table 2) measured under different pH values using solutions containing mixtures of all tested amino acids at 503 K. The data for the first-order decomposition rate constants are not shown to minimize the number of presented figures. For leucine, isoleucine, phenylalanine, serine, threonine, and histidine, it was found that the higher pH value enhanced the stability of such amino acids by lowering the decomposition rate. By contrast, methionine, tyrosine, lysine, and arginine were more stable in the acidic media. Among the tested amino acids, the decomposition rate of aspartic acid could not be determined at near-neutral pH (because of a high rate of decomposition at this pH) and showed high stability at basic or acidic pH values. From these results it could be concluded that most amino acids are labile at acidic and near-natural pH and more stable in the highly basic pH, which represents the ionized form. It is well-known that, under the subcritical water condition, the ion product increases dramatically and so in turn does the hydrogen ion concentration. Accordingly, the power of the hydrolysis reaction increased due to the increase in acid catalytic effect. Thus, in the same way, at lower pH the hydrolysis reactions were enhanced accordingly. Such a conclusion is in agreement with what has been reported elsewhere²⁶ for alanine decomposition under subcritical water conditions in a flow reactor.

Thermodynamic Data. Both activation energy, E_a and entropy, ΔS , were calculated from the Arrhenius plot and Eyring equation, respectively, using the calculated values of the decomposition rate constants measured at three different temperatures (503, 533, and 563 K, respectively) at pH 6.2 (Figures 10 and 11). The obtained values are listed in Table 4. For a mixture of amino acids, the serine recorded the highest activation energy of decomposition under the subcritical water condition while the lysine recorded the lowest. For aspartic acid, glutamic acid, and arginine the activation energy could not be calculated because of their high rates of decomposition under the testing conditions.

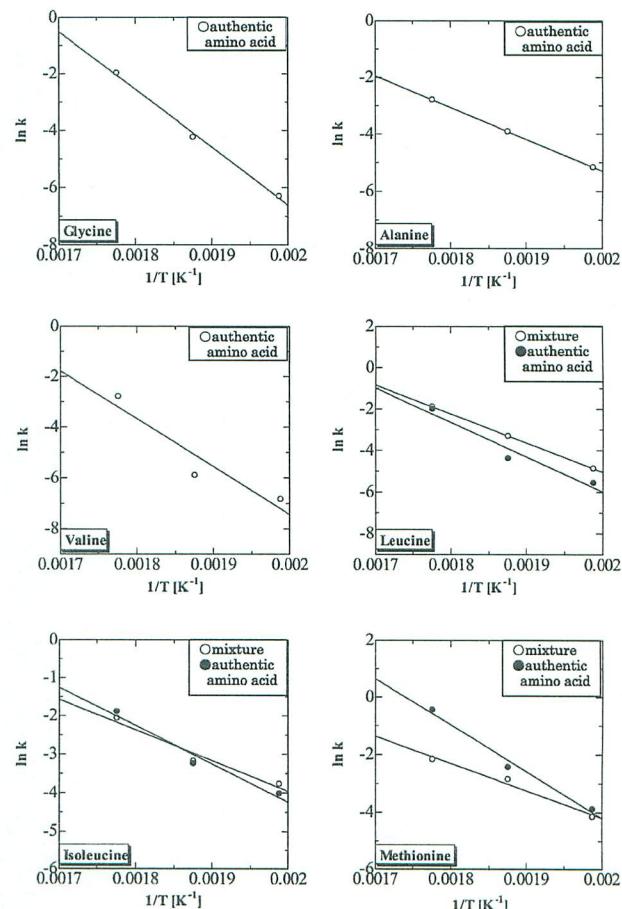


Figure 10. Arrhenius plot of amino acids having a hydrophobic (nonpolar), aliphatic R group in a mixture of amino acids (closed circle) and the solution of an authentic amino acid (open circle) at pH 6.2.

Table 4. Effect of the Presence of Amino Acids in a Mixture of Amino Acids and the Solutions of Authentic Amino Acids on the Energy of Activation and Entropy of the Decomposition of the Amino Acids at pH 6.2

R group type	amino acid	activation energy, E_a [kJ/mol]		activation entropy, ΔS [kJ/K·mol]	
		authentic	mixture	authentic	mixture
hydrophobic nonpolar aliphatic	glycine	169.8	—	-7.6	—
	alanine	93.2	—	-149.9	—
	valine	157.0	—	-40.0	—
	leucine	138.5	116.6	-64.8	-100.9
	isoleucine	83.1	66.4	-161.3	-192.2
	methionine	134.1	78.2	-58.7	-170.6
hydrophobic aromatic	phenylalanine	125.3	103.6	-91.6	-126.1
hydrophilic aromatic	tyrosine	69.3	111.3	-186.1	-110.5
hydrophilic polar aliphatic	serine	112.2	151.9 ^a	-89.8	-21.9 ^a
	proline	49.3	—	-243.9	—
positively charged	lysine	46.4	51.8	-229.0	-218.9
	histidine	110.2	81.7	-114.1	-162.3

^a Calculated based on two values of the reaction rate constants at two different temperatures.

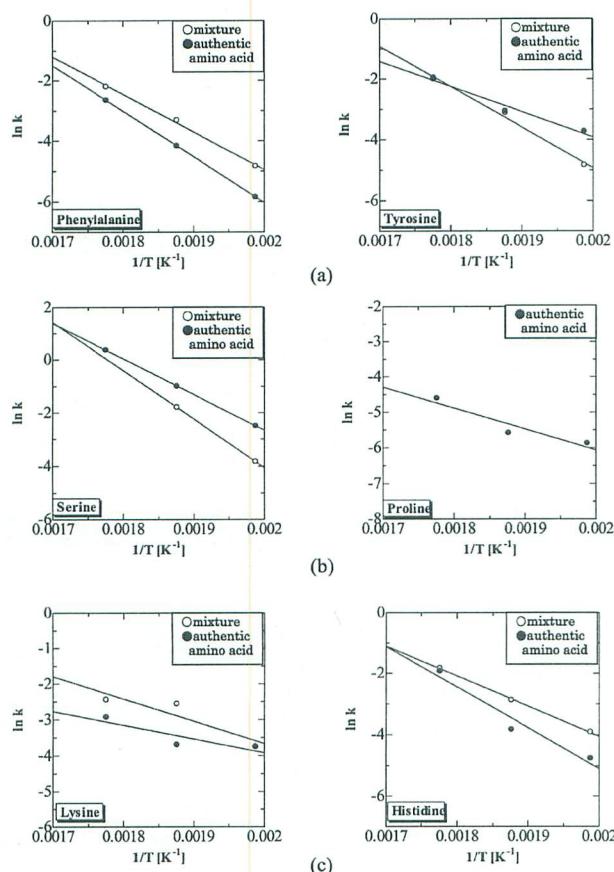


Figure 11. Arrhenius plot of an amino acid having a hydrophilic or hydrophobic aromatic (a), a hydrophilic (polar), aliphatic R group (b), or a positively charged R group (c) in a mixture of amino acids (filled circles) and the solution of an authentic amino acid (open circles) at pH 6.2.

Comparing the activation energy values for the decomposition rates of amino acids measured in the case of being in a mixture of amino acids and singly decomposed in a solution revealed no big difference in the case of lysine. However, for other amino acids a significant difference could be observed. Except in the case of tyrosine, the results showed that the activation energy in the case of single amino acids was higher than that in the case of a mixture of amino acids. These results were reflected again when we explored the values of the activation entropy. These values give information about the relative structure of the transition state compared to the ground state of the reactants.

Generally, the activation energy and entropy increased in the case of using a single amino acid, which indicated that the transition state became more ordered compared to the ground state (reactant state) with higher stability, but the opposite was observed in the case of tyrosine, which showed a higher stability when present in a mixture of amino acids.

Conclusions. In short, the obtained results in this study measured under the saturated subcritical water condition showed that the existence of amino acids together in a mixture decreased their overall stability. Moreover, most amino acids are labile at acidic and near-natural pH and more stable in highly basic pH, which represents the ionized form. Such observations lead to the conclusion that the extraction of amino acids from proteinaceous material should be done under very mild temperature conditions at either near-natural or basic conditions depending on the target amino acids required to be extracted.

Future Work. In the very near future we are going to present a detailed study for the pathway of the reactions of amino acids under saturated subcritical water conditions.

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